

ISSN-0973-9122 (Print) • ISSN-0973-9130 (Electronic)

Volume 16

Number 3

July-September 2022



Indian Journal of Forensic Medicine & Toxicology

Website: www.ijfmt.com

Official Organ of Indian Association of Medico-Legal Experts (Regd.)

Indian Journal of Forensic Medicine & Toxicology

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Print-ISSN:0973-9122 Electronic - ISSN: 0973-9130

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Website: www.ijfmt.com

"Indian Journal of Forensic Medicine & Toxicology" is peer reviewed quarterly journal. It deals with Forensic Medicine, Forensic Science, Toxicology, DNA fingerprinting, sexual medicine and environment medicine. It has been assigned International standard serial No. p-0973-9122 and e- 0973-9130. The Journal has been assigned RNI No. DELENG/2008/21789. The journal is covered by EMBASE (Excerpta Medica Database). The journal is also abstracted in Chemical Abstracts (CAS) database (USA). The journal is also covered by EBSCO (USA) database. It is now official publication of Indian Association of Medico-Legal Experts (Regd.).

Published at

Institute of Medico-legal Publications

Logix Office Tower, Unit No. 1704, Logix City Centre Mall,
Sector- 32, Noida - 201 301 (Uttar Pradesh)



Indian Journal of Forensic Medicine & Toxicology

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Pattern and Distribution of Injuries from the Fall from the Height in Fatal Cases in a Tertiary Care Hospital, Chennai

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How to cite this article: Amirtha Sultana, Yuhesh Somasundaram, Shankar Subramanian et al. Pattern and Distribution of Injuries from the Fall from the Height in Fatal Cases in a Tertiary Care Hospital, Chennai, Indian Journal of Forensic Medicine and Toxicology 2022;16(3):1-5.

Abstract

Background: The second leading cause for the injury related deaths is fall from height. The injuries fatalities depends on the height of fall and its impact surface. Landing position is also considered as an important parameter.

Aim: The aim of the study is to study the pattern and distribution of injuries from the fall from height in fatal cases.

Methodology: This study includes 100 cases of victims brought to the tertiary care hospital with the history of fall from the height. The cases were brought from the accident site or after death as a consequence of fall in this tertiary care hospital. During autopsy all the relevant details like basic details and the injury details like dimensions, injury types and the primary impact site were taken. Blood and urine examination were done for finding alcohol and drugs. Data entered in MS excel and analysis done in SPSS 23 software. P value <0.05 is considered to be significant.

Results: Majority of the study participants belongs to 31-40 years of age group(30%) followed by 41-50 years of age (18%). Male predominance was observed in our study (90%).34% were daily labours. The most common external injury is abrasion noted in 90% of the study participants. The most common primary impact site was head (50%) followed by backside (12%).

Conclusion: Fall from heights cause significant morbidity and mortality. It is important to create awareness among the workers and to provide safety gears for the persons working in construction sites.

Keywords: Fall; height; morbidity; mortality; Impact.

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Introduction

According to the International classification of the disease fall is defined as An event which results in a person coming to rest inadvertently on the floor or the ground or the lower level. Next to the Traffic accidents, it is the fall from the height which is considered as the major cause of the trauma in both developing and the developed countries. Each year 646000 falls occurs approximately. In Low income countries and the middle income countries the falls related fatalities occurs in 80% of the population. Falls accounts for 60% of deaths in the south east asia region and the Western Pacific region. In the year 2019 according to the NCRB (National Crime Records Bureau accident and suicide statistics) it was observed that around 12048 cases of falls have been registered in India.¹ Of which the male female ratio was found to be 5.4:1 where 11997 were fatal cases. The most common victims belongs to 30-45 years of age.²

Fall from the height is the most common cause of severe blunt trauma in India. In Present situation the falls from the height was found to be increased in urban areas due to the increased construction activities. It occurs generally in the accidents, suicides and to less extent in homicides. Soon after the fall there is a damage in the body due to the energy absorption at the impact site. This energy is similar to the kinetic energy which will cause the injuries to the body parts. The velocity will depends on the height of fall to some extent. The pattern of injuries depends upon many factors like the height of fall, velocity, the primary impact site, the contact tissue viscosity and elasticity and ground surface etc.³ Around 37.3 million falls requires medical attention due to the severity of the injury. Such falls will also leads to DALY (Disability adjusted life years) lost. Falls most commonly occurs in 65 years or more followed by 15-19 years of age and <15 years of age. This study was aimed to study the pattern and distribution of injuries from the fall from height in fatal cases.

Methodology

Study setting:

Hospital based cross sectional study was conducted in the Department of Forensic Medicine, Madras Medical College, Chennai which is a tertiary

care centre. The study was done for a period of one year, from August 2016 to August 2017.

Sample Size:

All the victims brought from the accident site or after death as a consequence of fall during the study period were included. The sample attained is 100.

Data Collection:

The following parameters were documented in the study proforma.

1. Personal particulars like Name, Age, Gender, occupation, nature of fall, site of primary impact, nature of floor on which they fall, height from which they fall, period of survival were documented from the investigating officers.
2. In fall from height cases, the height was determined by visiting the crime scene and taking measurements.
3. Survival period was calculated from the treatment findings and the autopsy findings.
4. Precipitating factors like mental illness, epilepsy, natural diseases and the use of drugs or alcohol were found with special efforts.
5. Data related to the injury types (internal or external), nature of injury, its dimensions and location where also recorded. Primary impact injuries photo was taken and noted.
6. Blood and urine examination were done for analysis of alcohol or drugs if suspicion.
7. For the treated patients clinical data was obtained.
8. Autopsy was conducted by Letulle's method of an en masse removal of viscera and dissection of organs.
9. Dissection of head, scalp, dura, vault and base of skull were examined for the head injuries. To find out the kind of hemorrhage like Sub dural Haemorrhage (SDH), Extra dural Hemorrhage (EDH), Sub Arachnoid Hemorrhage (SAH), Intra ventricular Haemorrhage (IVH) and the Intra cerebral Haemorrhage (ICH), brain was dissected carefully. The blood infiltration areas were cleaned with the help of water, then it is dissected and examined to assess the nature of injury and its extent.

Statistical analysis:

After collecting the data, it was entered in MS excel Windows 10. Statistical analysis was done in SPSS 23. Continuous data were expressed in terms of Mean±Standard deviation and Categorical variable were expressed in terms of numbers percentages. P value of <0.05 is considered as significant.

Results

Table 1: Age and sex of the Victims

Age of the victim	Male	Female	Total
<10	0	1	1
11-20	7	2	9
21-30	16	0	16
31-40	27	3	30
41-50	18	0	18
51-60	16	2	18
>60	6	2	8
Total	90	10	100

Among the study participants majority were male (90%) followed by Females (10%). Most of male study participants belongs to 31-40 age category followed by 41 to 50 years of age. Among the female majority belongs to 31-40 years of age.

Table 2: Occupational status of the victims:

Occupation	Number
Daily Labour	34
Construction worker	15
Unemployed	14
Student	10
Others	9
Painter	7
Driver	5
Farmer	2
IT worker	2
Own business	2

In our study 34% were Daily labourers, 15% were construction workers and 14% were unemployed.

Table 3: Distribution of primary impact injuries:

Part of the body injured	Primary impact injuries (N)
Head	50
Back	12

Chest	10
Neck	9
Shoulder	7
Foot	7
Face	5

More than 50% of the study participants have primary impact on Head followed by Back 12% and in turn by Chest 10%.

Table 4: Pattern of injuries:

Nature of injury	Number(N)
Abrasions	90
Contusions	11
Lacerations	31
Fractures	131
Intracranial Hemorrhage	82

In our study for majority of victims Fractures were noted 131 Followed by Abrasions which is observed in 90 cases. Intracranial hemorrhage is noted in 82 Cases. Among fractures noted the most common site of fracture is Skull which alone noted in 40 cases followed by the Base of the skull fracture which is noted in 37 cases.

Table 5: Distribution of the fatal injuries:

Site of injury	Number of cases (N)
Head injury	34
Multiple injury	31
Spinal injury	17
Cerebrospinal injury	11
Pelvic injury	4
Blunt injury abdomen	2
Multiple	1
Total	31

Most of the study participants in our study had head injury 34% followed by multiple injuries 31%. 17% had spinal injury and 11% had cerebrospinal injury.

Discussion

In our study out of the 100 cases with the history of fall from the height 90% were male and the remaining 10% were female. These results were supported by the studies done by Mukesh et al.⁴, Lalwani S et al.⁵ and Kumar JVK et al.⁶ The maximum cases belonged to 31-40 years of age (30%) followed by 41-50 years (18%) and 51-60 years of age (18%). Majority of the victims belongs to 21-50 years of age

(64%). Similar results were observed in Mukesh et al. study, Prathapan V et al.⁷ and Kohli A⁸ et al. Most of the female victims belongs to 11-20 years of age.

In present study the primary impact site was head (50%) followed by back of the body (12%). Similarly Prathapan V et al.⁷ also in his study on fall from the heights observed that 53% of his cases have head as primary site of impact. Similarly 46.6% of the cases have head as the primary site of impact in study done by Kumar JVK et al.⁶

The most common external injury observed in our study is Abrasions 90%. Contusion was least in our study 11%. Similar results was also observed in Mukesh et al.⁴ study where he found abrasion alone in 16(40%) and abrasion with other wounds in 27(67.5%). Contusion reported in 3(7.5%) of his cases.

In Fatal injuries head, Spinal and Cerebrospinal injury together constitute 62%. Intracranial hemorrhage was noted in 82% of our study participants. Sub arachnoid hemorrhage was common type noted followed by subdural type. Similar results was observed in Mukesh et al. study⁴ and also in the Hartshorne et al.⁹ and also in the Goonetilleke et al.³

Liver is the common organ to be injured in the abdomen. Heart damage and Kidney's damage is not observed. Heart is not damaged as it has rib cage to protect it. Kidney's due to its small size and its location it narrowly escapes from the damage. The results of the Hartshorne et al. was different from our results. He stated that that chest injury and the abdominal injury were very rare. We can explain that if the height of the fall increases the injury to chest

and abdomen will increase.

Limitation:

The main limitation of the study is it is a single centric study and we didn't compare rural and urban population. Secondly in our study we didn't mention the height of the falls. Thirdly the time interval between the injury and death is not noted in our study. Substance abuse history and pattern was not elicited.

Funding:

No funding is given for any of the authors

Competing Interests:

There is no Competing Interest

Acknowledgement

The authors like to thank the Head of the Department of Forensic Medicine, Medical College, Dibrugarh.

Conclusion

It is concluded in our study that most of the fall from height occurs in workplace. The patterns were different from that of the ground level fall or of pedestrian injuries. Skull fractures involves vault and the base. Intracranial and intraventricle hemorrhage also noted along with the fractures. Head injury is followed by spinal injury and cerebrospinal injury. Most of the victims were daily labourers followed by the construction workers.

Recommendations:

It is recommended to do a multicentric study all over the country to study all patterns of injuries. Awareness camps should be conducted to the public about the risks involved in the rooftops and about the safety measures for the prevention of falls. Proper lighting and safety railing should be present in all balconies and roofs. For workers involved in construction of multistorey building it is essential to give safety gears like helmet, Airbags and nets. Pre employment risk assessment is essential before placement to rule out comorbidities like hypertension and the diabetes.

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A Case Report on a Dead Man Walking in a Rural University of South Africa

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How to cite this article: BL MEEL. A Case Report on a Dead Man Walking in a Rural University of South Africa. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):6-10.

Abstract

Background: Abuse of power in rural universities is less visible to the public but widely prevalent. Myriad forms of corruption are prevalent in the rural university concerned. Productive staff is generally appreciated and awarded in most universities but in this rural university, they get punished, terminated, or subjected to disciplinary inquiries.

Objective: To report on the abuse of power and difficulties of an academic in a rural university of South Africa.

Case History: X was an employee of the department of health and affiliated with the local University in a dual system of employment where he has served the university for over two decades (1996 to 2018). He underwent persistent harassment, bullying, and dehumanization in his early period of employment. It progressively evolved to a more serious form with disciplinary inquiries, suspension, stoppage of salary, and birthday bonus. The managers of this rural university tried hard repeatedly to carry out constructive termination but failed. Mr. X retired after his completion of age 65 with respect and appreciation. The history, duration, and different designs of abuse of power have been described in this report.

Conclusion: It is guilty, to be honest, and hardworking in a rural university in South Africa as it functions like a secretive society.

Keywords: Power abuse; management; dehumanization.

Introduction

South Africa came on the world map when the first heart transplant was carried out in 1967.¹ Recently, it came into the limelight when the Omicron virus was discovered by a South African scientist on 24 November 2021.² South African universities and their academics are doing excellent research to maintain their high standards and ranks amongst universities worldwide.³ These academic institutes are highly competitive and strive to maintain their high grading therefore academics work hard to

maintain research output of high quality. Some well-known universities have the policy to publish a minimum of two research articles by their academic staff in a year and on that basis, they are promoted and awarded. The management of these universities motivates and awards their staff. There is a rural university that also has academics but to carry out the research is analogous to committing a crime-riddled with malice and professional jealousy. You are subjected to various forms of punishment in the form of reduced salary, no promotion, and disciplinary enquires in extreme cases. These so-called

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“managers” of the university are pseudo-academics. They feel entitled to authorship without intellectual contribution simply because they are in positions of power. They hold meetings and discuss the subject of research requirements but in fact are themselves obstacles to research.⁴

Mr. X is a medical specialist registered with the Health Professions Council of South Africa, who works in a public hospital as well as in the university’s medical school (joint establishment). He has received no annual increase in his basic salary.⁵ The occupational specific dispensation (OSD) scale was implemented in 2009, but thereafter it has not been revised.⁵ Mr. X’s earnings have consequently dropped to 40% less than those of his professional colleagues.⁵ Mr. X has written several letters to express his grievances to the human resource department of the university but has received no satisfactory answer.⁵ The main purpose of this case report is to highlight the problem of abuse of power and lack of accountability in the rural university of South Africa.

Case history

The report is a monumental example of victimization. Mr. X has undergone all sorts of pain

and suffering but he has confidence that he will clear all the charges. Mr. X was an employee in the department of health as well as the local university, a dual system of employment inherited from the former university. He has served the university and the department of health dutifully from 1996 to 2018. In fact, he was primarily employed by the department of health as they contributed to a salary of more than 60% plus overtime allowance. Mr. X has not understood the culture of the university concerned. He thought that hard work and research publications would earn him promotion, appreciation, and respect. This thought process was his fatal flaw that ultimately led to his unfair treatment. He underwent persistent harassment and dehumanization in his initial phase of employment but tolerated it as he had limited choice. The team of management changed but not the mindset. The rural university was behaving like a secretive society and only a few can get benefited. As time progressed the treatment became more vicious with disciplinary inquiries, suspension, stoppage of salary, and birthday bonus. The managers of this rural university tried hard to carry out constructive termination but failed. Mr. X retired after his completion of age 65 with respect and appreciation.

Table 1: Calendar of abuse of power from 2008 to 2018 in a rural university in South Africa.

Date	Charges	Origin	Inquiry commissioner	Outcome	Duration
01.08.2008	DC1: plagiarism	E-mail of a girlfriend through the University of FortHare	A top labour law-practitioner (external)	Found not guilty on 22.12.2009	1 year & 4 months and 22 days
04.08.2010	DC2: self-plagiarism with Suspension	Organized emails from editors by the FHS dean	appointed staff (internally)	Withdrawn (13.06.2011) Suspension lifted, 18.10.2010	11 months and 9 days
2010	Professional Death sentence	No reason	-----	Withdrawn by a letter 28.03.2014	Almost 4 and half years
11.07.2013	Forensic audit	No basis for audit	Auditing firm	Continued till date	Continued till retirement
24.02.2016	DC3: absence from duty	Absence without permission	A lecturer from the law faculty	Discontinued	About a month
26.02.2016	Block salary	Dean emailed HR	Blocked by Murano	Paid later	1 month
15.09.2018	Stopped Birthday bonus	HR instructions just after the meeting of the department of health and University	Kanile	Paid half in October 2018	-----
December 2018	Group Insurance not refunded	It was refunded to fellow Prof. Cisco	Kanile	It is still not paid	No explanation
Personal record file in HR lost	Revealed in a meeting with faculty and HR	Requested an inspection of personal record file but was refused.	Murano	Still no explanation of this missing file	The letter is written on 03.07.2015.

Discussion

It is well known that professional jealousy is prevalent in universities, but hardly to the level of maliciousness endured by Mr. X who was tormented and pressurized to resign from his post and leave the university. It was a series of persistent DCs, a reduction in the salary of an employee, and no promotion.^{6,7} Higher management generally protects a productive staff from internal as well as external threats in a university but in the case of Mr. X, they played an active role.^{6,7} The workers union was the only entity that helped Mr. X, otherwise, his forceful premeditated resignation may have come to fruition. The maliciousness is self-evident by the repeated consecutive similar DCs, each one accompanied by harsher punishment with paradoxically less serious allegations and charges. Mr. X. brought a case of a university employee in the same faculty who had, in fact, duplicated an article. This fact was blatantly ignored, and no disciplinary inquiry was instituted against the concerned individual. The initiator of this DC was also head of the faculty at the time and refused to carry out an inquiry pertaining to the other staff member with the duplicated article. He was adamant in his quest to only institute a DC inquiry and punish Mr. X. This serves Mr X's case of intentional premeditated and pre-orchestrated academic and financial ruin by the heads, managers, and administrators of this rural university. He spent many hours of his time and his golden years doing research and academic work at this university which led to the recognition of this relatively unknown institution by the scientific committee both locally and internationally. The lack of recognition and respect by the managers is both surprising and disheartening to Mr. X. It also highlights not just disrespect towards Mr. X but also the attitude of this pseudo-academic institution towards academia. In my opinion, such institutions should be blacklisted by the scientific world.

This report highlights a unique case of mistreatment of Mr. X by a rural university. Mr. X not only survived but also cleared his charges before his retirement in 2018. After facing a decade-long, persistent, malicious disciplinary inquiry, suspensions, forensic inquiry, low paid salary, and birthday bonus from 2008 to 2018 in a rural university in South Africa, X was cleared of the malicious charges forced on him prior to his retirement. Two other senior staff members were also informed of the imminent disciplinary inquiry, but they resigned and left the university before the inquiry ensued. The human resource department (HR) is a puppet of the so-called managers and actively colluded against

Mr. X. It is weak and helpless and does not deserve the title of "human resource" department. This was also pointed out by an external assessor of this report.⁸

Disciplinary inquiries

Three consecutive disciplinary inquiries, aimed at constructive dismissal were carried out from 2008 to 2018. The first two DCs were based on plagiarism, the so-called *double jeopardy* in legal terms. Third DC was manufactured through the department of health where they showed that the department of health is the primary employer of Mr. X, in dual employment. It is true that the department of health contributes a major portion of his salary. Mr. X is sandwiched between two employers. Dual employment is complicated.^{6,7} Such an employee needs greater protection as the primary employer, in this case, the department of health, paid the salary of Mr. X through a second employer and in this regard ended up exploiting him.^{6,7}

The first DC was serious, and the allegation was that Mr. X plagiarised material of his publication from someone else but there was no suspension. Mr. X was not found guilty. When Mr. X asked that when he is not found guilty then the complainant must be guilty, and his degree must be revoked but the university refused. This is the position of the rural university as they may not have a policy document to revoke the degree.

The second DC was less serious, Mr. X was suspended, wherein, Mr. X was alleged to have plagiarised his own material and had duplicated publication. The managers could not succeed in manipulating the outcome of the first DC, as the inquiry commissioner was independent and submitted the report truthfully. Subsequently in the second DC, they were very careful in appointing their own commissioner, a foreign staff from the rural university. They were appointed telephonically without any terms of reference. This fact came into the picture during the process of DC when the union representative asked the head of the institution to be present as a witness. The head was invited because he told a team of union representatives that Mr. X is guilty prior to the completion of the inquiry. The telephonically appointed commissioner told the union representative that the head is a powerful, "big" man and therefore, he cannot call him to appear as a witness. This commissioner comes from a country where there is a system of hierarchy and "concepts of the big man and small man exist" and nobody can challenge or question the "big" man. At this time, the union representative asked the appointed commissioner to show his official letter of appointment, and the commissioner failed to do so.

Third DC was started in 2016 and managers were assured by the appointed commissioner that he will "fix" Mr. X. Mr. X went on leave which was approved by his HOD as per the university practice, but the head manager of faculty also wanted Mr. X to get his approval before leaving. This was not in the rules of the faculty. The faculty manager was new to his job. The commissioner of inquiry was appointed and before the inquiry started this commissioner told his colleague about DC against Mr. X and that he will "fix" Mr. X. This colleague was a union member and part of the inquiry later. This conversation was disclosed to Mr. X by the union member in writing to protect Mr. X and to carry out fair justice. It was surprising to know that the appointed commissioner was a past convict and had served jail time. The punishment entailed a lack of pay for a period of one month to Mr. X as he was under suspension.

1. Suspensions

Generally, a reasonable university carries out an explanation from an employee if there is some dispute or irregularities but in the case of Mr. X, there was no explanation asked by the immediate head of the faculty. Everything was decided unilaterally. It was an unforgettable experience that Mr. X has come across in his life for the first time and even to this day he experiences regular flashbacks during his retirement period. No option of internal inquiry was utilized by the head of the rural university in the case of Mr. X. It was a blessing in disguise as a credible commissioner of inquiry was appointed in first DC, which probably they regretted later.

The suspension was not even required but was awarded to Mr. X by the head of the institution. It was so sudden that when Mr. X came to work in the morning and he found that the locks of his office had been replaced. When he enquired then the person in charge, told him that he has done it on the instruction of HR. When Mr. X went to HR, they issued a letter of suspension. All this came as a surprise to Mr. X. He had just finished his first DC and the second DC with suspension was more rigorous than the first one. The charge was that he had duplicated his article in two journals. Mr. X reported to his union, and they were surprised by it but firm to help Mr. X. Mr. X has found duplications of articles among four staff members in his faculty and at least one had clearly duplicated his work. The union member went to the head of the faculty who had started the malicious DCs and showed him this duplicated article. They asked him to suspend

him as well, but this head of the faculty refused to suspend him. He said that it is only Mr. X who was guilty as we wanted to punish him. It is sad. Surprisingly the suspension was lifted within two and half months as they needed Mr. X to conduct the 4th year MBChB students' examination.

2. Professional death sentence

Mr. X utilizes his work material in his writing and if he does not work, then he cannot carry out research. Therefore, they stopped him to carry out research only, and was not allowed to enter the laboratory. The managers of the university coerced the head of the laboratory not to allow Mr. X to use the laboratory, a so-called professional death sentence in legal terms. This was because one cannot get material for publication without a laboratory. This was their intention to stop him from working so that Mr. X cannot publish. This sanction existed for greater than 4- years. It is sad that universities are motivating their staff to do research and this rural university stopped Mr. X from publishing.

3. Forensic audit

The managers of the university still could not stop their thirst to stop the maliciousness against Mr. X. They started a forensic audit to find out something about Mr. X so that he can be chased out of the university. Unfortunately, they failed to find anything, and still, the report is pending. A letter was written to the university head by the research office regarding a report of the forensic audit, but they could not produce it to date.

4. Freezing research accounts

The forensic auditor could not find anything, but the frozen research funds were not released. A letter from the research office of a rural university was written to the head of an institution, and then a few approved claims were released from duplicate copies. There were approved claims from the office of the research directorate that were lost, and those claims were never received.

5. Stoppage of salary

Stoppage of salary for one month with suspension was associated with this third DC. Subsequently, Mr. X was near retirement, and he was worried about his pension. Mr. X has written several letters and emails to the head of the rural university, but it was not his priority to solve any aggrieved staff issue. He was also behaving like a tourist in university with paid holidays as he was not concerned about anything.

6. Stoppage of birthday bonus

There was a meeting organized by the department of health, and the university regarding the issue of Mr. X. Mr. X was presented with a lawyer and a union member. It is two days before the middle of the birthday month of Mr. X. The University HR representative returned to his office and ordered to stop the birthday bonus of Mr. X. This was a surprise to Mr. X, and he reported to the head of an institution, but he has not taken any action.

7. Discrimination in payment of Group insurance refund

Mr. X was not refunded the unauthorized deduction of group insurance money from his salary but another professor in the same rural university was refunded. When Mr. X raised the issue, then he was told how another professor can leak this confidential information to Mr. X.

8. Personal record has been lost from the human resource department

It will be surprising to hear that a personal record file of an employee has been lost before his retirement and no action has been taken against the HR director or clerk who is the custodian of a record. It is a serious offense and must be registered with the police for an investigation. This is probably because the file belongs to Mr. X.

9. Trend in promotion in a rural university

Generally, a promotion in a university is decided based on the academic profile of the staff, but this is not a trend in the rural university. Research publications are merely a requirement on paper for a promotion. Most of the appointed professors at the rural universities either have few or no publications and as such are not worthy of such a title. There is a new trend to getting promoted to Professorship at this university, whereby, a staff member is invited to deliver a 1- hour lecture, the so-called professorial lecture. The invited lecturer is a pre-specified staff member who does not need to have any research publication and it takes just one hour to bypass the system and get promoted to a professorship. This was a new addition to academic corruption.

Conclusion

Mr. X has undergone a series of humiliation and financial ruin because of this ongoing one after other,

endless inquiries and suspension. Mr. X has done the highest amount of research work culminating in a plethora of publications. His work brought this less well-known university onto the world map. His research has been recognized and awarded nationally and internationally. Mr. X can recover financially but the pain and suffering are unforgettable. He is walking like a dead man.

Ethical Issue

This is the case report that was kept anonymous and confidential. The author has used a fictitious name and tried to keep the anonymity of the university and managers. If it is linked in some way, it is not the intention of the author as it is not an individual problem, system degradation, and failure.

Funding: Self-funded

Conflict of interest: None

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Assess Severity of Organophosphate Poisoning by Peradeniya Organophosphorus Poisoning (Pop) Scale

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How to cite this article: Brinda Mevada, Sangita Parikh, Kaushika Chaudhari et al., Assess Severity of Organophosphate Poisoning by Peradeniya Organophosphorus Poisoning (Pop) Scale. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):11-16.

Abstract

Background: OP compound poisoning is an important indication for emergency admission in most hospitals throughout India. WHO estimates that approximately 3 million pesticide poisonings occur worldwide and causing more than 2,20,000 deaths. Peradeniya Organophosphorus poisoning scale grades the severity of OP poisoning and assess the prognosis of patients. The present study aims to correlate serum cholinesterase level and the clinical criteria score described by the POP scale at initial presentation and the severity of poisoning with need for ventilation and outcome.

Methods: A hospital based cross sectional observational study was conducted over period from October 2017 to October 2019 at tertiary care hospital, Ahmedabad. Total of 75 patients were evaluated for POP scale and serum cholinesterase levels for assessment of severity of poisoning. POP scale was studied to predict the need for ventilatory support, duration of hospital stay and outcome.

Conclusion: In our study males were most commonly involved (61%). Most commonly affected age group was between 25 to 40 years. 72% had mild, 25.33% had moderate, 2.77% had severe OP poisoning according to POP score. Respiratory failure was more common in moderate and severe OP poisoning, 68.42% and 100% respectively. Prolonged ICU stay (more than 5 days) was required in moderate and severe group of OP poisoning, 63% and 50% respectively as compared to mild group (16.67%). 100% mortality was noted in severe group, 47.36% was noted in moderate group.

Keywords: OP poisoning; POP score; severity.

Introduction

OP compound poisoning is an important indication for emergency admission in most hospitals

throughout India. OP compounds are used as pesticides, herbicides, chemical warfare agents in form of nerve gases.^{1,2} The widespread availability

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of these compounds has increased the likelihood of poisoning. WHO estimates that approximately 3 million pesticide poisoning occur worldwide and causing more than 2,20,000 deaths.³

OP acts by inhibiting the enzyme cholinesterase, results in accumulation of acetylcholine at synapses and myoneural junction leading to cholinergic over activity.⁴ Salivation, Lacrimation, Urination, Defecation, Gastric cramps, Emesis (SLUDGE) symptoms occur acutely within minutes to hours. Mortality ranges from 4-30% in Indian studies. Respiratory Failure is the most common complication of OP poisoning leading to death.⁵ Early recognition and prompt ventilator support may improve survival.

Peradeniya Organophosphorus poisoning scale is a clinical scale developed by Senanayake et al. at the university of Peradeniya, Srilanka to assess the severity of OP intoxication.⁶ This study is an attempt to grade the severity of OP poisoning using POP scale, categories them into mild (0-3), moderate (4-7) or severe degrees (8-11) and also to assess the prognostic value of this scoring by comparing with various clinical parameters. This might enable clinicians to identify patients at high risk of dying soon after presentation, allowing more intensive monitoring and treatment.

Peradeniya OP compound scale has not been studied much in Indian scenario. The present study aims to correlate serum cholinesterase level and the clinical criteria score described by the POP scale at initial presentation and the severity of poisoning with need for ventilation and outcome.

Aims and Objectives

1. Study clinical profile of patients with OP poisoning.
2. To assess the correlation of POP scale and serum cholinesterase level in predicting
 - a). The clinical severity, need for ventilator support and duration of ICU stay
 - b). To predict outcome of patients with OP poisoning.

Materials and Methods

A hospital based cross sectional observational study was conducted over period from october 2017 to october 2019 at tertiary care hospital, Ahmedabad.

Type of Study: Cross sectional, observational study.

Sample size: 75 cases.

75 patients of Acute Organophosphorus Poisoning who were above the age of 18 years admitted in medicine department.

Selection Criteria

(a) Inclusion Criteria

- Patients with history of exposure to organophosphorous compounds with in previous 24 hours with characteristic clinical manifestations of organophosphorous compound poisoning.
- Patients who give valid consent.

(b) Exclusion Criteria

- Patients who receive treatment with atropine before admission.
- Patients with history of OP poisoning with other substances.
- Patient with history of serious systemic illness which alter the clinical course and outcome.

Methodology

The study was done on the patients admitted in medicine department of tertiary care hospital, Ahmedabad during the period of study (i.e. oct 2017 to oct 2019). After obtaining the informed consent, patients with history of exposure to organophosphorous compounds within 24 hours were included in study according to aforementioned inclusion and exclusion criteria. Epidemiological and clinical data were recorded in proforma. Patients were evaluated for Peradeniya organophosphorous poisoning scale and serum cholinesterase levels for assessment of severity of poisoning. Peradeniya oraganophosphorous poisoning scale was studied to predict the need for ventilatory support, duration of hospital stay and outcome. Patients were observed during hospital stay for possible complication and respiratory failure. Data was symmetrically arranged in microsoft excel sheet and the data was further analysed by SPS software and inferences were drawn.

Observation and Results

In our study, male patients were 46 (61%) out of 75, and female patients were 29(39%). In this study, male to female ratio is 1.58:1. So study suggested male preponderance.

Majority of the patients were from age group between 25 to 40 year. 35(47%) patients of the 75 were from this age group. Total 68% patients were below 40 years. 24(32%) of the 75 were above 40 years of age.

Table 1: Age vs Outcome

Age	Survived No (%)	Expired No(%)
18-25	15(93%)	1(7%)
25-40	29(83%)	6(17%)

Age	Survived No (%)	Expired No(%)
>40	20(83%)	4(17%)
Total	64(85.34%)	11(14.66%)

When mortality rate was analyzed in different age group, A lower mortality rate was noted in age group of less than 25 years compared to age group above 25. However, mortality rate was similar between 25 to 40 years and above 40 years of age group patients.

Table 2: Time interval b/w consumption and hospitalization vs Outcome

Time interval (hours)	Survived no (%)	Expired no(%)	Total no. of patients(n=75)
Less than 1 hr	22(91.67%)	2(8.33%)	24(32%)
1 - 3 hr	31(88.67%)	4(11.33%)	35(46.66%)
3 - 6 hr	11(68.75%)	5(31.25%)	16(21.33%)
More than 6 hr	—	—	—
Total	64	11	75

Significant lower mortality was noted in patients who came to hospital in less than 3 hrs after ingestion of poisoning. There was 8.33% mortality in patients who were admitted within 1 hr after ingestion of poison which was significantly lower than those who were admitted after 3 hrs (31.25%).

Nausea, vomiting (100%) were present in all patients admitted with op poisoning. Other most common symptoms were breathlessness, altered sensorium. Seizure was noted in 5.33% of cases. On

examination finding, most consistent sign was miosis, noted in all patients admitted with poisoning. Out of 57 patients who had breathlessness, mechanical ventilator support was required for 21 patients suggesting respiratory failure.

When peradeniya scoring was applied, majority of patients were in mild to moderate POP score group and only 2.77% patients were in severe POP score group. Maximum patients (72%) were admitted with mild Peradeniya score.

Table 3: POP score vs ventilatory support and duration of ICU stay

Score	Ventilator support		Duration of ICU stay		Total no of patients (n=75)
	Yes	No	Less than 5 days	More than 5 days	
0-3	6(11.11%)	48(88.88%)	45(83.33%)	9(16.67%)	54
4-7	13(68.42%)	6(31.57%)	7(36.84%)	12(63.16%)	19
8-11	2(100%)	0	1(50%)	1(50%)	2
Total	21(28%)	54(72%)	—	—	75

Out of 75 subjects involved in study, 21(28%) of them developed respiratory failure and required mechanical ventilation support. When patients distributed according to Peradeniya score, out of 54 in mild group 6(11.11%) of them required mechanical ventilator support which was lower than in moderate group (68.42%). All patients from severe pop score group were presented with respiratory failure and required ventilator support.

Duration of ICU stay was noted during study, and classified patients according to duration of ICU stay more than 5 days or less than that. So in mild pop score group poisoning, only 16.67% subjects required more than 5 days ICU hospitalization as compared to the moderate and severe group this was significantly low. Respiratory failure was most common complication noted in our study. Total 21 Patients who were admitted with respiratory

failure. One patient who was initially on mechanical ventilator support, subsequently improved and extubated, but on the 3rd day of hospitalization, he had respiratory failure due to intermediate syndrome and subsequently he did not survive despite all efforts. There were 11 deaths out of total 75 patients. 9(47.36%) patients expired from moderate pop score

group and 2(100%) were in severe poisoning. There was no mortality noted in mild poisoning even when 6(11.11%) patients developed respiratory failure. So this data suggested that higher mortality was observed among moderate to severe poisoning as compared to mild poisoning according to POP score.

Table 4: Serum cholinesterase level on admission Vs outcome

Level	Survived no (%)	Expired(%)	Total
<10%(<465 U/L)	0	2(100%)	2(2.66%)
10%-20%(465-930U/L)	9(60%)	6(40%)	15(20%)
20%-50%(930-2330 U/L)	25(89.28%)	3(10.71%)	28(37.33%)
>50%(>2330 U/L)	30(100%)	0	30(40%)
Total	64(85.33%)	11(14.66%)	Total=75

In this study, 40% patients had serum cholinesterase level more than 50%(>2330 U/L) which was normal and all of them survived. 28(37.33%) patients had serum chE level between 20-50%, out of

them 3(10.71%) patients expired, and 40% mortality rate was noted who had serum chE level between 10 to 20%. 2 patients admitted with less than 10% serum chE level and both of them expired.

Table 5: Comparison of severity according to serum cholinesterase level Vs POP score

Pop score	Serum cholinesterase level				Total
	< 10% (Severe)	10-20% (Moderate)	20-50% (Mild)	>50% (Normal)	
Mild(0-3)	0	0	24	30	54(72%)
Moderate(4-7)	0	15	4	0	19(25.33%)
Severe(8-11)	2	0	0	0	2(2.66%)
Total	2(2.66%)	15(20%)	28(37.33%)	30(40%)	75

When severity of op poisoning was compared according to serum chE level and POP score, 40% patients had normal serum chE level and 37.33% patients had mild level poisoning according to serum chE level. 72% patients were from mild op poisoning group according to POP score. So this mild group of patients according to pop score was comparable with normal to mild group according to SchE level. According to pop score 25.33% patients and according to Serum chE level 20% cases were noted in moderate group of poisoning. In severe group, according to POP score and serum chE level, 2.66% patients were noted. Hence severity according to POP score was comparable to that according to serum chE level.

Discussion

This study was conducted in medicine department of a tertiary care hospital from October 2017 to October 2019. Total 75 subjects were studied. The clinical and diagnostic finding of our study

is compared with other studies as follows. In our study, majority of the patients were within age group 25-40 yr (47%). This is in comparison to study done by goel et al.⁷ Basavraj et al.⁸ vernekar et al.⁹ This study reveals male preponderance with 46(61%) males involved from total 75 subjects. Female patients accounting for 29(39%) cases. The male to female ratio in this study is 1.58 : 1. This results are in comparison with study done by vernekar et al.⁹, Goel et al.⁷ Most of the patients in this study were from mild group of pop score. 54(72%) patients reported in mild pop score, whereas only 2(2.77%) patients from severe pop score. 19(25.33%) patients presented with criteria of moderate pop score. This is comparable to study done by Basavaraj et al.⁸ Respiratory failure was the most common complication which may develop within 24 h after exposure. Early onset of respiratory failure is due to cholinergic over activity, whereas late onset respiratory failure has been attributed to respiratory infections and intermediate syndrome.

In our study, the chances of developing respiratory failure and requiring ventilator support was highest in the severe group. The probability decreases as you move towards the mild group. This indicates peradeniya score as a tool for early prediction of respiratory failure. 21 of the 75 patients required ventilatory support, out of them 13(68.42%) patients were from moderate pop score group. 6 (11.11%) patients out of 54 in mild pop score group required ventilatory support. All patients from severe pop score group required ventilatory support. This results are comprable to study done by Basavraj et al.⁸ and pavan et al.¹⁰

Another outcome was noted in moderate to severe op poisoning with higher rate of prolonged hospitalization compared to mild group. In our study 2 patients who had severe op poisoning, 1 of them died within one day and another one was intubated for 7 days and then expired. From moderate group, 63.17% patients were admitted in icu for more than 5 days compared with mild group, only 16.67% patients were admitted in icu for more than 5 days. This results are comparable with study done by V Vernekar et al.⁹

We observed 100% mortality rate among severe op poisoning according to pop score. Among moderate pop score with total 19 subjects, 9(47.36%) patients expired. There was no mortality noted in mild pop score poisoning group, despite initial mechanical ventilator support. So these results were suggests that patients with moderate to severe pop score on admission having higher mortality than patients with mild score. This results are comparable with study done by Basavraj et al.⁸ Overall mortality in our study was 14.66%. Total 11 patients expired from total 75 subjects in our study. All patients died because of respiratory failure. So, respiratory failure was most common complication with op poisoning in our study. This mortality rate is comparable to study done by Goel et al.⁷, V vernekar et al.⁹ Malarvizi et al.¹¹ There was less mortality in patients who came within 1 hour. Mortality was highest (31.25%) when patients were admitted after 3-6 hours following ingestion of pesticide. In patients who were admitted between 1 hour to 3 hours following ingestion of poison, the mortality was 11.33% and in patients who were admitted within 1 hr, mortality was 8.33%. Our findings are consistent with malrvizi et al.¹¹ who reported increased mortality with increasing time interval between hospital admission and consumption

of poison. Serum cholinesterase activity estimation is a reliable diagnostic test in OPC poisoning. Observations from this study shown that patients with higher Serum cholinesterase activity on day of admission has a better prognosis than with lower enzyme values. Initial estimation of Serum cholinesterase activity can be used to predict the prognosis of patients. In this study, higher mortality rate was observed in patients who had low level of serum chE level than compared to >50% level. 2 patients who had less than 10% serum cholinesterase level and severe pop score, subsequently died. All patients, who had serum cholinesterase level above 50%(normal), survived. 10.71% mortality was observed in patients who had 20 to 50% serum cholinestearse (mild) and 40% mortality was noted in serum cholinesterase level between 10 to 20%. This study results are comparable to study done by Basavraj et al.⁸

Conclusion

Op poisoning is the most common suicidal mode of death in india. There is good correlation between POP Scale and serum cholinesterase levels on admission and severity of poisoning. There is good correlation between POP score and subsequent respiratory failure. The Peradeniya score (POP) applied at admission is useful to predict the outcome of the subjects in terms of both morbidity and mortality. The results of our study agreed with other studies, which had similar results and hence it is safe to assume that POP score which is an easy, quick and inexpensive method can be used for all patients presenting with OP poisoning as a predictor of outcome.

Ethical Clearance: Taken from IRB

Source of Funding: Self

Conflict of Interest: Nil

Abbreviations:

Ach Acetylcholine

AChE Acetylcholinesterase

OP Organophosphorus Poisoning

POP Peradeniya Organophosphorus Poisoning scale

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Toxicological Analysis by Computational Assessment Tools of Herbal Medicine Compounds Urushiol II and Bhilawanol-B from *Semecarpus Anacardium*

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How to cite this article: K. Jyothi Prasad, Sivakumar. Vulava, Vijaya Chandra Reddy Konda et al., Toxicological Analysis by Computational Assessment Tools of Herbal Medicine Compounds Urushiol II and Bhilawanol-B from *Semecarpus Anacardium*. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):17-21.

Abstract

Background: *Semecarpus anacardium* used in many Indian folklore medicine as 'Ballataka' or 'Bhilwa'. They are also known to cause pathological conditions by the presence of toxic principles in nut and roots of plants and possible pharmacokinetic and toxicological profiling of known toxic principles are explored by computational tools.

Aims & Objectives: The aims of the study were to study toxicological active principle Urushiol II and Bhilawanol-B by computational analysis and prediction tools.

Materials & Methods: This study was investigated on web-based tools PubChem to extract the chemical structure, followed by authentication and validation with the chemical formula. The two-dimensional structures are further converted to three-dimensional (3D) structure with ChemSketch software; the derived 3D structures are then screened for molecular properties followed by absorption, distribution, metabolism, elimination, and toxicity through admetSAR software. The reports are analyzed and predicted for pharmacokinetic and toxicity characters of Urushiol II and Bhilawanol-B from *S. anacardium*.

Results: The compounds screened Urushiol II and Bhilawanol-B from *S. anacardium* both had drug likeness score of MLOGP>4.15, the LD 50 values of urushiol II is comparatively more 4000 mg/kg/d than bhilawanol 2700 mg/kg/d for rat oral route testing and positive predictive results for skin irritation and allergic dermatitis. The genotoxicity battery assay was negative for both Urushiol II and Bhilawanol-B.

Conclusion: The computational analysis predicts the uroshiol II and bhilawanol-B present in *S. anacardium* belongs to class II Toxic hazard classification.

Keywords: *Semecarpus anacardium*; folklore; toxicology; urushiol II; bhilawanol-B.

Introduction

In folklore and traditional medicine, mostly many medicinal preparations are extracted from various

plant sources.¹ Complementary and Alternative Medicine (CAM) plant preparations are widely used when disease treatment not affordable and amenable to treatment by modern method².

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Semecarpus anacardium Linn. Belongs to family: *Anacardiaceae* distributed in sub-Himalayan region, tropical and central parts of India. The plant well-known for its medicinal value in Ayurvedic and Siddha system of medicine utilizes this plant for its medicinal value and commonly known as marking nut (dhobi nut) with vernacular names as 'Ballataka' or 'Bhilwa'.^{3,4} The available literature review revealed presence of various components from *S. anacardium* such as phenolic compounds, biflavonoids, sterols and glycosides.⁵⁻⁸

The constituents of our study interest is present in the fruit pericarp called as marking nut include 1-(2,3-dihydroxyphenyl)-8-pentadecene called Urushiol II and o-dihydroxy compound called bhilawanol. Previously reported pharmacological activity of *S. anacardium* includes anti-atherosclerotic activity⁹, anti-inflammatory activity of SA for both immunological and non-immunological origin¹⁰, antioxidant activity¹¹, hypoglycemic effect¹² and anti-spermatogenic effect¹³ in various animal published studies. The present study is an attempt to explore toxicological aspects and pathogenesis predictions of Urushiol II and Bhilawanol-B using standardized computational tools. Utilization of computational research methods and analysis is economical, time saving with advantage to possibilities to predict toxicological properties avoiding animal and humans exposure and provide results to understand pathophysiological reasons to guide further wet lab research.

Aims and Objectives

The Study aims to screen pharmacokinetic and toxicological properties of known biological active compounds Urushiol II and Bhilawanol-B from *S. anacardium* by standardized computational analysis tools.

Materials and Methods

Hardware and Software

The selected compounds molecular properties of chemical structure from leaf extract of *A. paniculata* extract are carried out in Lenovo 2021 Model installed with windows 11 software, java enabled with updated plugins.

Data Set

The chemical structures of Urushiol II and Bhilawanol-B from *S. anacardium* with two-dimensional (2D) pictures were collected from accredited indexed published journals and other sources such as PubChem, ChEMBL, ChemPDB, and Asinex Ltd. After a detailed review, the structures are developed with ChemSketch, followed by PHASE software module was used to convert the 2D structures into three-dimensional (3D) structures.¹⁴

Virtual Screening

Pharmacokinetic and Toxicological Prediction

The 3D structures developed are explored virtually using online prediction software¹⁵. Results from computational analysis of compounds on absorption, distribution, metabolism, excretion, and toxicity are acquired in the virtual screening workflow protocol.

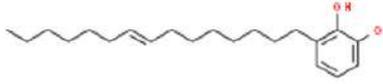
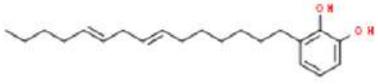
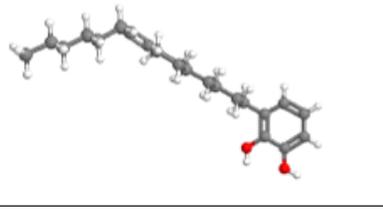
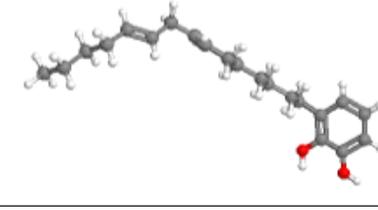
Statistical Methods and Calculation

Interactive molecular properties calculator applet (MolSoft L.L.C. San Diego, CA, USA) is used for molecular volume and drug-likeness score. The study is done in the department of Forensic medicine, Pharmacology and the college digital library using online tools during Dec 2021 to Feb 2022. The study was self-funded conducted at SVIMS University, Tirupati, Andhra Pradesh, India. The study is considered under the category for exemption from institutional ethics committee approval as it does not involve animals and humans and done by computational bioinformatics tools.

Results and Discussion:

The present study is designed to study and analyze Toxicological properties of Urushiol II from *S. anacardium* followed by a prediction of pharmacokinetic parameters based on results obtained by bioinformatic experimental models. From the detailed review of literature from earlier studies Urushiol II and Bhilawanol-B from *S. anacardium*, the 2D chemical structure were retrieved from PubChem online compound database platform and the 2D structures converted to 3D structure by ChemSketch software [Table 1].¹⁶ The 3D structures are the processed with MOLSOFT L.L.C Software, and the molecular properties are predicted [Table 1].

Table 1: *S. anacardium* compounds 2D, 3D structure & Physiochemical properties

Sl.No	Characters	Urushiol II	Bhilawanol-B
1.	2D Structure		
2	3D Structure		
3	Chemical Formula	C ₂₁ H ₃₄ O ₂	C ₂₁ H ₃₂ O ₂
4	Molecular weight	318.49 g/mol	316.48 g/mol
5	Num. H-bond acceptors	2	2
6	Num. H-bond donors	2	2

The results predict 2 hydrogen bond acceptor and donor site in the structure of both molecules which could be analysed to have better absorption and permeation. The Pharmacokinetics prediction profile of Urushiol II revealed similarly to have high gastro enteral absorption but cannot cross the blood

brain barrier and not a substrate of P-Glycoprotein [Table 2]. With metabolism prediction with CYP4503A4 a most common pathway for xenobiotics Urushiol II did not inhibit where as Bhilawanol -B found to enzyme inhibitor, which may be expected to cause drug interactions.

Table 2: ADME predicted profile of *S. anacardium* nut active compounds

Sl.No	Pharmacokinetic Parameters	Urushiol II	Bhilawanol-B
1.	G.I Absorbtion	High	High
2	BBB	No	No
3	P-gp Substrate	No	No
4	CYP450 3A4 Inhibitor	No	Yes
5	Log K _p	-2.90cm/s	-3.37cm/s
6	Druglikeness	Yes; 1 violation: MLOGP>4.15	Yes; 1 violation: MLOGP>4.15
	Bioavailability Score	0.55	0.55

This prediction of permeability coefficient (K_p) for the transport of compounds through mammalian epidermises is based on the linear model by Potts RO. and Guy RH. Based on the Computational analysis the results of uroshiol II and bhilawanol-B were -2.90cm/s and -3.37 cm/s respectively[Table 2]. The analysis of reports shows very high penetrability over topical

application of both molecules.¹⁷ The Predictions results revealed drug-like physicochemical properties maintained by both uroshiol II MLOGP>4.15 and bhilawanol-B MLOGP>4.15 with good bioavailability score of 0.55 [Table 2] which is analyzed as sufficient for oral absorption as described by Lipinski's rule (Lipinski CA (December 2004)).¹⁸

Table 3A: Toxicology prediction profile of *S. anacardium* nut active compounds

Sl.No	Species	LD50(mg/Kg/d)		Reliability Index	
		Urushiol II	Bhilawanol-B	Urushiol II	Bhilawanol-B
1.	Rat Oral	4000	2700	0.52	0.53
2.	Rat Intraperitoneal	180	130	0.28	0.39
3.	Mouse Oral	790	520	0.44	0.27
4.	Mouse Intraperitoneal	130	190	0.77	0.81
5.	Mouse Intravenous	31.91	6.92	0.28	0.29
6.	Mouse Subcutaneous	180	140	0.25	0.29

Reliability index: <0.3 = Not reliable prediction quality; 0.3-0.5 = borderline prediction quality; 0.5-0.75 = moderate prediction quality; >0.75 = high prediction quality. LD-Lethal dose.

Acute toxicity LD50 prediction models over rat oral and mouse intra peritoneal analysis revealed highly predictable index and the LD 50 values of

urushiol II is comparatively more 4000 mg/kg/d than bhilawanol 2700 mg/kg/d for rat oral route testing[Table 3A].

Table 3B: Toxicology prediction profile of *S. anacardium* nut active compounds

Sl.No	Toxicology Profile	Urushiol II	Bhilawanol-B
1.	Toxic hazard classification by Cramer	Class -II	Class -II
2.	Skin irritation(rabbits)	Positive	Positive
3.	Allergic contact dermatitis (human & guinea Pig)	Positive	Positive
4.	Respiratory Sensitization	Negative	Negative
5.	Teratogenic potential (human Model)	Negative	Negative

Table 4: Genotoxicity prediction profile of *S. anacardium* nut active compounds

Sl.No	Parameters	Urushiol II	Bhilawanol-B
1.	Structural Alerts for DNA Reactivity	Negative	Negative
2.	Ames test in <i>S. typhimurium</i> (in vitro)	Negative	Negative
3.	Mutagenicity consensus	Negative	Negative
4.	Micronucleus Test in Mouse Erythrocytes	Negative	Negative
5.	Comet Assay in Mouse	Negative	Negative

The toxicological prediction of skin irritation (rabbit model) and allergic contact dermatitis(in human & guinea Pig model) was positive as compared with literature review [Table 3B]. This explains that when applied externally, the juice causes irritation and a painful blister, which produces eczematous eruptions of the neighboring skin with which it comes into contact, and there is itching. The lesion resembles a bruise (artificial bruise). To fabricate wounds (usually bruise) by external application of juice over the skin is a common criminal practice by malingers.¹⁹⁻²¹

Histopathologic study is considered gold-standard for diagnosis of skin lesions, either neoplastic or inflammatory, most of the times macroscopically when a blister is less than 0.5 cm, it is called a vesicle

and when it's greater than 0.5 cm, it is called a bulla. In humans, high concentrations of an irritant will produce marked ballooning of keratinocytes in the upper epidermis with variable necrosis ranging from a few cells to confluent areas of the epidermis.

Neutrophils are found in the areas of ballooning and necrosis, and mild spongiosis is also present in the adjacent epidermis. If low and medium concentrations of an irritant are applied, the histopathological spectrum of the reactions produced often mimics that seen in allergic contact dermatitis, with epidermal spongiosis, mild superficial dermal edema, and a superficial, predominantly perivascular infiltrate of lymphocytes. Pustular reactions show sub corneal vesicles with neutrophils, cellular debris, and a fibrinous exudate. There are also some neutrophils in the upper dermal infiltrate.²²

Both uroshiol II and bhilawanol-B prediction analysis revealed negative to respiratory sensitization and Tearatogenic potential to human models and the battery of genotoxicity profiling was also negative and falling in class II hazardous chemicals probably explains the use in traditional medicines with the indigenous ways of detoxification and use as medicinal substances.[Table 3B & 4].

Conclusion

The use of computational bioinformatics tool is a test method that substitutes traditional animal models with non-animal systems such as *insilico* standardized models benefits in the Reduction, Refinement, and Replacement of animals used in research and testing. Skin contact with the acrid juice *Semecarpus anacardium* is known to cause skin and mucosal irritation, inflammation, vesication, and ulceration²³. The present computational analysis predicts the presence of uroshiol II and bhilawanol-B in juice can cause skin sensitization with better dermal penetrability and ability to cause allergic dermatitis A variety of nut extract preparations from this source are effective against many diseases, viz., arthritis, tumors, and infections and so on in ayurveda and siddha medicine. The negative teratogenic ,genotoxic potential and compounds are predicted to be class II toxic hazard classification by Cramer. However, the mechanism of the pharmacological action and its scientific validation in traditional use of its nut can be greatly aided by the isolation of its active principle and determination of structure–function relationship for pharmacological and toxicological activity by further wet lab studies.

Conflict of Interest: None declared.

Ethical clearance: No Humans or animals involved in study.

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Questionnaire Based Survey on Medicolegal Autopsy among Medical Students in Sikkim

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How to cite this article: Karma Mingur Diki Bhutia, Sankha Subhra Debbarma. Questionnaire Based Survey on Medicolegal Autopsy among Medical Students in Sikkim. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):22-25.

Abstract

Background: Contemplating the curriculum of an Indian Medical Undergraduate, demonstration of autopsy is still considered to be the cornerstone in Forensic Medicine. Medicolegal autopsy has always been perceived negatively by the public either due to their religious beliefs or due to their preconceived notions or fear. Considering these prejudices, a study was undertaken not only to assess the knowledge, attitude & perception of a medical undergraduate but also to understand the reflection of these societal stigma on a medical student.

Material and Methods: A cross sectional survey was done on 200 medical students pursuing medicine in SMIMS, Gangtok, Sikkim who participated voluntarily in the study wherein structured questionnaire in google form was distributed through google link validated through their mail i.d. & registration number.

Results: Out of 191 medical students, the number of students were predominantly females with the ratio of 1:1.30 (M: F). 87.96% of the students knew that the medicolegal autopsy is done in all sudden, suspicious & unexpected death. 90.05% strongly affirmed that the medicolegal autopsy ascertains the cause of death. The spectrum of ease with which the students watched the autopsy was almost along the same wavelength, 27.75% students were comfortable, 25.13% were uncomfortable, 17.80% were indifferent while 29.32% were not yet exposed (due to online teaching). Majority of students (93.72%) are interested in watching watch more of postmortem examination.

Conclusions: The attitude of the students were optimistic towards autopsy & awareness among the participants was found to be acceptable. However, there were still a lot of grey areas that needed to be addressed.

Keywords: Knowledge; attitude; perception, Survey; medicolegal autopsy.

Introduction

Autopsy is known to determine the cause of death since the beginning of time. In 44 B.C, Roman jurist Antistius performed an autopsy on Julius Caesar & declared that out of 23 stab wounds sustained, Caesar

succumbed to his chest injury leading to the rupture of aorta. This application of medical knowledge to homicide investigation in Cesar's death was possibly the history's first autopsy report with an opinion as an expert witness.¹ But the first medicolegal autopsy on record was performed in Bologna (Italy) in 1302, by

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Bartolomeo De Varignana. While in India, it was only in the 18th century that the first medicolegal autopsy was performed officially by Dr. Edward Bulkley.²

“Autopsy means postmortem examination of the corpse to establish identity; determine the cause, manner & time since death; collect physical evidence & also to determine the question of live birth & viability”.² Based on consent, there are 2 types of autopsies: medicolegal autopsy & clinical or academic autopsies. In the former, consent is given by the state on behalf of the deceased while in the latter consent has to be obtained from the legal heir. In an editorial autopsy case report, an author Ameer Hamza has clearly highlighted the worldwide decline in clinical autopsy rate for the last few decades despite its evidentiary importance & high value.³ In non-medicolegal/ clinical autopsies, the fright of trial in court, shortage of expert manpower, cost effectiveness, non-compliance from patient or relatives, communication failure, increased objections based on optics & emotions along with the medical practitioner’s attitude & knowledge were few of the reasons identified to cause the decline in number in clinical autopsies.^{4,5} In medicolegal cases, consent is not required & autopsy is mandatory under the order of competent legal authority.

On receiving the Inquest report, Body Challan & Requisition form for autopsy from the Investigating Officer/ Executive Magistrate, scientific examination of a deceased is carried out in all sudden, suspicious & unnatural death under the laws of the State.^{6,7}

Material and Methods

Study design: A survey based cross-sectional study was designed to assess the knowledge, attitude and perception on medico legal autopsy among the Indian Medical Undergraduates of Sikkim Manipal Institute of Medical Sciences, Sikkim Manipal University, Gangtok, Sikkim. The questionnaires were structured, self-administered, pre-designed⁸⁻¹⁰ and modified in context of Sikkim.

Data collection: The primary data were obtained using google form & shared via google link. The data was then compiled, organized & analysed.

Inclusion criteria:

1. Willing to give consent.
2. Active participants.
3. Knowledge on medicolegal autopsy.

Exclusion criteria:

4. Refusal.
5. Incomplete form submission.
6. Repetition.
7. Forms without validation (like email id or registration no.)
8. First year MBBS students since their curriculum for Forensic Medicine begins only from 2nd year.

Results

This study was conducted on MBBS students & Interns willing to participate & give their consent for the study. Out of 191 students, 108 were females & 83 were males participating in the study from different batches (Table 1).

Table 1: Total number of participants from different batches

Batch	No. of participants
2015	02 (1.05%)
2016	14 (7.33%)
2017	29 (15.18%)
2018	51 (26.70%)
2019	95 (49.74%)

A cross sectional survey based on 25 questionnaires was designed to gauge the in-depth knowledge, positive/ negative attitude & perception of the medical students towards medicolegal autopsy.

It was observed that the students have sound knowledge & positive attitude towards medicolegal autopsy.

However, the 25th question was mostly designed to assess the irrationality or the superstitious belief that they have experienced or have heard of in their daily life. For which an array of answer was observed, to name a few:

“Presence of ghosts near autopsy hall”

“The paranormal activities are common near the autopsy hall and we usually don’t walk that way at night!”

“The dead souls haunts”

“Autopsy is done for harvesting organs”

“Body becomes impure after postmortem”

“Post mortem disturbs passage to afterlife”

“Person will be born disfigured In Next life and he’ll haunt others after death”

"Post-mortem examination will bring no good to anyone"
"Salvation is not found after post mortem"

These irrational phobias or superstitious beliefs mentioned by the students has a negative connotation towards autopsy. Leastways, it is fair to say that the social stigma associated with the dead is not unknown to these students too. The idea of teaching forensic medicine should not only be limited to the knowledge part of the education but also to make an impact in changing the negative attitude & perception of the students towards autopsy with scientific reasoning, cautious explaining & holistic approach to promote the sense of well-being.

Discussion

This study was carried out among 191 medical students of SMIMS in order to assess their knowledge, attitude & perception towards medicolegal autopsy. A questionnaire based cross sectional survey with the inclusion & exclusion criteria was self-administered among volunteered students. Out of 191 medical students, the number of students were predominantly females with the ratio of 1:1.30.

On the knowledge-based front, 87.96% of the students know that the medicolegal autopsy is unavoidable in all sudden, suspicious & unexpected death which is similar to study done in Ahmedabad & Haryana.^{10,11} 90.05% strongly affirmed that the medicolegal autopsy is done to know the cause of death which is concurred by other studies too.^{8,10,11} 87.43% students agreed that the law enforcement agency orders medicolegal autopsy. Majority of the students choose inquest form as prerequisite for medicolegal autopsy (53.93%) & only 32.98% knew that the consent of family members is not required in a medicolegal autopsy. Similar findings were also observed in a study conducted in Haryana.¹¹ 33.51% of the students knew that viscera can be removed for chemical analysis in medicolegal autopsy without consent. The approximate findings were also observed in studies done in Uttar Pradesh, Telangana & Ahmedabad.⁸⁻¹⁰ 39.27% believed that the expenses incurred during medicolegal autopsy is borne by the government/ teaching hospital. 41.36% agreed that a surgically disposed organ does not need an autopsy. 65.45% of students knew that the Postmortem report is handed over to investigating officer. 49.21% of students agreed that not all medicolegal cases end up in going to the court.

On assessing the attitude of the students towards demonstration of medicolegal autopsy, it was observed that 27.75% students were comfortable, 25.13% were uncomfortable, 17.80% were indifferent while 29.32% were not yet exposed (due to online teaching). Majority of students (93.72%) are interested in watching more of postmortem examination. The attitude of the students was mostly positive towards medicolegal autopsy, similar to the studies done in Uttar Pradesh, Telangana, Haryana & Ahmedabad.⁸⁻¹¹ In contrast to the study done in Sweden, where 75% of the students were uneasy with the thought of performing autopsy on oneself.¹² 67.01% agreed on performing autopsy on self & offering it to relatives too which concurred with studies done in Haryana, Ahmedabad, Uttar Pradesh & Sweden.⁹⁻¹¹ Most of the students (83.25%) assented that postmortem examination in a medical education should be mandatory. Many of them also wanted to watch autopsy while only 16.75% did not want to watch it which is in accordance with studies by different authors.⁹⁻¹¹ 84.29% agreed that didactic teachings with autopsy demonstration would provide an opportunity for students to discuss the issues related to autopsy & the results coincide with the observation made in a study conducted in Haryana.¹¹ Contrary to the similar study done in Haryana, 41.36% disagreed that it causes disfigurement of body.¹¹ 59.69% of the participants agreed that autopsy delays the funeral. Only 39.79% were confident on performing the medicolegal autopsy independently after completion of their graduation. The source of information for most of the students (77.49%) were from Lecture & Books similar to study done in Haryana.¹¹

As far as perception of the students towards medicolegal autopsy is considered, 85.86% of the students does not think that postmortem examination is disrespectful in any way. Similar to studies done in different medical colleges.⁹⁻¹¹ Most of the students 61.26% of the students does realise that autopsy is not merely a legal obligation in concurrence with other studies.^{8,9} Majority, 60.73% of the students does not feel that autopsy is against religious belief. 52.36% believes that autopsy does not cause emotional disturbances.

Conclusion

Overall, the participants do have sound knowledge on medicolegal autopsy & its implications. However, the attitude of future

practitioners is formed by the societal experiences, customs, traditions, culture, religious rites & rituals which predisposes an individual psychologically to conform to the societal norms. The knowledge in cognitive domain, optimistic attitude can facilitate the societal perception without prejudices with holistic approach of explicit learning, awareness, cautious & scientific reasoning to facilitate the social being of an individual.

Conflict of interest: Nil

Source of funding: Self

Ethical clearance: The present study was carried out using the google form-based questionnaire & does not involve any risk to humans in any form. However, the consent from the participants is taken & it is also ensured that the response would be anonymous and solely used for research purpose only.

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Pattern of Injuries in Fatal Head Trauma Due to Road Traffic Accidents

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How to cite this article: M. N. Rajamani Bheem Rao, A. Nirmala, R. Raguram. Pattern of Injuries in Fatal Head Trauma Due to Road Traffic Accidents. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):26-30.

Abstract

Background: Head trauma is considered to be the major cause of death in road traffic accidents. According to world health organisation, about 1.24 million deaths occur due to road traffic crashes. Particularly, the treatments for head injury in the older aged people seems to be more complicated when compared to younger and middle age groups. The aim of this study is to analyse and identify the significance and outcome of head trauma due to road traffic accidents of various age groups.

Methods: This study was conducted on Madras Medical College, in the Department of Forensic Medicine, Chennai. A total of 200 fatal head injury case autopsies were performed. After obtaining the necessary and relevant information about the deceased, a thorough autopsy was performed and the findings were recorded.

Result: Out of 200 cases, 83% were men and 44.5% were recorded to be between 31-50 age group. Fissure fracture of skull was found to be most prevalent (40.5%) and subdural haemorrhage was the common findings in head injury (95%).

Conclusion: The results of our study showed that most of the people who had accidents were pedestrian and two wheeler driving persons.

Keywords: Head injuries; intracranial hemorrhages; road traffic accident; skull fracture.

Introduction

According to WHO estimates, about 1.24 million deaths occur each year as a result of road traffic crashes. Around 20 to 50 million more people suffer non-fatal injuries, with many incurring a disability as a result of their injury. In India, according to Ministry Of Road Transport and Highways (MORTH), during the year 2020, there were around 5 lakh road accidents,

which resulted in deaths of 134,513 people and injured more than 5 lakh persons in India. It is the leading cause of mortality for young adults of age less than 45 years. Among all the regional injuries, the injury to the head is the most important in forensic practice. The present study aims to analyze the significance of nature of injuries, part of the brain injured, duration of survival and intracranial hemorrhages noted in fatal road traffic accidents.

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Materials and Methods

The study was conducted on 200 fatal head injury cases brought for autopsy and conducted at the Mortuary, Department of Forensic Medicine, RAJIV GANDHI GENERAL HOSPITAL Medical College, Chennai. A complete autopsy was done and all the findings were recorded in detail. The dissection technique of scalp, skull and dura was in accordance with the procedures suggested by Gresham GA and Turner AF and the brain dissection as suggested by Ludwig J. All the statistical analysis was done using EpiInfo 3.4.3 (2007) software. Descriptive statistics of categorical data were presented as proportions for comparison.

Results

General distribution of fatal RTA Cases. The most vulnerable age group was those in 31-50 years followed by the age group of 21-30 years. The reason being that they form the most active group of the society and hence are prone to road traffic accidents.

(83%) Were males and (17%) were females, males were more prone to head injuries in road traffic accidents since they are more into outdoor activities like driving vehicles, working outdoor posing them risk due to accidents.

Females succumbed to road traffic accidents were mainly due to them being pillion riders without helmet and pedestrians. of the 200 cases studied scalp contusion was present in 190 cases. Diffuse scalp deep contusion of the scalp was commonest in 79 cases (39.5%) followed by scalp contusion of right-fronto- temporo-parietal regions of scalp. laceration of the scalp was present in 20 cases i.e., 10% of cases. Two wheeler drivers accounted for 80 cases 40%, pedestrians accounted for 80 cases 40% followed by two wheeler pillion riders 19 Cases (9.5%).

Table 1: Percentage of different types of fracture of the skull.

Fracture_group	Frequency	Percent
Bony and Dural Defect	12	6.0%
Comminuted Fracture	8	4.0%
Diastatic Fracture	1	0.5%
Depressed Fracture	6	3.0%
Fissure Fracture	81	40.5%
Intact	87	43.5%
Others	5	2.5%
Total	200	100.0%

As in table-1, fissure fractures were the commonest type (81 Cases) 40.5% followed by Comminuted fracture (8 cases) 4.0% and depressed fracture (6 cases) i.e. 3.0%. Skull vault was intact in 43.5% of the case, i.e. 87 cases.

Table 2: Region Wise Distribution of Extradural Hemorrhage in Fatal RTA Cases.

EDH	Frequency	Percent
Parietal	19	9.5%
Frontal	1	0.5%
Temporal	6	3.0%
Occipital	1	0.5%
No	173	86.5%
Total	200	100%

As in table 2, extradural hemorrhage was more commonest in parietal region 19 cases 9.5% followed by temporal region and least common in frontal and occipital regions 0.5%.

Table 3: Distribution of Subdural Hemorrhage in Fatal Head Injury Cases in RTA

SDH	Frequency	Percent
RTFTP	37	18.5
LTFTP	34	17.0
Diffuse	119	59.5
No	10	5.0
Total	200	100.0

As in table 3, the most common extra axial hemorrhage was subdural hemorrhage. Subdural hemorrhage was more common in both the cerebral and cerebellar hemispheres of brain 59.5% followed by the surface of fronto- temporo-parietal region of right cerebral hemisphere of brain, 37 cases (18.5%).

Table 4: Distribution of Subarachnoid Hemorrhage in Fatal Head Injury Cases.

SAH	Frequency	Percent
RTFTP	33	16.5
8LTFTP	25	12.5
Diffuse	111	55.5
No	31	15.5
Total	200	100.0

As in Table 4, Subarachnoid haemorrhages were present in 169 cases. The most common site for

subarachnoid haemorrhage was on the surfaces of both the cerebral and cerebellar hemispheres of brain (55.5%) followed by the surface of fronto temporo-parietal region of the right cerebral hemisphere of brain (16.5%).

Overall Incidence of Intracerebral Hemorrhages

Subdural hemorrhage was present in 190 cases 95%, followed by subarachnoid hemorrhage 84.5%, extradural hemorrhage 13.5%, intraventricular hemorrhage 13.5%, intracerebral hemorrhage 9.5% and brain stem hemorrhage 6.5%.

If the type of hemorrhage is considered in isolation, then cases having subdural haemorrhages were the highest in number 190 cases (95%) followed by subarachnoid haemorrhages in 169 cases (84.5%), extradural haemorrhages in 14 cases and intracerebral haemorrhages 9.5%. brain stem haemorrhage was present in 17 cases (8.5%).

Table 5: Base of Skull Fractures

Base skull	Frequency	Percent
ACF	5	2.5
Comminuted Fracture	9	3.0
IGH	1	.5
Intact	112	56.0
MCF	47	23.5
MCF -PCF	1	.5
MCF ACF	2	1.0
NIL	1	.5
PCF	17	8.5
Right Middle and Posterior Cranial Fossa	1	.5
Right Temporal Bone to Left Temporal Bone	1	.5
Surgical Defect	1	.5
The Right Bone and Temporal to Occipital Bone	1	.5
The Right Temporal Bone	1	.5
Total	200	100.0

As in table 5, Fracture of the base of the skull was noted in 78 cases. middle cranial fossa is the commonest with 47 cases (23.5%), Fracture of the posterior cranial fossa is observed in 17 cases (8.5%) and fracture of the anterior cranial fossa noted in 5 cases (2.5%).

Discussion

According to Ministry of Road Transport and Highways (MORTH)², India pedestrians,

bicyclists and two-wheeler riders comprise the most unprotected road users, accounting for around 40% of all fatalities. In our study also, we found that pedestrians [80 cases (40%)], pillion riders [19 cases (9.5%)] and drivers of two-wheelers [80 cases (40%)]. pedestrians were the most vulnerable group followed by drivers and pillion riders of two-wheelers.

In a study done by Menon et al.¹ 682 victims of road traffic accidents who died due to injuries sustained to the head were autopsied at District Wenlock Hospital, Mangalore, India over a period of 5 years between January 1999 and December 2003. Skull fractures were present in 88.88% of the cases. Fractures of the vault were found in 88%, base of the skull in 35.97% and a combination of both in 35% of cases. In most of the cases, fissured fractures were found (23%). Among intra-cranial haemorrhages, subdural haemorrhage was found in 52.63% and subarachnoid haemorrhage in 27.27% of cases. Contusions and lacerations of brain were found equally in 35% of cases.

When analyzing the contributing factors for the occurrence of road traffic accidents in our study, out of the 99 victims who were drivers or pillion riders of two-wheelers, 80 had not used helmet and hence succumbed to head injuries.

According to WHO, wearing helmet can reduce the risk of deaths by almost 40% and the risk of severe injury by over 70%. Hence strict laws should be enforced to make helmet use mandatory for the drivers of two-wheelers and pillion riders.

In the present study of 200 cases, maximum number of persons sustained fatal head injuries in the day time. Preponderance of occurrence of fatal head injuries in road traffic accidents during day time can be explained by the fact that active work is done at day time.

Another common risk factor for RTAs is drunken driving. In a systematic review done by Das A et al.², 2-33% of the injured and 6-48% of killed RTA victims had consumed alcohol or drugs. In our study however only five victims were reported to have consumed alcohol. This should be because of under reporting and lack of data regarding alcohol use.

Recently there has been a marked increase around the world in the use of mobile phones by drivers that is becoming a growing concern for road safety. But in our study, no data was available regarding the use of mobile phones by the accident victims. Other risk factors like bad illumination, rain, pet or domestic animals and natural diseases contributed very less to the occurrence of RTAs in our study.

Majority of the patients were managed conservatively and only 23 cases (11.6%) had undergone surgery. The period of survival was less than a week in the majority of cases. In approximately 22% of the cases, the period of survival was > 7 days.

The maximum number of deaths occurred during the first 24 hours can be explained by the fact that intracranial haemorrhages, contusions of the brain, laceration of the brain and edema of the brain which are not compatible with life can occur immediately.

In 16 cases (8%) of the fatal head injury cases death occurred on the spot. Among the type of road users, two wheeler drivers accounted for 80 Cases (40%), pedestrians accounted for 80 cases 40% followed by two wheeler pillion riders 19 Cases (9.5%). In a study by Bayan et al.³ in Pune also, they had found that pedestrians were the most vulnerable group followed by drivers and pillion riders of two-wheelers.

In a study done by Ogleznevetal.⁴ on craniocerebral trauma in road traffic accidents, the most severe form of brain compression was multifactorial compression (27.6%) and its most common form was compression with subdural hematoma (35.3%). In over half the cases (62.6%), BI due to RTA was associated with extracranial lesions, leading to diagnostic problems. The pattern and site of lesions were related to the type of a transport vehicle and to the role of a victim as a traffic participant. Multiple extracranial lesions were mostly frequently encountered in victim pedestrians (30.3%), BI concurrent with chest damage was common in drivers (12.8%), BI concurrent with "whip" injury of the cervical spine was found in drivers and passengers though such combinations were also seen in pedestrians (1.5%-5 cases).

In the present study, among scalp injuries contusion of the scalp was the commonest scalp injury, followed by lacerations and abrasions. Frontal, parietal and temporal regions of the scalp were the commonest regions to be involved.

When the fractures of the skull vault are analyzed, linear fractures were the commonest type (81) followed by comminuted (8) and depressed (6) fractures. This correlates with the study done by Jacobsen et al.⁷ in Copenhagen, where linear fracture was the commonest type followed by comminuted, depressed, ring and spider-web fractures. In the Jaipur study by Goyal et al.⁸, linear fractures were the commonest followed by depressed and then the comminuted fractures. This correlates with data as given in Anil Aggrawal⁹ who concluded linear fracture as the commonest type of fracture. Considering the predominant site of the skull fractures, frontal and temporal fractures were much more common than parietal and occipital fractures. This is because of the mechanism of most road traffic accidents exposing the fronto-temporal region to risk of trauma than the parieto-occipital region.

The incidence of the fractures of the base of skull in the present study was 78 cases which was much higher when compared with the previous studied [Goyal et al.⁵ (< 1.1%).

In the majority of the cases of fatal head injury, there was a combination of fracture of the vault of the skull, intracranial haemorrhages and fracture of the base of the skull. This can be explained by the fact that, fracture commences at the region of maximum impact and then radiates downwards to the base of the skull.

The most common extra axial hemorrhage in our study was subdural 190 cases, followed by subarachnoid 169 cases, whereas in most of the previous studies [Jacobsen et al.⁶, Bhat VJ et al.⁷], subarachnoid hemorrhage was the commonest. Subdural haemorrhage was observed in 190 Cases. Subdural haemorrhage was the most common intracranial haemorrhage (95%) followed by subarachnoid haemorrhage (84.5%), extradural haemorrhage (14%) and intracerebral haemorrhage (9.5%). Our observations are in correlation with the above findings.

In the present study, it is evident that subdural haemorrhages is one of the commonest intracranial haemorrhages followed by subarachnoid haemorrhage, extradural haemorrhage, intraventricular haemorrhage and intracerebral haemorrhage.

In the present study of 200 cases of fatal head injuries in road traffic accidents, in addition to other parts of the body were also involved in addition to cranio-cerebral injury. Chest injury was noted in 11 cases, rib fractures in 6 cases and fracture of the extremities in 8 cases.

Conclusion

1. The most vulnerable age group was 31-50 years followed by 21-30 years, 83% of the victims were men.
2. 80% of the victims were pedestrians or two-wheeler riders. Majority of the two-wheeler victims were not wearing helmets at the time of the accident and hence sustained significant head injuries.
3. In majority of the cases (78%), the period of survival was less than a week.
4. The maximum number of accidents causing fatal head injuries were observed during day time 134 cases as compared to night time 66 cases.
5. The motor cyclist and pedestrian were the commonest group of victims in vehicular accidents 160 cases (80%) followed by two wheeler pillion riders.
6. Two wheeler was the commonest offending vehicle followed by four wheeler.

7. Scalp injury with fracture of the skull, fracture of the base of the skull and intracranial hemorrhage was the commonest presentation in fatal head injury cases of road traffic accidents.
8. Totally 96 cases fractures of the vault of the skull were detected in the autopsy. Linear or fissure fractures were the most common type seen in 81 cases, 8 cases were comminuted fractures and 6 cases were depressed fractures.
9. On considering the most common site of fractures in the skull vault, temporal bone was the commonest bone to be fractured followed by frontal bones.
10. Fracture of the base of the skull was observed in 78 cases. The floor of middle cranial fossa was most commonly fractured 47 cases (23.5%) followed by floor of posterior cranial fossa 17 cases (8.5%)
11. Subdural haemorrhage was the most commonest intracranial hemorrhage observed in 190 cases (95.0%) followed by subarachnoid haemorrhage 169 cases (84.5%). Brain stem hemorrhage was found in 13 cases (6.5%)
12. Contusion of the scalp was the commonest scalp injury 190 cases followed.
13. Laceration of the brain parenchyma was found in 20 cases. (10%)
14. In addition to head injury, chest, upper limbs and lower limbs are commonly involved in road traffic accidents.
15. Two wheeler drivers and pedestrians were more commonly involved in Fatal head injuries in road traffic accidents followed by two wheeler pillion rider.
16. As regard to the type of vehicle, two wheeler was the common offending vehicle.

Recommendations

1. A national level registry must be established for registering all road traffic accidents all over the country. It will give insight about the epidemiological correlates and risk factors of RTAs, which will help in taking appropriate preventive measures.
2. Modifiable risk factors contributing to the occurrence of RTAs should be brought under control. Use of helmets for two-wheeler riders should be strictly enforced by law. Drunken driving and use of mobile phones while driving should be strictly prohibited. Use of seat belts should be made compulsory. Traffic rules including the lane discipline should be enforced strictly. Bad roads should be repaired and adequate lighting should be provided in all the roads.
3. Emergency contact numbers should be provided in all the roads. Health care facilities should be improved to provide timely interventions to RTA victims. Adequate ambulance facilities should be made available.
4. Standardised national level guidelines should be developed for the management of RTA victims in order to improve their survival.
5. Strict actions should be enforced against the negligent drivers for rash driving.
6. Drivers to be carefully scrutinised before issuing driving license.
7. Traffic rules to be enforced strictly.
8. Use of seat belts and wearing of helmets to be made compulsory for the occupants of vehicle.

Conflict of Interest: Nil.

Source of Funding: Self.

Ethical Clearance: Taken from Institutional ethics committee, Madras Medical college.

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The Influence of Chest-Knee Position on the Appearance of anal Physical Findings in Comparison with other Clinical Positions during the Examination of Sexual anal Assault Victim

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How to cite this article: Mohanad S. O. Jaber, Shrouq S. Al-Sabaileh. The Influence of Chest-Knee Position on the Appearance of anal Physical Findings in Comparison with other Clinical Positions during the Examination of Sexual anal Assault Victim. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):31-35.

Abstract

Background: Sexual anal assault is an important and critical issue worldwide. Usually the clinical examination reveals subtle findings to prove the incident of sexual anal assault. Thus for approaching a proper diagnosis, the victim needs to be examined by a trained and experienced forensic physician who is officially involved in dealing with such issues.

Methods: Prospective study of 82 victims of sexual anal assault transferred by the local prosecutor to the forensic medicine department.

Results: The majority of victims were females (82%), most of them were younger than 18 years (53.8%). The findings obtained by other clinical positions regarding the distribution of anal cutaneous folds revealed that (82%) of victims showed normal distribution, and (18%) showed abnormal distribution of the folds. While after chest-knee positioning (33.3%) of victims showed normal distribution of the anal cutaneous folds and (38.5%) showed abnormal distribution along with (28.2%) of victims showed absence of folds. Regarding inspection of anal sphincter by other clinical positions; (84.6%) of victims showed normally contracted sphincter and (15.4%) showed less contractility of the sphincter, whilst by chest-knee positioning (35.9%) of victims showed normally contracted sphincter with the majority of victims (64.1%) showed abnormal contraction. Concerning injuries in the anal region represented mainly by contusions and abrasions; they were noticed only in (12.8%) of victims by other clinical positions, while were present in (29%) of victims examined with chest-knee position.

Conclusions: During the clinical examination of victims of sexual anal assault, and in comparison with other clinical positions used, the application of chest-knee position showed overall more conspicuous physical findings, especially regarding the distribution of anal cutaneous folds, the inspected degree of contractility of anal sphincter, and the presence of injuries in the anal region represented mainly by contusions and abrasions.

Keyword: Sexual Anal assault; clinical Positions; sodomy; forensic Pathology.

Introduction

Sexual anal assault is an important and critical issue worldwide, especially in term of diagnosis¹, it happens in different ages and in both genders.

And it is always considered a medico-legal case in all countries. Usually the anal clinical examination reveals subtle findings to prove the incident of sexual anal assault^{2,3}, and for approaching a proper diagnosis, the victim needs to be examined by a

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trained and experienced forensic physician who is officially involved in dealing with such issues.

Globally, many policies regarding examination are present, some forensic centers depends on subjective methods, others relay on objective ones, and a third party uses both methods.^{1, 4-7} In Jordan and Palestine, the subjective method is widely applied. However, the subjective method - since being affected by many different factors⁸ - implies a high probable variation in its results between the examiners, perhaps for the same case. A significant factor of these is the way of patient positioning during the clinical examination.

Our study aims to analyzing the influence of chest-knee position on the appearance of anal physical findings in comparison with other clinical positions during the examination of sexual anal assault victim.

Materials and Methods

Prospective study of 82 victims of sexual anal assault transferred by the local prosecutor to the forensic medicine department at Al-Ahli and Hebron Governmental Hospitals in the period from July 2017 to March 2022.

Table 1: Distribution of cases according to the age and the gender:

Age correlation to the gender	Male	Less than 18 years	18%	82 Cases (100%)
		More than 18 years	0%	
	Female	Less than 18 years	53.8%	
		More than 18 years	28.2%	

Clinical examination results

Table 2 correlates the distribution of cases with chest-knee position and with other clinical positions regarding the appearance of the following physical findings; the distribution of anal cutaneous folds, the Inspected contractility of anal sphincter, and the Injuries in the anal region as following: with other clinical positions most of victims showed normal distribution of anal cutaneous folds during examination (82%), while with chest-knee

positioning, most of victims showed abnormal and even absent folds during examination (66.7%). Anal inspection revealed a normally contracted anal sphincter (84.6%), whereas after changing the victim to chest-knee position, this normality had changed to abnormally looking contraction of the sphincter (64.1%). Injuries in the anal region were present in (12.8%) of victims with other clinical positions and the percentage had elevated to (29%) with chest-knee position.

Results

Needed data was collected from the victims by a thorough history related to the incident, and were analyzed and arranged in three tables as following: the age correlated to gender, the anal physical findings and the photographical results with chest-knee position and with other clinical positions.

In general, the number of victims of sexual anal assault who were transferred by the local prosecutor to the department of forensic medicine during the period from July 2017 to March 2022 was 82. Data were analyzed according to the age correlated with gender, and the appearance of anal physical findings with chest-knee position in relation to other clinical positions used.

History

Table 1 shows the distribution of cases regarding the age and gender as following: most of the victims were less than 18 years old (71.8%), most of them were females (53.8%), and all male cases being less than 18 years old (18%).

Table 2: Distribution of cases according to anal physical findings.

Clinical examination of anus			Total & percent	
Appearance of anal cutaneous folds	Other clinical positions*	Normal*	82%	82 (100%)
		Abnormal*	18%	
		Absent	0%	
	Chest-knee position	Normal*	33.3%	82 (100%)
		Abnormal*	38.5%	
		Absent	28.2%	

Clinical examination of anus			Total & percent	
Inspection of anal sphincter contractility	Other clinical positions*	Normally contracted*	84.6%	82 (100%)
		Abnormally contracted*	15.4%	
		Normally contracted*	35.9%	82 (100%)
		Abnormally contracted*	64.1%	
Injuries the anal region Mainly contusions and abrasions	Other clinical positions*	Presence of Injury	12.8%	82 (100%)
		Absent of Injury	87.2%	
	Chest-knee position	Presence of Injury	29%	82 (100%)
		Absent of Injury	71%	

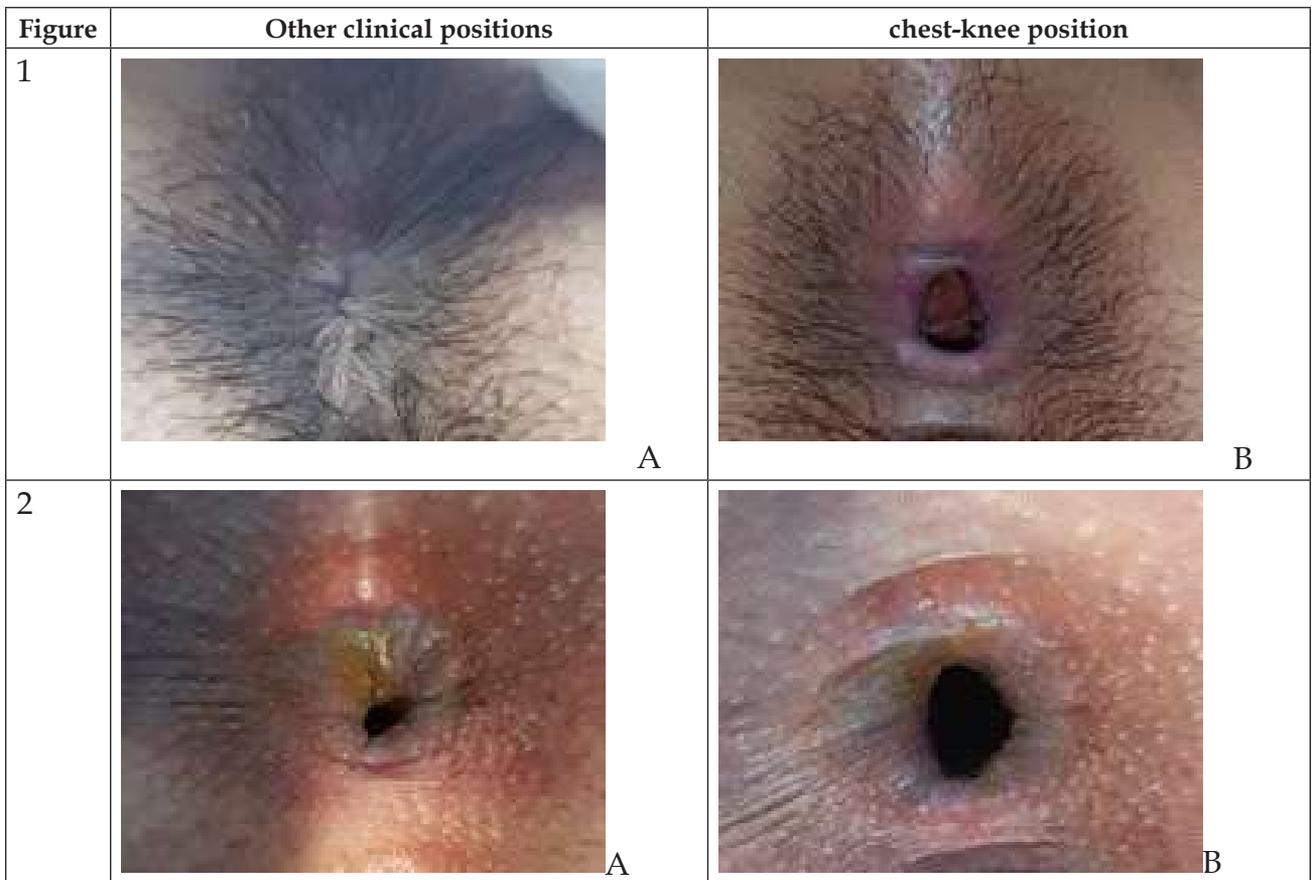
- * Other clinical positions: lateral position, Sim’s Position (lateral recumbent position), Standing - up position, and Kneeling position.
- * Normal appearance of anal cutaneous folds: Circumferentially and evenly existed folds around the anal orifice.
- * Abnormal appearance of anal cutaneous folds: Unevenly distributed folds and being obliterated in some areas around the anal orifice.
- * Normally contracted anal sphincter: Fully closed orifice by inspection assessed after performing gentle separation of buttocks.
- * Abnormally contracted anal sphincter: less than full contraction of the sphincter ; with different degrees of laxity.
- * Injury around anus: Mostly (contusion and/ or abrasion).

Photographical results of sexual anal assaults

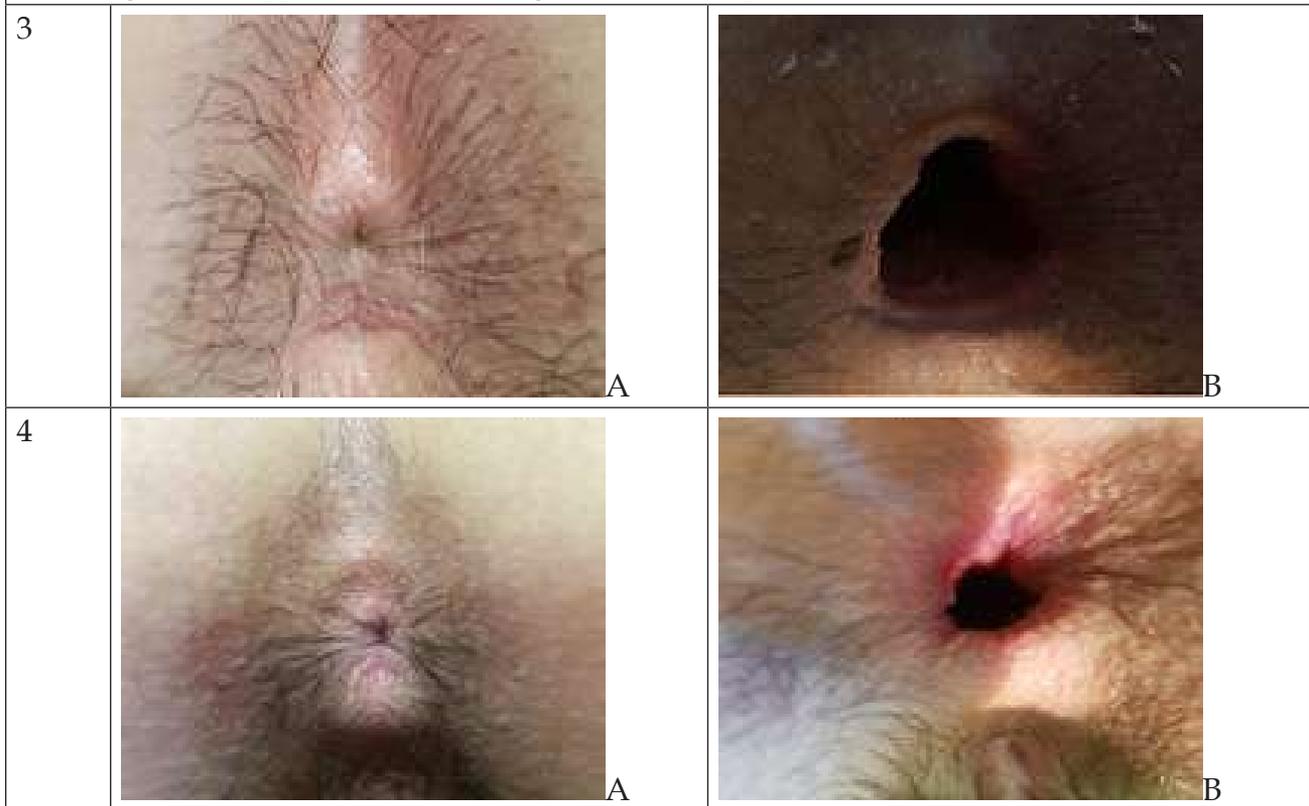
Table 3 shows the photographical results of four different victims of sexual anal assaults when clinical examination conducted first with other clinical positions and then with chest-knee positioning as following: with other clinical positions, most

of victims showed normal findings during the examination, while after chest-knee positioning, all victims showed abnormal findings regarding the distribution of anal cutaneous folds, the inspected state of anal orifice contraction, and the presence/ absence of anal injuries.

Figure 1: The appearance of anal findings with chest-knee position in relation to other clinical positions used



Contd.. Figure 1: The appearance of anal findings with chest-knee position in relation to other clinical positions used



Figures 1, 2, 3 and 4 (A): Show the degree of anal sphincter contraction when applying other clinical positions, note the distribution of anal folds in figures 1,3 and 4.

Figures 1, 2, 3 and 4 (B): Show the changes in the degree of anal sphincter contraction, and the distribution of anal folds after chest-knee positioning which became much less contracted and obliterated respectively.

Discussion

Sexual abuse is unwanted sexual activity, with perpetrators using force, making threats or taking advantage of victims not able to give consent. Most victims and perpetrators know each other. Immediate reactions to sexual abuse include shock, fear or disbelief. Long-term symptoms include anxiety, fear or post-traumatic stress disorder.⁹

Results of a physical examination will be within normal limits in 80% of child victims of sexual abuse¹⁰, since the mucosal tissue is elastic and may be stretched without injury.¹¹ It worth noting that conducting the physical examination must take place as much early as possible since the anal physical injuries can heal rapidly due to high blood supply of the area; superficial abrasions and fissures can heal within 24 to 48 hours. Unfortunately, many victims of sexual abuse do not seek medical care until weeks or months after the incident.

In our study, most victims were under the age of 18 years old. This finding increases the necessity of continuous parental supervision, and stressing on the vital duty of schools in notifying the relevant authorities of any possible violation of children's rights or suspected cases of child abuse of any form, in addition to the critical need of supporting the societies with governmental facilities specialized in family protection programs.

Females regardless the age, showed to be affected more than males. This largely lies within the child abuse issue, and the rest lies within the context of domestic wife violence.

Clinical examination of the anal region may be performed with the patient in the supine, lateral recumbent or prone position with gentle retraction of the buttocks. These clinical positions have been experienced in our clinic, However, It turned out that they were not beneficial in showing the full picture of the truly existing signs of anal assault. While examining the patient in chest-knee position showed more precise and obvious results. We emphasize the importance of explaining the position intended and its value to the victim prior to the clinical examination. Since Physical comfort and psychological acceptance of the chest-knee position by the victim is vital for achieving the best results of the examination.

The degree of anal sphincter contractility showed by inspection revealed an obvious difference with chest-knee position comparing with other clinical positions used (table 3).

Noticing that the percentage of results of the distribution of anal cutaneous folds showed to be approximate to the results of inspection of anal orifice contractility in both chest-knee position and in other clinical positions, this approximation is caused by the anatomic linkage between the cutaneous folds and the voluntary muscle of the external sphincter which is responsible for the sphincter contractility, since these folds are formed by the effect of contraction of the corrugator cutis ani muscle; which radiate from the superficial portion of the external sphincter muscle to the deep aspect of the perianal skin causing puckering of that skin (folds). The clinical importance of the distribution of these folds lies in their obliteration in case of chronic sexual anal assault.

Among the different physical signs of anal sexual assault, the mentioned signs in table (2) were the mostly affected by the clinical position used, and were more obvious with the chest-knee than being in other clinical positions used.

Conclusions

During the examination of victims of sexual anal assault, and in comparison with other clinical positions used, the application of chest-knee position showed overall more conspicuous physical findings, especially regarding the distribution of anal cutaneous folds, the inspected degree of contractility of anal sphincter, and the presence of injuries in the anal region represented mainly by contusions and abrasions. And for obtaining the best possible results, patient's physical comfort and psychological acceptance of the clinical position must be achieved by explaining the position intended to him/ her prior to examination.

Compliance with Ethical Standards

Conflict of Interest: Authors A and B declare that they have no conflict of interest.

Ethical approval: All procedures performed in studies involving human participants were in accordance

with the ethical standards of the institutional and/or national standards.

Informed consent: International regulations allow using data obtained from forensic department to be used in prospective/archival studies, without the need to obtain an explicit research committee.

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An Analysis of Firearm and Explosives Injury in Imphal: A 2 Years Autopsy Based Descriptive Study

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How to cite this article: Nani Gopal Das, Thoidingjam Bijoy Singh. An Analysis of Firearm and Explosives Injury in Imphal: A 2 Years Autopsy Based Descriptive Study. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):36-40.

Abstract

Deaths due to high end machineries like guns and sophisticated explosives are on increasing trends with throughout the world. Such deaths lead to high morbidity and mortality in the human society. The present study was conducted to explore various socio demographic characters of victims and medicolegal aspects related to firearm and explosive injuries death. This was a descriptive cross sectional autopsy based study of all firearm and bomb blast injury deaths brought to mortuary of Regional Institute of Medical Sciences, Imphal Manipur during the period from October 2013 to August 2015. In this study a total of 46 cases of firearm and explosives death during the study period was analysed in various medico-legal aspects. Rifled firearm and bomb blast was commonest weapon employed. Multiple injuries and head injuries were commonest cause of death. The trends of death might help the law enforcement authorities and Government to take proactive steps to curb this menace.

Keywords: Firearm; bomb blast; Homicide; suicide; injury; weapon.

Introduction

Firearms and explosives are one of the most dreaded weapons used by human being to kill themselves. Ever since the medieval history to present day, firearms of different types have resulted in great morbidity and mortalities. Firearms as a means of homicide or suicide are relatively becoming common in most places of the world, whereas road traffic accidents are relatively more common among young people.¹ And thus it depicts the frequency with which law and order situation in the society have been compromised. Firearm injuries account for high death rates but also lead to long term physical

and mental morbidity for individuals, families, and societies at large.⁴

According to statistical data from the National Crime Report Bureau, total victims murdered by firearms during 2014 in India including all states and union territories was 3,655, out of which 540 were killed by licensed firearms and 3,115 by unlicensed firearms. Major case load were detected to be from Uttar Pradesh and Bihar state.¹⁶

With the progression of human civilization, the urge for money and power has led to the demand for firearm and explosives weapons have seen an extraordinary rise in the present times.¹

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In North Eastern region of the Country, being surrounded with the international border around, firearm culture is known to exist since post independent era due to various reasons. However, there are very few detailed scientific analysis is available in this regard. The magnitude of problem out of such armed outfits is more or less present in all states of North Eastern region and Manipur is not an exception.

The present study was initiated to explore various socio demographic characters of victims and medicolegal aspects related to firearm and explosive injuries like type of weapon, motive behind the deaths, manner, intraday distribution, body parts involved and range of fire, cause of death etc.

Materials and Method

This was a descriptive cross sectional study of all firearm and bomb blast injury deaths brought to mortuary of Regional Institute of Medical Sciences, Imphal Manipur during the period from October 2013 to August 2015. Information was collected based on history of relatives, police information, requisition papers, and relevant reports produced by police. Type of firearm and bomb blast was determined on the basis of injury produced, burning of the skin, tattooing, entry and exit wounds. Manner of death as to homicidal, suicidal or accidental was based upon detailed examination of injuries and relevant history from police and relatives of the deceased and all other available documents.

Results and Observations

A total of 520 medico-legal autopsies have been conducted in the mortuary of Forensic Medicine Department, Regional Institute of Medical Sciences, Imphal during the period from October 2013 to August 2015. Altogether 46 (07.80%) cases were of firearm and explosives injury deaths during the study period and these cases were studied in details and comprehensively and thus the following results were observed. Out of 46 cases only 43(93.47%) cases were of males and 3(06.52%) cases were of females.

Maximum number of cases 21 cases (45.65%) was found in the age group 31-40 years and next in the frequency come the age group between 41-50 year with 15 (32.60%) cases and others in decreasing trends.

A maximum number of 27(67.50%) cases of firearm and blast injuries were observed from 6 p.m. - 12 midnight followed by 14(30.43%) cases from 6 a.m. to 12.00 noon. From 12 midnight to 6 am in the morning a total of 03(06.52%) cases were observed

and least number of cases in the study were recorded from 12 noon to 6 pm.

A total of 36 cases (78.26%) were from urban background while 10 cases (21.73%) were reported from rural areas.

A total of 19(41.30%) cases were by rifled firearm weapons while 17(36.95%) cases were by bomb or explosive blast, and other weapons as displayed in the table no 01.

In 36.94% cases, single entry wound was observed and double entry wounds in 10.08% cases of firearm injury and multiple entry wounds in 52.98% of the cases of total cases which mostly contributed by explosive injuries.

Out of all the cases analyzed during the study period, 21 injury cases (45.65%) were from distant range, 12 (26.08%) from close range and 13 cases (28.26%) were from contact range.

The most common motive behind death of reported firearm injuries was terrorism activity. Group and personal rivalry was second most common cause of firearm injuries followed by others. Only one case was reported where death occurred during dispersion of mob which was accidental in nature as mentioned in the table no 02.

On overall analysis of the data a total of 40 cases (86.95%) turnout to be homicidal, 05 cases (10.86%) were of suicidal nature while 01 case (2.17%) were accidental in nature as displayed in table no 3.

Multiple body parts was by far the most important site of injury with 14 cases (30.43%) followed by head including 09(19.56%) cases. Thoracoabdominal was the site of injury in 7(15.21%) cases and other sites in decreasing frequency as shown in table no 4.

Shock & haemorrhage was the most common cause of death and next comes the head injury as shown in Table no 05.

Table 01: Type of firearm used

Type of firearm	Number of cases	Percentage (%)
Rifled	19	41.30
Bomb or explosives	17	36.95
Shotgun	07	15.21
Country made gun	03	06.52
Total	46	100

Table 02: Motives behind the fatalities

Motives	Number of cases	Percentage (%)
Terrorism	22	47.82
Rivalry	11	23.91
Encounter	06	13.04
Depression	04	08.68
Property dispute	02	04.34
While dispersion of mob	01	02.17
Total	46	100

Table 03: Manner of death involved in various firearm fatalities

Manner of death	Number of cases	Percentage (%)
Homicide	40	86.95
Suicide	05	10.86
Accident	01	02.17
Total	46	100

Table no 04: Body parts affected

Body part affected	No of cases	Percentage (%)
Multiple body parts	14	30.43
Head only	09	19.56
Thoracoabdominal	07	15.21
Head & thorax	06	13.05
Abdomen only	05	10.87
Head & abdomen	05	10.86
Total	46	100

Table no 05: Causes of death

Cause of death	No of cases	Percentage (%)
Shock & haemorrhage	25	54.35
Head injury	14	30.43
Injury to the vital organs	07	15.22
Total	46	100

Discussion

Gunshot injuries due to firearms and explosives are prevalent worldwide. Globally such injuries have been reported in almost each and every part of the world. Suicidal firearm injuries are commonly reported from developed countries whereas homicidal firearm and bomb blast are fairly common in developing countries. As per latest data, India

ranks third all over the world in firearm related deaths, next only to Brazil and United States. Nearly 9 in 10 killed in India were men and most commonly involved people aged 20-24 years.¹⁵

According to the United Nations Organizations, young males were more vulnerable to the victims of such violent crimes due to their high risk taking behaviours ranging from street quarrels to drugs, from possession of weapons to gang memberships.¹³

It is observed that for the last 10 years in Manipur, there was a gradual declining trend of homicide after 2009 as per the data available in the archive of Department of Forensic Medicine, RIMS Imphal. There was 388(65.65%) cases of homicide in the year of 2009 which was mostly contributed to activities of miscreants and then number came down to 49(18.08%) in the year of 2014.

In our study, males outnumbered females with 93.47% deaths. More so, relatively young people in the age range of 31-40 years who are the bread earner of the family were the victims in the largest proportions (45.65%) as they are exposed to the outer world of competition whereas females are mostly housewives are confined in the domestic chores only. Similar results were obtained in the study conducted by Juglan S et al³, Dutta S et al⁵ and Kaul A et al.¹⁰

We found rifled firearms and bomb blasts were the most used weapon. Among explosives, improvised explosive devices (IED) were most commonly employed weapon. In contrast to our study findings, country-made weapons were the most common type used to produce gunshot wounds in Agra region according to Kumari S et al.² A possible reason for such difference is because there is easy availability of local manufacturers of country made weapons and shot guns in developing countries like ours as against extra technically sound rifled weapons.

In developed countries most common firearm weapon are rifled guns and similar patterns were observed in our study. Similar findings were observed by Moirangthem BK et al.⁷

Kumari S et al.² reports that 55% of cases were from rural background. In contrast to these findings, our analysis found a total of 36 cases (78.26%) from urban background.

In our study, mostly shots were aimed from distant range in majority of cases. In study by Sachan R et al.⁶ close range was found to be the commonest. However, in our study close range was the next after distant range. 17 cases of bomb blast were also observed in our study.

Majority of cases in our study were homicidal deaths. These included deaths from distant range and close range combined together. Only one case of accidental firing was observed. Similar observations were found by Kumari S et al.², Kohli et al.¹¹ and Singh BP et al.¹² In the study by Brain Guetsclow et al.¹⁴ suicidal deaths outnumbered homicidal deaths in past 30 years experience in United States.

In most of such homicidal cases single site entry wound marks were present. However, in 14 cases there were multiple injury marks which were due to firearm injuries associated with assault.

According to Hagraas et al.⁴ most firearm injury cases happened during night. Second in line were reported in afternoon while others were reported in morning. In contrast to that, in our study most of the cases were observed at late evening and early night time. The reason being early closure of all business and official activities in Imphal Manipur as compared other capital cities of a state. According to Kumari S et al.² 50% of the cases were observed in night. Such timings are more suited for silent and easy execution of incidents which can be partially attributable to intoxication or substance abuse which very much prevalent in this region.

Sachan R et al.⁶ reported that property dispute were the underlying cause in most of the cases to be followed by incidences like dacoities and personal enmity. In contrast to it, the present study we found terrorism and group rivalry were most common causes of fatalities.

Thoraco-abdominal involvement was more common in distant range shots of firearm injury and dispersion of pellet in bomb blast with homicidal manner and head was more commonly affected in firearm injury cases with suicidal manner. This is consistent with the principle of common knowledge that head and neck are the sites of easiest access for suicidal acts. Thoraco-abdominal injuries are more often involved in distant range shots and explosives due to greater surface area compared to head and neck. Similar observations were found by Patowary AJ⁹, Kohli A et al.¹¹ and Moirangthem BK et al.⁷

Shock & haemorrhage was the most common cause of death in our study and is in accordance with findings of Thube HE et al.⁸ and Patowary AJ.⁹ The reason could be because of multiple organs involvement in majority of cases leading to bleeding and shock out of it.

Conclusion

The present study highlights trends of firearm and explosives deaths in the developing country like ours where homicidal deaths are more than suicidal ones as against the trends in developed countries. It is of utmost important that government authorities put a strict vigilance and control over storage, production, distribution, circulation and licensing of such deadly weapons along with stringent measures to reduce such issues with commitment. And strengthening the social harmony and reducing interpersonal rivalries are to be tried upon at all levels to modify the mindset of the people in general.

Conflict of Interest: Nil

Source of Funding: Nil

Ethical Clearance: From Institutional ethical committee.

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Comparison of the Efficiency of Globalfiler™ IQC PCR Amplification Kit and Powerplex® Fusion 6C System with Half-Volume Reactions for Forensic Trace DNA Testing

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How to cite this article: Nattawoot Saokaew, Kewalee Junpan, Wawkan Duangshatome et al. Comparison of the Efficiency of Globalfiler™ IQC PCR Amplification Kit and Powerplex® Fusion 6C System with Half-Volume Reactions for Forensic Trace DNA Testing. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):41-47.

Abstract

Two major challenges in daily and routinely forensic genetics test are samples with low or very low quantities of DNA and high cost of processing. To overcome these obstacles, a reduction in reaction volume to half would offer a substantial benefit, but even so it should provide complete genetic profiles without conceding the quality of the results. The aim of this study is to test the robustness of two commercially available kits, GlobalFiler™ IQC PCR Amplification Kit (GF-IQC) and PowerPlex® Fusion 6C System (P-6C), with half volume reaction in accordance with SGWDAM guidelines. GF-IQC showed minimum and stochastic threshold of 45 and 610 RFU, while P-6C demonstrated 60 and 670 RFU of those values respectively. The P-6C can tolerate to many inhibitors including melanin, hematin, and humic acid. Both kits showed the same performance on case work profiling and the sensitivity of allele detection of P-6C was not significantly different from that of GF-IQC ($p=0.144$). The results of this study demonstrated that GF-IQC and P-6C with half volume reaction can produce good quality profiles and could be applied for forensic DNA examination.

Keywords: Forensic genetic; DNA typing; short tandem repeats; human identification; GlobalFiler™ IQC PCR Amplification Kit; PowerPlex® Fusion 6C System.

Introduction

The gold standard for human-based DNA profiling is Short Tandem Repeat (STR) fragment analysis, using multiplex PCR and capillary electrophoresis (CE).¹ In 2017, the Combined DNA Index System (CODIS) reported original 13 STR loci

and expanded with another 7 STR loci for human identification. Hence, at present commercial PCR kits consist of up to 20 loci for forensic DNA profiling.² It is necessary for a forensic laboratory to validate a commercial PCR kit before using it with casework, and do this following the SGWDAM Guidelines.³

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Many parameters such as baseline noise, sensitivity, inhibitor tolerance, accuracy and precision are used in the internal validation.⁴ Also, these parameters can be used to evaluate and compare commercially available kits.⁵⁻⁶

GlobalFiler™ IQC PCR Amplification Kit (GF-IQC) consists of 21 autosomal loci, amelogenin and 2 Y-chromosome markers with an additional IQC (internal quality control system) marker, whereas PowerPlex® Fusion 6C System (P-6C) consists of 23 autosomal loci, amelogenin and 4 Y-chromosome markers. Both kits include autosomal STRs from the CODIS core STRs.^{7,8} For successful DNA profiling, use of a high-performance kit is important because the trace DNA in forensic samples is often of low quantity and contains PCR inhibitors.⁹ The manufacturer's recommended PCR volume for most available kits is 25 µL, however several studies were successful in obtaining solutions with reduction in reaction volume to half.^{10,11} A reduction in reaction volume to half would provide many considerable advantages, such as complete genetic profiles even in low DNA samples or the presence of PCR inhibitors, and would be cost-saving in the daily forensic genetics routine. But optimization and comparison studies should be carried out to confirm the amplification efficiency and the quality of DNA profiles.

This study aimed to evaluate and compare the performance of two commercial PCR kits, GF-IQC and P-6C, with half-volume reactions. Multiple forensic parameters, including baseline noise, sensitivity and stochastic threshold, inhibitor tolerance and heterozygous balance, were investigated.

Materials and Methods

DNA samples

The samples used in this study were comprised of control DNA 1224, 1230 (InnoGenomics), 007 (Applied Biosystems), 2008M (Promega) and DNA left over from 31 real-casework samples (Table 1). Consent for their use was given by the Subdivision of Biology and DNA, Central Police Forensic Science Division. The DNA quantity was determined by Quantifiler™ HP DNA Quantification Kit (Applied Biosystems).

Table 1: Type of samples

Type of sample	Number of samples
Buccal swab	6

Contd... Table 1: Type of samples	
Blood	6
Tissue	1
Semen	2
Hair	1
Fingernails	2
Cigarettes	2
Vagina swab	2
Toothbrush	1
Touched DNA	8
Total	31

Amplification

For GF-IQC (Applied Biosystems), the components for a reaction consisted of 3.75 µL master mix, 1.25 µL primer set and 7.5 µL DNA template (total 12.5 µL; half reaction) with 29 cycles of 95°C for 1 min, 94°C for 10 sec, 59°C for 90 sec and 60°C for 5 min. As for P-6C (Promega), the components for a reaction consisted of 2.5 µL master mix, 2.5 µL primer set and 7.5 µL DNA template (total 12.5 µL; half reaction) with 29 cycles of 96°C for 1 min, 96°C for 5 sec, 60°C for 60 sec and 60°C for 10 min.

DNA electrophoresis

Prior to analysis, the samples were prepared by mixing 9.5 µL HiDi™ formamide, 0.5 µL size standard reagent and 1 µL PCR product. The samples were then denatured for 3 min at 95°C and applied to ABI 3500 Genetic Analyzer using POP-4 polymer and 36 cm capillaries with 1.2 kV 15 sec injection condition. The results were analyzed by GeneMapper® IDX v.1.4 (Thermo Scientific).

Baseline study

Nine samples of Low TE⁻⁴ were amplified with each kit with half-volume reactions. Then, the products were electrophoresed and examined at 1 RFU. The total results were computed to obtain an average of baseline peak height and a standard deviation (SD) of each fluorescent channel. These values were used to calculate the minimum threshold as average + 3*SD (Limit of Detection; LOD) and average + 10*SD (Limit of Quantification; LOQ) of each kit.¹²

Sensitivity and stochastic study

Serial amounts of input DNA, including 0.008, 0.016, 0.031, 0.063, 0.125, 0.25, 0.5, 1 and 2 ng per reaction of Control DNA 1224 were triply amplified with each kit. Then, the products were electrophoresed and examined at the minimum

threshold which was obtained from the base line study. Data were computed as percentage of allele detection, average heterozygous peak height, and peak height ratio (PHR). These results were used to assess the optimal input DNA, heterozygous peak balance and stochastic threshold for each kit.

Inhibitor study

Melanin, hematin and humic acid were dissolved in an appropriate solvent (melanin: 0.5 M NH₄OH; hematin: 0.1 M NaOH; humic acid: nuclease-free water).¹³ Each inhibitor, along with an optimal amount of input control DNA 1224, was used as half-volume for amplification. Then, the samples were tested with an ABI 3500 Genetic Analyzer and examined at 175 RFU. The percent of allele detection and average heterozygous peak height were calculated and used to assess each kit's tolerance to inhibitors.

Casework study

DNA samples from Table 1 were amplified and analyzed. For interpretation, results were determined at 175 RFU analytical threshold and the stochastic threshold from the above sensitivity study to assess kits' performance; percent allele detection was used to compare the results of the two kits.

Calculation and statistical methods

Results were organized and calculated using Microsoft Excel. Parameter calculations were as follows.

(1) Percent allele detection

$$\% \text{ allele detection} = \frac{\text{allele count}}{\text{total alleles}} \times 100$$

(2) Peak height ratio

$$\text{Peak height ratio (PHR)} = \frac{\text{RFU}_{\text{min}}}{\text{RFU}_{\text{max}}} \times 100$$

(3) Stochastic threshold

The highest surviving sister allele was defined as the stochastic threshold obtained from the results of serial input DNAs.¹⁴

(4) Wilcoxon rank test

Wilcoxon rank test was used for statistical comparison of percent allele detection and average peak height.¹⁵

Results

Baseline study

Limit of detection (LOD) and limit of Quantification (LOQ) that can be calculated from the average peak height of background noise of the GF-IQC and P-6C are shown in Table 2. GF-IQC gave its highest LOQ of 44.53 RFU in the green (VICTM) channel. Yet, P-6C's highest LOQ (55.16 RFU) was also in the green (JOE-6C) channel. The minimum threshold can be determined using the LOQs of each kit. GF-IQC and P-6C, with half-volume specimens, had 45 and 60 RFU as the minimum thresholds, respectively.

Table 2: LOD, LOQ and minimum threshold of GF-IQC and P-6C with half-volume reaction.

Dye channel	GF-IQC				P-6C			
	Dye	LOD	LOQ	Minimum threshold	Dye	LOD	LOQ	Minimum threshold
Blue	6-FAM TM	14.27	33.56	35	FL-6C	18.79	42.20	45
Green	VIC TM	20.14	44.53	45	JOE-6C	25.17	55.16	60
Yellow	NED TM	11.94	29.37	30	TMR-6C	12.94	29.30	30
Red	TAZ TM	18.36	41.23	45	CXR-6C	19.60	42.62	45
purple	SID TM	19.24	43.57	45	TOM-6C	16.75	37.19	40

Sensitivity and stochastic study

The relationship between percentage allele

detection and DNA input is shown in Figure 1. GF-IQC produced complete profiles when the range of DNA input was 125 pg - 2 ng. The allelic

dropout occurred when input DNA amount was 8 - 63 pg. For P-6C, the sensitivity was not found significantly different from that of GF-IQC ($p=0.144$).

Nevertheless, average heterozygous peak height of GF-IQC was significantly higher than that of P-6C ($p=0.008$).

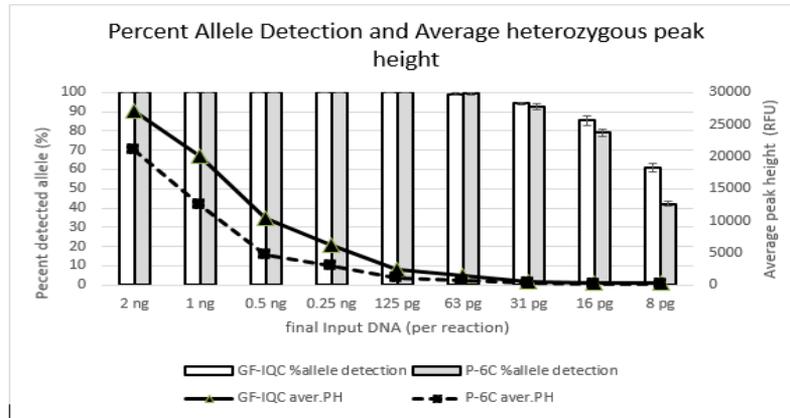


Figure 1: Percent allele detection (% allele detection) and Average heterozygous peak height (aver.PH) of serial input DNA, amplified by GF-IQC and P-6C.

Using half-volume reaction, GF-IQC performed up to 56% PHR in 0.5 - 2 ng input DNA (Figure 2). The lowest PHR was found at 10% in 31 pg input DNA. Whereas the P-6C had similar performance to

GF-IQC (Figure 3). In range of 0.5 - 2 ng, more than or equal 60% PHR was found. The lowest PHR was found at 8% in 31 pg input DNA.

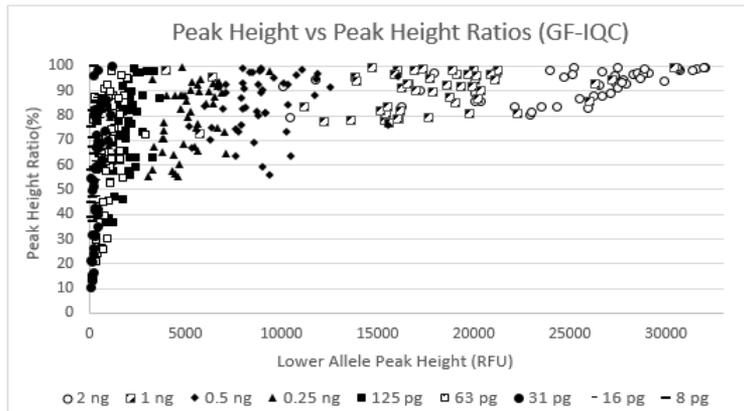


Figure 2: Average heterozygous peak of serial input DNA amplified by GF-IQC.

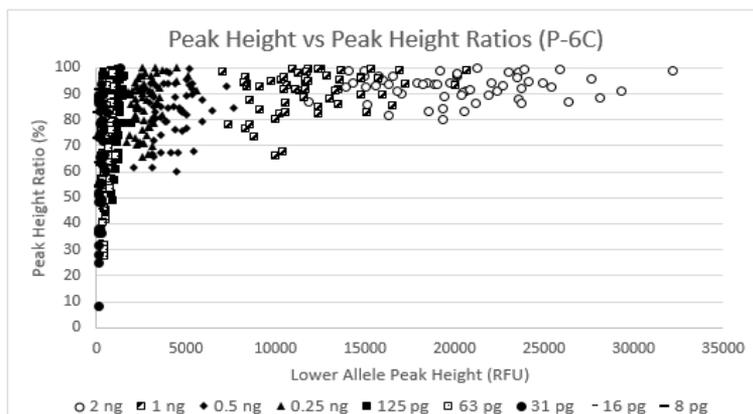


Figure 3: Average heterozygous peak of serial input DNA amplified by P-6C.

The evaluation of half-reaction performance of both kits showed that the optimal input DNA of both kits was 0.5 ng. This conclusion used three criteria: 1) revealing optimal peak height, 2) disappearing the pull up or off-scale peaks, and 3) providing a complete profile or lacking allele drop out⁽¹⁶⁾. Moreover, the stochastic thresholds of GF-IQC and P-6C were at 610 and 970 RFU, respectively. The highest surviving sister allele of GF-IQC was found at D19S433 (16 pg input DNA), while it was found at Amelogenin (63 pg input DNA) of the P-6C.

Inhibitor study

Overall, the peak height of DNA profiles was decreased when the concentration of inhibitors was high, and amplifying without inhibitors always

produced complete DNA profiles. As shown in Figure 4, melanin started to inhibit the activity of GF-IQC and P-6C at 60 and 80 ng/μL, respectively. Complete inhibition was found with melanin concentrations of 80 and 100 ng/μL. However, these melanin concentrations did not cause complete inhibition of the P-6C even at the higher concentration of 100 ng/μL. The hematin caused partial inhibition of amplification by GF-IQC at 750 μM, and complete inhibition at 1000 and 1250 μM (Figure 5). However, even the highest concentration of hematin did not inhibit the amplification of P-6C. Humic acid, at concentrations of 200 and 300 ng/μL, inhibited amplification activity of both GF-IQC and P-6C (Figure 6).

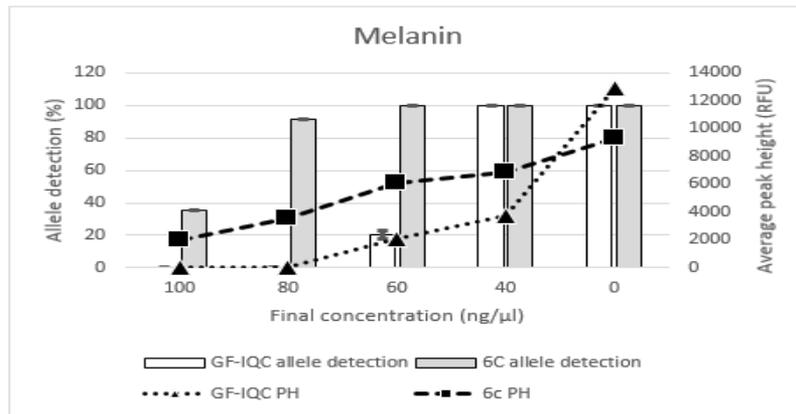


Figure 4: Percent allele detection and average heterozygous peak height with 5 concentrations of melanin in amplification using GF-IQC and P-6C. Bars and lines represent percent allele detection and the average heterozygous peak height, respectively.

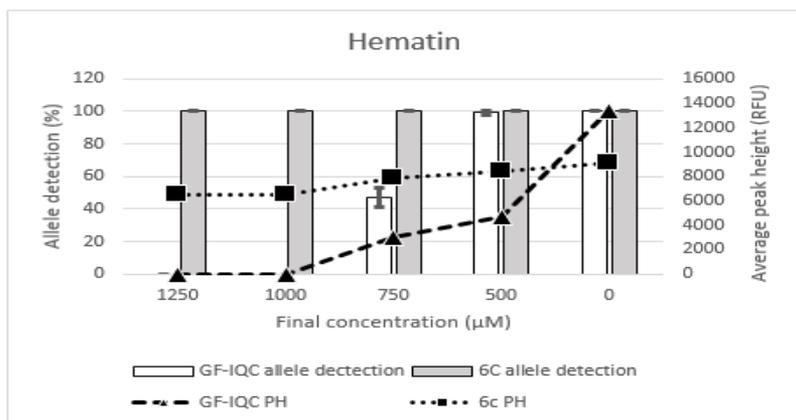


Figure 5: Percent allele detection and average heterozygous peak height with 5 concentrations of hematin in amplification using GF-IQC and P-6C. Bars and lines represent percent allele detection and the average heterozygous peak height, respectively.

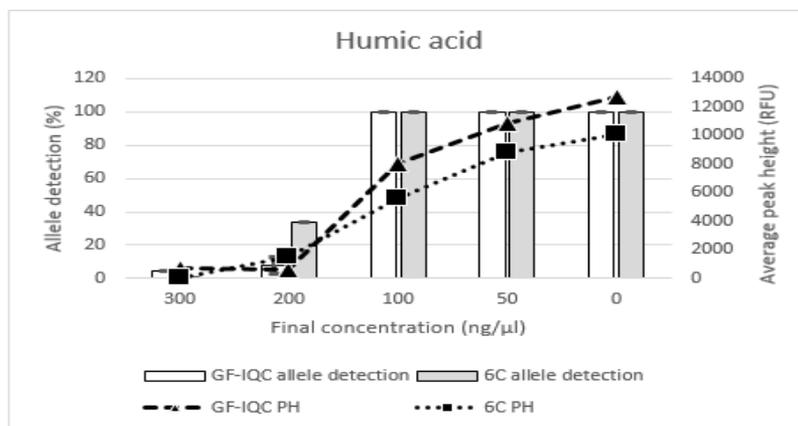


Figure 6: Percent allele detection and average heterozygous peak height with 5 concentrations of humic acid in amplification using GF-IQC and P-6C System. Bars and lines represent percent allele detection and the average heterozygous peak height, respectively.

Casework study

Among thirty-one samples, both kits detected 19 complete profiles, 4 partial profiles and 6 mixture profiles (data not shown). The kits' results were concordant except for sample S24 which originated from a fingernail. The GF-IQC gave a partial profile with 99% percent allele detection (SD=2), while the other kit gave a complete profile. Yet, both kits remained amplifying a concordant profile for this sample. Lastly, neither kit detected the trace DNA on a swab of a keyboard (code S20).

Discussion

This study is the first to evaluate and compare the performance of GF-IQC and P-6C, with half-volume reactions. P-6C generated higher baselines than did GF-IQC with these half-volume reactions, which is consistent with the results when full volume reactions are used.^{12,18} The GF-IQC with half-volumes showed minimum thresholds similar to previously published data with full volume reactions.^{5,17}

GF-IQC with half-volume reactions produced complete profiles with 0.125 ng of input DNA and there was no difference in sensitivity from that with full volume reactions.^{5,13,18} P-6C with half-volume reactions also produced complete profiles with 0.125 ng input DNA. This was consistent with full volume reactions reported by Cisana et al.¹⁹ but differed from the report by Feng et al.²⁰ the groups reported complete profiles with DNA amounts of 0.125 and up to 62.5 ng, respectively. GF-IQC and P-6C demonstrated similar results of percent allele detection, although the heterozygous peaks were significantly different. As described earlier, half-volume reactions of both kits amplified as well as full volume reactions. As a result, 0.5 ng was suggested as the optimal input DNA amount for both kits. The

stochastic threshold is crucial for DNA interpretation. The highest false-homologous peak which can determine stochastic threshold was observed in both kits with low input DNA. The thresholds with half-volume reactions were suggested at 610 RFU for the GF-IQC and 970 for the P-6C.

Performance in terms of generating balanced heterozygous peaks by the P-6C was slightly better than that of the GF-IQC. With optimal quantity of DNA (0.5 ng per reaction), Short Tandem Repeat amplification kits should perform more than 60% PHR.²¹ P-6C with half-volume reactions met this parameter adequately at 60%, whereas GF-IQC gave a slightly poorer PHR at 56%. Moreover, low input DNAs are likely to cause a decrease in PHR. In this study, the lowest PHRs were 10% and 8% for GF-IQC and P-6C, respectively (obtained from 31 pg input DNA).

Melanin, hematin and humic acid usually originate from hair, blood and soil, respectively, and are commonly found in evidence. In this study, P-6C was more tolerant to these three inhibitors (especially hematin) than was GF-IQC. Surprisingly, P-6C still showed complete profiles even when 1250 μM hematin was added into the reaction. Tan et al. reported that 2000 μM hematin cannot inhibit full volume reactions of P-6C.¹⁷ Thus P-6C with half-volume reactions can be used effectively with blood-related evidence. Moreover, both kits with half-volume reactions successfully amplified various types of samples from real casework, as shown by the results of 31 casework samples, and gave concordant results.

Conclusions

We evaluated and compared the performance of GF-IQC and P-6C with half-volume reactions. The optimal amount of input DNA was 0.5 ng for both kits. We suggest a 45 RFU minimum threshold and

610 RFU stochastic threshold for GF-IQC. We also suggest a 60 RFU minimum threshold and 970 RFU stochastic threshold for the P-6C.

In contrast, the GF-IQC was less tolerant to 3 inhibitors and produced imbalanced peaks more easily than did the P-6C. Yet, the GF-IQC produced significantly higher average peak heights however the kits had no significant differences in sensitivity, and performed with high robustness. Our overall assessment was that the GF-IQC and P-6C, with half-volume reactions, could both be applied productively for forensic purposes.

Ethical approval statement: The experimental protocol was approved by the Ethics Committee of Thammasat University (COA No. 024/2564).

Conflicts of interest: Nil.

Source of funding: Self.

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Changing of ATP and Its Metabolites in Blood Samples for Post Mortem Interval: In Vitro Study

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How to cite this article: Warangkool Chanpan, Churdsak Jaikang, Chaturong Kanchai. Changing of ATP and Its Metabolites in Blood Samples for Post Mortem Interval: In Vitro Study. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):48-52.

Abstract

Background: Postmortem interval (PMI) is an important in forensic practice. Many studies in blood and tissue of animals have revealed correlation between adenosine triphosphate (ATP) level and PMI. In this study aimed to explore the correlation between human blood ATP and its metabolites level and PMI in vitro model.

Methods: Ten milliliter of venous blood samples were collected from four healthy men and contained in EDTA tube. One milliliter of the blood sample was divided at 0, 1, 2, 4, 8, 12 and 24 h, respectively. The blood samples were extracted and measured ATP, adenosine diphosphate (ADP) and adenosine monophosphate (AMP) level by High performance liquid chromatography- diode array. Energy charge value was calculated to predict charge of ATP metabolites.

Results: The blood ATP, ADP and AMP levels increased in the first hour. The blood ATP, ADP and AMP levels did not obviously change before 12 hour. The blood ATP level trended to decrease but the blood ADP and AMP levels trended to increase after 24 hour. Energy Charge was not change in first 12 hour but trended to decrease after 24 hour.

Conclusion: The blood ATP level and its metabolites changed after 12 hour and apply for the PMI investigation.

Keywords: Post-mortem interval; ATP degradation; ATP metabolites; Forensic Science.

Introduction

The estimation of the time since death or postmortem interval (PMI) is an important step in criminal investigation and is the highest frequency question in forensic practice.¹⁻² Early stage of PMI investigation is based on algor, livor and rigor mortis for assessments.³ Ambient temperature, body

structure, cause of death, climate and diseases are associated factors and need evidences to describe the PMI processes.³ Many methods including molecular biology, spectroscopic technology, entomological and Thanatos chemistry, biochemical reaction and fluid concentration have been improved to help the PMI investigation.^{2,4}

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Adenosine Triphosphate (ATP) is an essential substance in cellular metabolism and widely uses for the time elapsed since death. The degradation of ATP in post-mortem tissue is an important biochemical reactions. The oxygen supply to the tissue is stopped after death and the normal physiological metabolic functions are damaged. Normally source of ATP comes from erythrocytes. Imbalance of ATP production and consumption appear after death. The ATP synthesis was stopped and rapidly degraded to adenosine diphosphate (ADP), adenosine monophosphate (AMP) which can be summarized as: $ATP \rightarrow ADP \rightarrow AMP$.⁵⁻⁶

An extensive metabolomics and biochemical change in all body tissue due to lack of circulating oxygen, altered enzymatic reactions, cellular degradation and cessation of anabolic production of metabolites after death. The biochemical changes provide information about the effect of death on cellular function especially ATP blood level. Blood sample has an advantage including easy sampling and sufficient specimens for the PMI estimation. There are many studies have revealed the relationship between ATP blood levels with PMI by using animal blood and tissues. In this study aimed to explore the correlation between human blood ATP levels and PMI in vitro model to provide a preliminary research basis for PMI investigation in forensic science.

Materials and Methods

Chemicals and reagents

Adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP) were purchased from Med Chem Express (New Jersey, USA) Ammonium hydrogen phosphate and methanol were purchase from BDH (Fontenay-Sous Boi, France). Chloroform was purchased from Labscan Limited, Thailand.

Subject and study design

Four healthy men Thai aged range 20-40 years were included in this study. The subjects who were the anemia and red blood cell diseases were excluded. Written inform consent was obtained. The Study protocol was approved by the Research Ethic committee Faculty of Medicine, Chiang Mai University (FOR 2564-07900).

Collection and specimen preparation

Venous blood approximately 10 mL was collected and contained in EDTA tube. The blood samples were stood at 25 °C through experiment. One milliliter of the blood sample were divided every 0, 1, 2, 4, 8, 12 and 24 hr. Each blood samples were extracted with methanol and chloroform in ration 1:2:2 according the method of Nagana *et.al.*⁷ Then, the solution was centrifuged at 4,000 g at 4 °C for 10 min. The organic solvent layers was collected and evaporated under nitrogen gas. The residue was re-dissolved with ammonium phosphate buffer pH 6.0 before HPLC analysis.

ATP and it metabolites measurement

ATP, ADP and AMP were measured by high performance liquid chromatography- diode array (Agilent 1260 Infinity Binary LC, Santa Clara, CA, USA) by apply the method of Menegollo *et. al.*⁸ The HPLC condition comprised of Purospher® Star PR-8 endcapped column (150 × 4.60, 5 µm). The mobile phase consisted of 100% 0.1 M ammonium hydrogen phosphate pH 6.0. Ten microliter of the sample was injected and the spectra were determined at 250 nm. The identification of the chromatographic peak was achieved by comparing the retention times and spectral characteristics (200-400 nm) of the eluted peaks with the standards.

For, energy charge value was calculated by as follows: $EC = [ATP] + 0.5 [ADP] / \{[ATP] + [ADP] + [AMP]\}$ ⁹

Statistical Analysis

Quantitative analysis of ATP and its metabolites were done by external standard curve under the same liquid chromatography condition. The data of ATP, ADP and AMP were expressed as mean ± standard deviation (mean±S.D). The data were evaluated using the SPSS version 22 (New York, USA) The One- Way ANOVA was evaluated for identifying significant level between ATP and its metabolites and time. The threshold for statistical significance was $p < 0.05$.

Results and Discussion

In this study, under HPLC-DAD condition, the ATP, ADP, AMP were appeared at 5.32, 5.96 and 8.59 min, respectively. Changing of ATP, ADP and AMP levels is demonstrated in Table 1 and Fig.1.

Table 1: Concentration of ATP, ADP and AMP levels in the blood at different post-mortem interval.

Time (hr)	ATP ($\mu\text{M} \times 10^2$)	ADP ($\mu\text{M} \times 10$)	AMP (Mean \pm SD)	Energy charge
0	21.14 \pm 3.56 ^a	18.45 \pm 3.92 ^a	46.18 \pm 22.69 ^a	0.94
1	48.32 \pm 5.27 ^b	37.03 \pm 2.07 ^a	48.72 \pm 7.77 ^a	0.95
2	8.03 \pm 0.19 ^c	15.23 \pm 1.74 ^b	1.78 \pm 0.15 ^b	0.91
4	15.94 \pm 0.17 ^d	25.51 \pm 1.93 ^b	18.80 \pm 0.33 ^b	0.92
8	13.51 \pm 0.22 ^d	24.06 \pm 1.72 ^b	11.27 \pm 8.23 ^b	0.91
12	16.25 \pm 0.21 ^d	20.71 \pm 11.09 ^b	17.43 \pm 0.21 ^b	0.93
24	10.31 \pm 0.24 ^e	49.49 \pm 1.30 ^c	112.61 \pm 9.44 ^c	0.78

The values are presented in mean \pm S.D. (n=4). Different small letters indicate significant difference between time in column at $p < 0.05$ which analyzed by One-way ANOVA followed by Bonferroni.

In the first hour, the ATP level significantly increased ($p < 0.05$) and the level did not changed during 4-12 h. After 12 h incubation, the ATP level significantly decreased comparing with the other times ($p < 0.05$). Highly activity ATPase enzyme and more glucose and nutrition contained in the blood can generate ATP molecule during the first period.¹⁰ Butt and his colleagues found that blood ATP level decreased during 0-72 h after death.⁴ The ATP level with in the first 8 h increased following not significant change within 8-16 h in skeletal rat muscle model.⁶ However, these results from in vitro model presented that the ATP decreased after 12 h and need to confirm in the crops study.

ADP is a by- product from ATP degradation in both the cells and in vitro models. In the first hour, the ADP level increased due to the ATP molecules were used in the cell resulting the ADP level rapidly decreased. We hypothesized that the ADP was used for the ATP synthesis by ATPase enzyme or it was degraded to AMP for energy production. Zhu et al. presented that ADP level did not significant change within the first 8 h but within 16-24 h the ADP increased.⁶ At the 2-12 h, the AMP level significant decreased after that the AMP level significant increased ($p < 0.05$). Zhu et.al showed that over all of the AMP level trended to increase in the first 144 h after death.⁶

Energy is an important biochemical molecule for the adequate turnover of the biomolecular structures and the functional metabolic viability in unicellular organisms. ATP, ADP and AMP level and energy charge (EC) reflect to the energetic status of the cell.¹² The EC value in physiological system presents in range 0.7-0.9 and reflects to a balance with the major ATP-consuming reaction. The value closely to 0.9 in living cell but it is dropped off provoking cell to die.^{13,14} Our results showed that the EC value in the first 12 h did not change but after 12 h the value was dropped indicating an imbalance of energy status. The ATP and its metabolites changed in the in human blood in vitro model. The ATP and its metabolites experienced “rise-decline-constant and increase within 24 h after starting experimentation. The possible reason is that residual enzymes in the blood continue to catalyze the decomposition of glycolysis and phosphocreatine and ADP continues to produce AMP and ATP ($2\text{ADP} \leftrightarrow \text{ATP} + \text{AMP}$).¹⁵ The degradation of ATP, ADP and AMP are not obvious and cannot be used to estimate PMI by itself during the first 12 h.¹⁶ Imbalance in energy homeostasis will be occurred after 12 h then determination of the blood ATP and its metabolites might be useful for PMI investigation. The correctly of PMI estimation has not significantly improved and less reliable methods.⁹ After death, a biochemical changes will occur under the influence of various intrinsic and environmental factors.⁶ These processes are non-reversible, progressive and time-sequence.

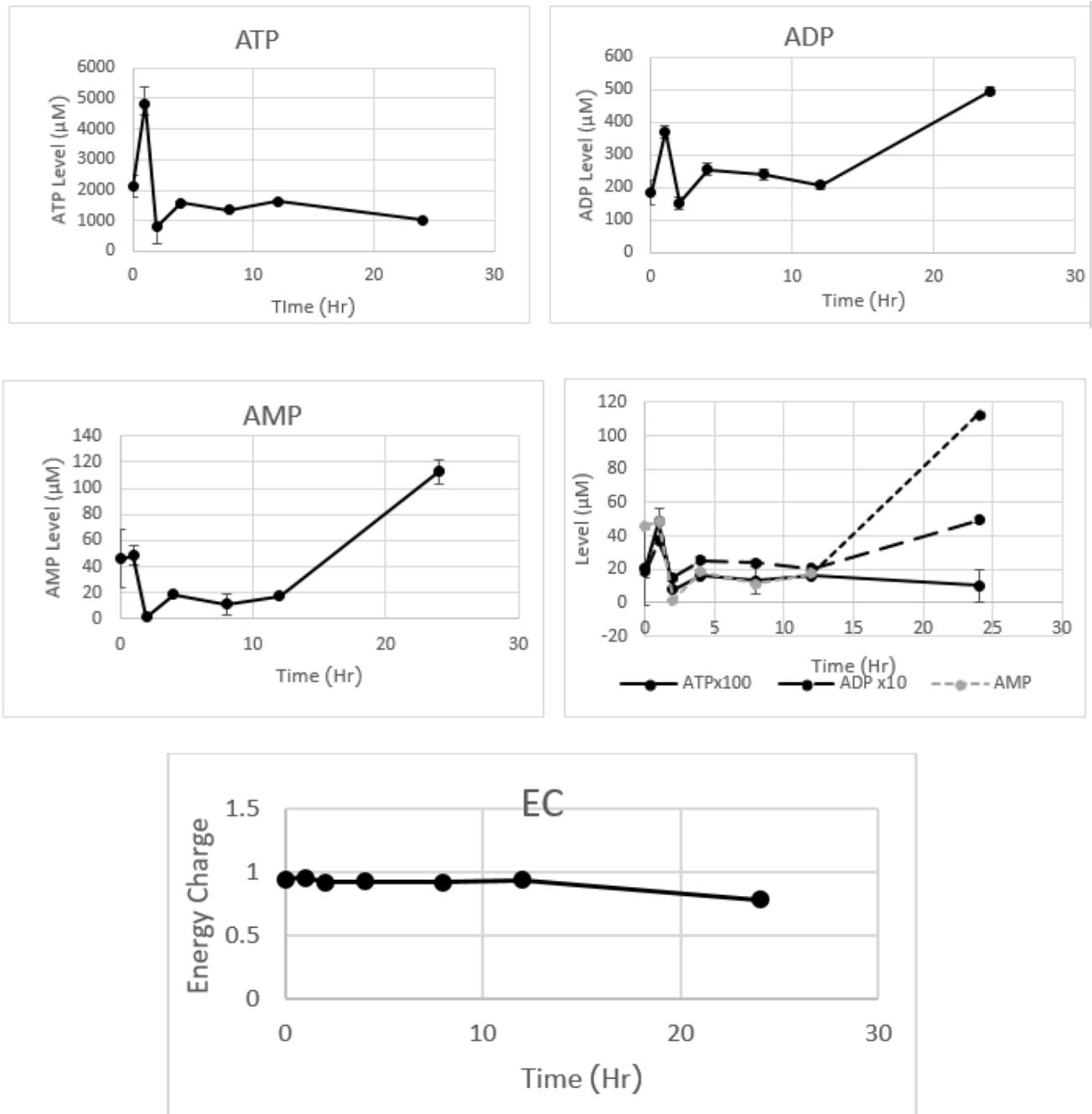


Figure 1: Change in the blood concentration of ATP, ADP AMP and energy charge in vitro model

Conclusion

For in vitro study, changing of ATP and its metabolites after death within 12 h might be applied

for postmortem interval investigation. However, study on human body should be studied further to give more information and the factors involved in postmortem interval interpretation.

Disclosure

The authors declare that there is no conflict of interest in this work.

Ethical Clearance

Taken from Faculty of Medicine, Chiang Mai University committee.

Source of Funding: Self

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Legal Aspects of Genetically Modified Food Product Safety for Health in Indonesia

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How to cite this article: Abdullah Khamdi, Dian Anggraini Wikamorys, Waty Anton et al. Legal Aspects of Genetically Modified Food Product Safety for Health in Indonesia. *Indian Journal of Forensic Medicine and Toxicology* 2022;16(3):53-57.

Abstract

Genetic Engineering is the transplantation of one gene to another, both between genes and across genes, to produce valuable products for living beings. The rapid growth of genetic engineering in Indonesia and Genetically Modified (GM) food products has raised many worries and issues about these foods posing a health risk when consumed. Concerns about GM food products are increasing, including the possibility of allergic reactions, gene transfer, and outcrossing. Indonesia is one of the countries that has accepted the Cartagena Protocol, a security protocol designed to protect biodiversity from the potential risk posed by genetically modified organisms created by contemporary biotechnology. The writer, through this study, would like to convey the extent to which the existing regulations in Indonesia regarding GM food product safety for public health and how the government's responsibility in GM food product safety is for public health. As a result of this study, GM food products had been regulated by law, and the establishment of a Biosafety Commission was one form of government responsibility for preserving the safety of GM food products for public health.

Keywords: Food; genetic Engineering; cartagena Protocol.

Background of Study

Technology in the food supply has developed rapidly, especially in developed countries, such as the United States, Canada, England, Australia, etc. The research discovered a breakthrough in the form of Genetically Modified Organisms (GMOs), which may be utilized to solve the problem of food shortages swiftly, but there are still safety concerns. Internationally, the European Union and international organizations such as the Codex

Alimentarius Commission (CAC) play a significant role in raising concerns about the consumption of Genetically Modified (GM) foods and the need for labelling and regulation of GM products (FAO, 2001). CAC is an intergovernmental organization founded by the Food and Agricultural Organization (FAO) and the World Health Organization (WHO) with 163 countries members.¹

Traditional and modern biotechnology are the two types of biotechnology. Traditional biotechnology

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is the use of microorganisms (organisms) to alter materials and the environment in order to get optimal results. The making process of Tempe (fermented soybean cake), Tape (fermented cassava) and bread are examples of traditional biotechnology. Meanwhile, modern biotechnology employs human abilities to manipulate biological organisms to create the desired result, such as through genetic engineering procedures.² Genetic Engineering is the transplantation of one gene to another, both between genes and across genes, to produce useful for living things. Initially, genetic Engineering was used to produce ideal creatures; for example, tomatoes that could not be grown in low-temperature locations were designed to become frost-resistant plants with a longer growing season and the ability to be cultivated in any weather.³ It is only done on plants to solve the problem of food shortages, and in its development, genetic engineering is used for plants and animals and has also evolved in humans. It is also commonly utilized in agricultural food technologies to increase production and quality and post-harvest improvements. Biotechnological plants authorized for use in food are modified to have traits such as (1) pest and disease resistance, (2) herbicide resistance, (3) nutritional content engineering, and (4) improved shelf life (Manuhara, 2006).⁴

Controversy over genetically modified products (from now on abbreviated as GM products) is still happening today. Several worldwide challenges have surfaced, including the dangers of genetically engineered foods for health. In 1996, WHO discovered a new type of chemical contained in transgenic organisms and their products. These chemicals resulted in new illnesses, such as the AAD gene, which was discovered in transgenic cotton and may be passed on to bacteria that cause gonorrhea.⁵ GM product continues to bring up pros and cons in the community. According to agreed people, GM products can be useful in reducing the use of plant chemicals, overcoming food shortages, and being able to produce good quality food, yet, on the other hand, the disagreed people see the safety of GM products cannot be proven for consumption since it is thought to have a detrimental impact on health when ingested, as well as a destructive impact on the environment and the inability to improve lucrative yields.⁶

Imported genetically modified crops currently circulating in Indonesia include soybeans, corn, and

potatoes. Most of the population in Indonesia daily consume Tempe (fermented soybean cake) and tofu as vegetable side dishes. According to Young and Lewis (1995), there was very little information on the effects of changes in the nutritional composition of GMO (Genetically Modified Organisms) foods from both plants and animals, such as nutrient interactions, gene interactions, bioavailability/absorption of nutrients, potency nutrition, nutrient metabolism, and gene expression in situations in which nutrients are altered.⁷ Based on this information, it is suspected that there is no single study that guarantees genetically modified food is 100% safe for consumption. According to Dresbach et al., not all of the effects of GMOs have been examined. Thus there is no certainty about their safety.⁸ Based on the above description, and the writers want to investigate the legal regulation of the safety of GM products for health in Indonesia and the government's responsibilities to the people who consume GM products.

Discussion

1. Legal regulation of genetically modified (GM) food products in Indonesia

Genetic Engineering of living things, whether in plants, animals, or people, generates moral debate among scientists and legal controversy.⁹ The United States, the European Union, China, Africa, Australia, and the Philippines have regulated the circulation of GM food products. According to the Biosafety Clearing-House database, food safety has been declared for 117 Genetically-Modified Corn, 33 Genetically-Modified soybeans, and 99 Genetically-Modified potatoes. Meanwhile, based on the Center for Environmental Risk Assessment database, currently, there are around 184 types of GMO declared as safe food.¹⁰

The government regulates the safety of genetically modified food products, referring to the results of the convention on biodiversity in the world. The Cartagena Protocol, an agreement between various parties that regulates the procedures for deliberate cross-border movement, including the handling and utilization of a living organism produced by modern biotechnology from one country to another by a person or entity, has been ratified by the Indonesian government.¹¹ The Cartagena Protocol is a further implementation of the Convention on Biological Diversity (Protocol Cartagena on Biosafety) which aims to ensure a level of protection in the safe transfer,

handling and use of genetically modified organisms (GMO). GMO food products such as corn, soybeans, and potatoes are extensively distributed in Indonesia. Thus, the government must anticipate it, particularly those that threaten biodiversity and human health, by ratifying the Cartagena Protocol into Law No. 21 of 2004 on Ratification of the Cartagena Protocol on Biosafety to The Convention On Biological Diversity (from now on referred to as the Cartagena Protocol Law). Article 11 paragraph (1) of the Cartagena protocol states that the party making the final decision regarding domestic use within fifteen days after the decision is made must notify the parties through the Indonesia Biosafety Clearing House. The party shall submit a written copy of the material to the National Focal Point of each party, indicating that it does not have access to the Biosafety Clearing House in advance.¹² One of the Cartagena Protocol Law functions is to obtain optimal benefits from the safe use of modern biotechnology without harming biodiversity and human health.

According to Law No. 36 of 2009 on Health (from now on referred to as the Health Law) Article 109, anyone who distributes GMO products must guarantee their safety for the community or consumers and the surrounding environment. Meanwhile, based on Article 77 paragraph (1) of Law No. 8 of 2012 on Food (from now on referred to as the Food Law), there is a government authority in charge of issuing a permit from the GMO produced to regulate its circulation in public. Then Article 78 paragraph (1) explains that those who develop GMO products must adhere to the government's guidelines to ensure the product's quality to be assured and safe for the general public consumption. For those who violate the provisions of the requirements set by the government, administrative consequences in the form of fines, temporary stoppage of operations, production, and circulation, withdrawal of food from circulation by producers, compensation, and license revocation will be imposed, as specified in Article 79.

As referred to as in Government Regulation No. 28 of 2004 on Food Safety, Quality and Nutrition Article 14 paragraph (2), the safety checks for GM food products must include genetic information, description of donor organisms, description of genetic Engineering, characterization of genetic Engineering, and information on food safety.

Government Regulation No. 69 of 1999 on food labelling and marketing was established by the

government in order for the people to know whether the food they eat is the result of genetic engineering or not. According to Article 35 of Government Regulation No. 69 of 1999, GMO food must have a genetically modified food label, information on GMO food and a distinctive logo arising from genetic engineering.

The requirement to include information on genetically modified foods is informative because, in general, food products circulating in the market are safe products for consumption, free of harmful ingredients and processed well to ensure their safety.

Supervision and control of GMO food products are regulated in Article 25 of Government Regulation No. 21 of 2005 on the biosafety of Genetically Modified Product. Supervision and control of PRG products are carried out by the Minister or the authorized Head of LPND. After the Minister or head of the LPND receives the report, they will assign the Biosafety Commission to check the truth of the report. If the GMF product is found to have a harmful impact on health or the environment, the relevant Minister has the authority to cancel the GMF product's circulation.

2. Government responsibility for the safety of genetically modified food (GMF) products for health.

On the other hand, health insurance for GMO products such as soybeans is not yet guaranteed. One of the experts in genetic engineering, Jeffrey M. Smith in *Seeds of Deception and Genetic Roulette*, said there are at least 65 serious health risks due to consuming GMF products. In fact, according to Smith, between 1994-2001, there was a phenomenon where food-related diseases had doubled along with the circulating GMF products. According to Mae Wan Ho, genetically modified plants are useless and harmful to health.¹⁴ The issue of food safety, including genetically modified food, has become a concern in several developed countries such as Australia, New Zealand, and several European countries.

Some countries are trying their best to protect their citizens from minimizing the impact of GMF products. However, it is not the case in developing countries like Indonesia, which focus solely on food sufficiency and not on food security. While developing countries have not been able to ensure the safety of food products in the form of agricultural commodities, the state must still ensure information disclosure and security.

Genetically modified food is derived from genetically modified living things. In general, food is sourced from plants; currently, genetic engineering in plants is being developed. GMO plants are widely used as food ingredients which are commonly known as genetically modified food products. The usage of MGO products raises worries and issues that these foods may pose a health risk when consumed, including the possibility that GMO food products may cause allergic reactions, gene transfer, and outcrossing.

1. GM food products may affect allergic reactions.

Many GMO food products contain unknown or untested microorganisms as possible allergy contributors. Genes from non-food sources and new gene combinations can trigger allergic reactions in people who consume them or exacerbate existing ones. According to Nordlee et al. (1996), Brazil nuts, one of the MGO food products, were banned from circulation because they induced allergies in consumers. The allergic reaction is thought to be caused by specific gene engineering.¹⁵ In principle, gene transfer from allergenic-caused food is undesirable unless proven that the protein produced by gene transfer is not allergenic. Although traditionally produced food products have not been tested for allergies, GMO food products must go through a range of testing procedures. FAO and WHO have prepared a testing and evaluation protocol for GM food products to be distributed. There has been no evidence of an allergenic reaction to GMO food products on the market.

2. Gene Transfer

The transfer of genes from GMO food products into body cells raises concerns if the transferred genes can cause harm to human health. It is possible if antibiotic-resistant gene transfer is used in the GMO food product manufacturing process, but it is pretty unlikely. In the body of transgenic organisms, it allows antibiotic resistance marker genes to be inserted into certain plants and transferred to disease-causing microbes in the intestines of humans or animals that consume GMO food products. This phenomenon had the potential to result in antibiotic-resistant microorganisms in live communities, as well as antibiotic-resistant

human health issues (Bettelheim, 1999; Hileman, 1999).¹⁶

3. Outcrossing

Outcrossing is the transfer of genes from genetically modified plants to plants of other related species in nature, for example, mixing post-harvest products from conventional plant seeds with genetically modified plant products. Some countries have implemented a separation strategy between agricultural land for genetically modified crops and agricultural land for conventional crops.

With public concern about PRG, it is necessary to apply the precautionary principle and a structured system that regulates and assesses the safety and risk factors of GM food products. On the notion that alien, foreign genes might alter the nutritional value of foods in unexpected ways, reducing or increasing some nutrients and other nutrients, GM food products are assumed to be the cause of numerous diseases. Therefore, the government conducts a series of safety assessments of genetically engineered products as a responsibility to the community.

Procedures for assessing, releasing, distributing, and using GMO food products are regulated in Chapter V of Government Regulation No. 21 of 2005. The assessment is based on a written application submitted by the applicant to the authorized Minister or the head of the authorized Non-Ministerial Government Institution (LPNK). The head of the authorized Non-Ministerial Government Institution (LPNK). If applying for a permit for release to the environment, then the authorized Minister or the head of the authorized LPNK submits a request for a recommendation for the environmental safety of PRGs to the Minister. Authorized Minister is the Minister who is responsible for the environment. In the context of providing recommendations for the biosafety of GMO food products, the authorized Minister or the Head of the authorized LPNK is assisted by the Biosafety Commission (KKH) and the Biosafety Technical Team (TTKH) to conduct a review of technical documents and further tests if necessary. The Biosafety Clearing-House (BKKH), as the Biosafety Commission (KKH) apparatus, announces the receipt of the application, process, and summary of the assessment results in an accessible place for the public to provide an opportunity for the community to submit their responses. Biosafety Commission (KKH) submits recommendations for environmental safety

to the Minister, recommendations for food safety and feed safety to the authorized Minister or the Head of the authorized LPNK. The authorized Minister or the head of the authorized LPNK must base their decisions on the biosafety recommendations of the Minister or the Chair of the Biosafety Commission (KKH). The authorized Minister or the Head of LPNK issues release and distribution permits following applicable laws and regulations for GMO food products that have received biosafety recommendations and can be used for needs in various fields following their designation permits.¹⁷

Conclusion

1. Legal regulations for genetically modified products in Indonesia are already very strict with the ratification of the Cartagena protocol; it is Law no. 21 of 2004 on Ratification of the Cartagena Protocol on Biosafety to The Convention on Biological Diversity and also strengthened by Law No. 8 of 2012 on Food.
2. The government has a role to play in ensuring the safety of genetically altered products for public health by conducting a series of studies on genetically modified food products before being distributed to the public by establishing a Biosafety Commission.

Suggestion

1. There is a need for biosafety regulatory analysis and decision-making on biosafety evaluation in Indonesia.
2. There has to be a massive public education campaign about the benefits and risks of genetically modified food products.

Ethical Clearance: Nil

Conflict of Interest: Nil

Source of Funding: Self Funding

Acknowledgement: Nil

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The Expression of Neogene TIGD3 that Derived from DNA Transposons in Colorectal Cancer Cell lines

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How to cite this article: Ahmed Arnaoty, Hiba. M. Ahmed, Yves Bigot et al. The Expression of Neogene TIGD3 that Derived from DNA Transposons in Colorectal Cancer Cell lines. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):58-62.

Abstract

DNA transposons are exposed to a molecular domestication process, which results in the formation of neogenes, which may play a role in human genetic instability. TIGD3 (Tigger-derived [TIGD] family of proteins) is one of these Neogene, and its role in the human genome is unknown.

Aim: The expression of Neogene TIGD3 in colorectal cancer cell lines and its putative function in carcinogenesis are being investigated.

Method: The protein expression of the TIGD3 gene was investigated using the western blot method in twelve colorectal cancer cell lines (HCT116, SW48, LOVO, DLD1) that are microsatellite instable MSI, (SW480, SW620, HT29, LS123, COLO205, T84, SW403, SW1463) that are microsatellite stable MSS, and in healthy colon tissue as a control in our study.

Results: The expression of the TIGD3 protein was found in all twelve colorectal cancer cell lines, with varying degrees of expression and numerous isoforms, which was not found in healthy colon tissue.

Conclusion: There may be a link between colorectal cancer evolution or progression and TIGD3 gene expression.

Keywords: DNA transposons; domestication; neogene; TIGD3; microsatellite instable; microsatellite stable.

Introduction

Carcinogenesis and tumor growth are linked to a breakdown in the genome's integrity, which is translated by genetic instability. Transposable elements, including DNA transposons that have had their mobility inactivated during evolution, make up half of the human genome.^{1,2}

These DNA transposons usually appear as inactive DNA fragments that are epigenetically silenced by the host genome to inhibit transcription and further transposition.³⁻⁵

After then, such elements are subjected to low selective pressure and develop sequence variation (mutations) over time. However, it has recently been

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discovered that some transposable elements manage to evade host cell silencing and get domesticated by host genomes, resulting in the beginning of novel genes (neogenes) that encode proteins.⁶⁻⁹

Recent human genome investigations have revealed that several of these proteins are involved in a variety of biological processes that contribute directly or indirectly to genome stability (cellular proliferation, cellular cycle progression, chromatin modification, transcription control).^{8,9}

These domesticated elements are also involved in placental development, viral resistance, chromatin structure, DNA recombination and repair, gene control, apoptosis, and brain development, among other cellular and developmental roles.⁶

RAG1, RAG2 are two proteins that play a role in recombination of genes for immunoglobulin and T cell receptor genes in vivo and can also serve as a transposase in vitro under certain conditions.¹⁰ The TIGD3 neogene, which was chosen for investigation, has a DNA binding domain as well as a catalytic domain.¹¹

In the current work, an in vitro model of human epithelial colorectal malignant cell lines was used to analyze the expression of TIGD3 neogenic protein using the western blot method, with the protein extracted from these cancer cell lines and antibodies produced by Arnaoty et al.¹¹, that allow us to investigate and analyze the numerous isoforms of neogenic recombinase corresponding to our TIGD3 neogene produced from DNA transposons.

The goal of this work is to demonstrate TIGD3 protein expression in colorectal cancer cell lines with two phenotypes, MSI and MSS, and to determine if this Neogene TIGD3 has a role in genetic instability and, as a result, in the initiation, promotion, or progression of cancer.

Materials and Methods

This work was carried out in the GICC (Genetics, Immunity, Chemistry, Cancer) unity of research department of CNRS (National centre of scientific research)/ Tours/ France.

Cell lines culture

Twelve colorectal cancer cell lines were included in this study, (HCT116, SW48, LOVO, DLD-1, SW480, SW620, HT29, LS123, COLO205, T84, SW403,

SW1463). These cell lines were grown in OptiMEM medium plus 10% FBS, streptomycin/penicillin 5.5µg/ml. Hela cell line was also used for achieving our transfection of our plasmids TIGD3. Culture conditions for all at 37 °C in a humidified 5% CO₂. All of these cell lines were kindly provided by INSERM U915 /Tours/ France. Healthy gut tissue was taken from a healthy individual while achieving routine colonoscopy examination/ department of gastroenterology/ Trousseau Hospital/ France.

Cell lines proteins extraction and Dosing

Whole protein from all cell lines were extracted with using lyses buffer (SDS 20%, NaCl 100mM, BetaMercaptoEthanol 10mM, Protease inhibitor), heating at 65°C for 5 minutes then breaking the DNA by ultrasound wave for 20 seconds and centrifuging the tube in 15,000 rpm at 20°C for 10 minutes, taking the supernatant and the isolated protein was quantified by a commercially available modified Bradford assay by UV spectrophotometer.

Western blot assay

Western blot protein samples were prepared by boiling the isolated protein with denaturing sample, balanced amounts of cell proteins (40 µg) where placed in each well. The protein was then separated by SDS-PAGE on a 10% polyacrylamide gel and transferred to a PVDF (polyvinylidene difluoride membrane) (Bio-Rad, Richmond, USA). The membranes were blocked with 5% non fat dry milk in TBS and 0.5% Tween 20 for 1 hour and probed with the appropriate primary antibody that synthesized by us, for 2 hours at room temperature, then the membrane was washed 3 times with TBS and 0.1% Tween 20 for 10 minutes, and incubated with the appropriate horseradish peroxidase-conjugated anti anti mouse secondary antibody (Abcam) for 1 hour at room temperature. The membrane was then washed 3 times with TBS and 0.5% Tween 20 for 10 minutes and protein bands visualized by using a commercially available enhanced chemiluminescence kit (Amersham Biosciences) according to the manufacturer's instructions, the membrane was exposed to film for 1 and 30 min.

Results

Expression of the protein TIGD3 in colorectal cancer cell lines

The western blot analysis of the protein expression of the gene TIGD3 in 12 colorectal cancer cell lines

revealed four different products of expression of this gene, which correspond to four different TIGD3 isoforms (90, 60, 52 and 50 kDa) (figure 1). Among the 12 colorectal cancer cell lines studied, no cell line shows the four isoforms of TIGD3 (90, 60, 52 and 50 kDa). The expression of three isoforms (90, 60 and 52 kDa) was observed only in two cell lines DLD1 and T84. The expression of two isoforms (60 and 52 kDa) was observed only in five cell lines HCT116, SW620, DLD1, SW1463 and T84. The expression of the isoform (50 kDa) was observed only in SW620 cell line. The isoform 52 kDa (a molecular weight equal to that of the *TIGD3* transposase) was common between them (all 12 colorectal cancer cell lines) and it was strongly expressed in all these cell lines except in SW480 (MSS). The most notable finding in this study is the lack of TIGD3 gene protein expression in sample C2, which corresponds to non-cancerous tissue collected from a healthy person. (Fig. 1,2,3). Figure 1 shows the signals of protein expression for the TIGD3 gene on protein extracts from colorectal cell lines, as captured by the PVDF membrane chemiluminescence reaction. By hybridizing the membranes with a particular monoclonal antibody, the amount of the housekeeping protein actin in each lane was determined. (Anti actin antibodies/ Abcam). As shown in figures 2 and 3, these levels were estimated using the Multigauge analysis program for signals taken from each cell line split by their contents or amount of protein actin.

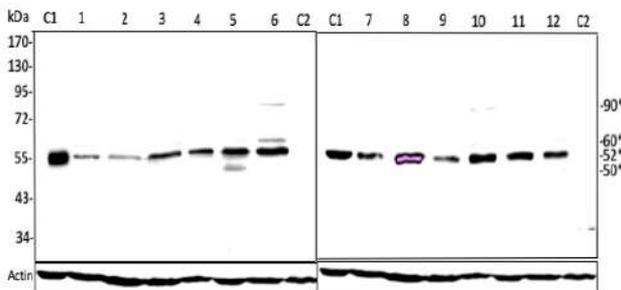


Fig. 1: Western blot analyses of TIGD3. Lanes 1 to 12 correspond to protein extracts from the human colorectal cancer cell lineages. C1 correspond to protein extracts from HeLa transfected with pVAX-TIGD3. C2 corresponds to an extract of human healthy gut. * indicates the 90, 60 and 50 kDa isoforms of TIGD3; **, indicates a 52 kDa isoform with a molecular weight equal to that of the TIGD3 transposase. Hybridizing the membranes with a specific monoclonal antibody actin in each lane. Molecular weights are indicated in the left margins. Molecular weights of the TIGD3 isoforms are indicated in the right margin.

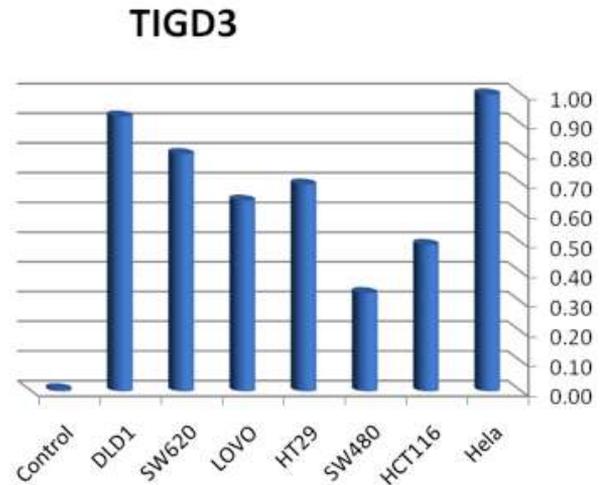


Fig. 2: TIGD3 expression (Isoform 52 kDa) in different colorectal cancer cell lines (HeLa transfected with pVAX-TIGD3, HCT116, SW480, HT29, LOVO, SW620, DLD1 and Control(an extract of human healthy gut)) respectively.

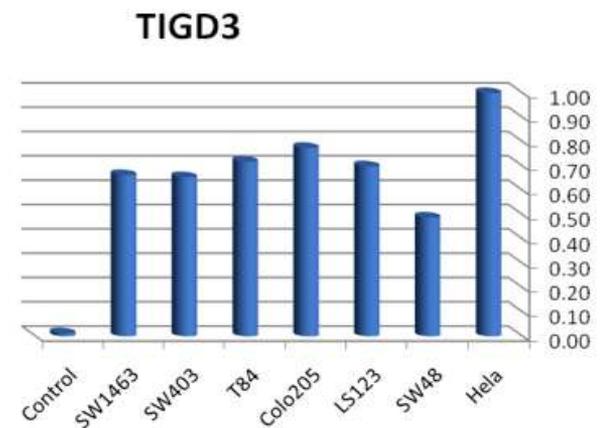


Fig. 3: TIGD3 expression (Isoform 52 kDa) in different colorectal cancer cell lines (HeLa transfected with pVAX-TIGD3, SW48, LS123, COLO205, T84, SW403, SW1463 and Control (an extract of human healthy gut)) respectively.

Discussion

For the first time, we were able to demonstrate the protein expression of the TIGD3 gene in these colorectal cancer cell lines, which reflects the expression of a domesticated DNA Neogene on such cell lines. No other study achieves the same level of success as this one, owing to the lack of a particular antibody aimed against this type of domesticated DNA Neogene.¹¹ Despite the fact that commercial antibodies available for testing the protein expression of the TIGD3 gene in these cell lines are unable to provide any of these results.¹¹ Also, there is no data in the previous bibliography that shows the exact molecular weight of TIGD3 protein by western blot method, but our prior work revealed the molecular weight of this protein by western blot to be 52 kDa.¹²

Multiple isoforms of protein expression of the TIGD3 gene, which were discovered in our work by western blot in different cell lines and could be explained by the possibility of post translational alteration of this protein, were previously unknown. The findings, which were acquired using the anti-TIGD3 antibody that we developed in partnership with In Cell Art, reveal new information on TIGD3 gene expression.¹¹

All of the colorectal cancer lines tested expressed the 52 kDa isoform. It's worth noting that the expression of this isoform was higher in cell lines (SW620, DLD1, LOVO, HT29, LS123, Colo205, T84, SW403, SW1463) that had either metastatic or advanced colorectal cancer, with the exception of the LS123 cell line, which had non-advanced colorectal cancer (Dukes B). No expression was discovered in the C2 for any of the four isoforms identified in our study (protein extract from healthy gut tissue; figure 1).

All of these data could point to a link between this gene's level of expression and the stage of cancer progression, with high expression in cell lines associated with advanced cancer or metastasis. This could be due to either the gene playing a role in cancer progression or the highly advanced tumour expressing more of this gene; however, more research and confirmation is needed.

All these cell lines (SW620, DLD1, LOVO, HT29, LS123, Colo205, T84, SW403, SW1463) which were highly express the 52 kDa isoform of TIGD3 gene represent a status of MSS (Micro Satellite Stable) except for the DLD1 and LOVO which are status of MSI (Micro Satellite Instable). This link between gene expression and cell line MSS status could indicate that either nucleotide stability (MSS) has a favorable effect on TIGD3 gene expression or that this gene plays a function in nucleotide stability inside the nucleus of these cell lines. The possibility of an inverse relationship between nucleotide instability (MSI) and gene expression level, on the other hand, may support the idea that nucleotide stability has a favorable effect on gene expression. Unfortunately, there is no material in the bibliography that attempts to demonstrate this probable link between MSS status at the nucleotide level and TIGD3 gene expression. We need more research and work on this gene before we can approve this possible link.

As well as, the isoform which correspond to the molecular weight of 60 kDa which is observed in cell lines (HCT116, SW620, DLD1, SW1463) was highly expressed in the cell line DLD1 than other cell lines. Also the isoform 90 kDa which is observed in only two cell lines (DLD1 and T84) was higher in DLD1

(MSI) than T84 (MSS). The high expression of these two isoforms (90, 60 kDa) in DLD1 with a status of MSI may suggest a relation between the micro satellite instability and the level of expression which is seen only in these two isoforms (90, 60 kDa) but not seen in the isoform 52 kDa which was highly expressed in most cell lines with a status of MSS. This could suggest a link between nucleotide stability and the expression of distinct isoforms of this gene. In other words, the expression of distinct isoforms for the same gene depends on whether the cell line is MSS or MSI, and the development of these four TIGD3 isoforms in these cell lines could be related to nucleotide stability or instability.

Our research was carried out on cell lines that have the chromosomal region 11q13.1, which contains the TIGD3 gene and is not deleted, implying that our gene expression analysis is complete.¹³⁻¹⁹

In addition, the data observed in colorectal cancer series indicate that chromosomal deletions type LOH (Loss of heterozygosity) are less common on the 11q chromosome arm than the other arm.^{20,21} Therefore, the expression of a TIGD3 gene is not related to allelic loss on chromosome 11q.

To our knowledge, no studies have been reported on the study of gene expression TIGD3 in these human cancer cell lines.

Conclusion

The presence of protein expression for the TIGD3 gene in all colorectal cancer cell lines, with higher expression in cell lines that had progressed or metastasized, and absence in healthy tissue, could indicate a strong relationship between cancer evolution or progression and gene expression, which could lead to a possible role for this gene in colorectal cancer.

Source of Funding: This work was funded by the C.N.R.S, the I.N.R.A., the Groupement de Recherche CNRS 2157, and the Ministère de l'Éducation Nationale, de la Recherche et de la Technologie. It also received funding from a research program grant from the Cancéropôle Grand-Ouest grants from Amgen and the French National Society of Gastroenterology.

Ethical Clearance: Samples were taken from the department of histopathology/Trousseau hospital/Tours/France. Patients had been informed of using their samples for research purposes and their consents to participation in this type of research had been collected.

Conflict of Interest: There is no conflict.

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Covid-19 Risk Factors and Radiographic Severity Index Description in Covid-19 Patients Dr. Mohammad Hoesin Palembang 2021

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How to cite this article: Alfian Hasbi, Budi Santoso, Anjeli Primeisa. Covid-19 Risk Factors and Radiographic Severity Index Description in Covid-19 Patients Dr. Mohammad Hoesin Palembang 2021. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):63-68.

Abstract

Background: COVID-19 is a pandemic disease caused by droplet infection from SARS-CoV-2. Due to its rapid transmission and high case fatality rate, the identification of risk factors and prognostic factors is important. Obesity is a risk factor for poor outcomes in COVID-19. It is associated with chronic inflammation, disorders of the immune system. Obesity can be determined based on BMI. Chest X-Ray is supported in establishing the diagnosis and prognosis of COVID-19 patients. Assessment of the severity index of Chest X-Ray radiographs can use the Modified Chest X-Ray Scoring System of RSUP Dr. Soetomo. This study was conducted to analyze the relationship between BMI and chest radiography severity index in hospitalized COVID-19 patients at dr. Mohammad Hoesin Palembang in 2021.

Methods: This research used a cross-sectional analytic observational design. Sampling was done using a consecutive sampling technique with 70 samples and obtained from the patient's medical record. The data were analyzed by univariate and bivariate (Chi-Square) using IBM SPSS Statistics 26 software.

Results: Patients with BMI Overweight-Obesity had more in Moderate-Severe (18.6%) radiographic severity index scores (18.6%) than Normal-Mild (15.7%). Chi-Square bivariate analysis, BMI ($p=0.033$; $p\text{-value} < 0.05$) had a significant relationship with the chest radiographic severity index with Odds Ratio 3,00, 95% CI (1,073-8,386).

Conclusion: There is a significant relationship between body mass index and chest radiography severity index in COVID-19 patients. Overweight-Obesity BMI patients have a 3-fold chance of having a Moderate-Severe category of radiographic severity index compared to Underweight-Normal BMI patients.

Keywords: COVID-19, Obesity; IMT; Chest-X-ray; radiography severity index.

Introductions

Coronavirus Disease-2019 (COVID-19) is an infectious disease caused by a species of coronavirus,

known as Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). Globally, as of 20 July 2021, a total of 190,671,330 confirmed cases of COVID-19, including 4,098,758 deaths, were reported

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to WHO.¹ Shortness of breath, cough and fever are the most common clinical symptoms of COVID-19.² COVID-19 is capable of being transmitted and spreads through droplets that come out through the nose and/or mouth when a person infected with COVID-19 is talking, coughing or sneezing.³

Patient who has a high risk and severe complications is the elderly group, this age factor is related to COVID-19 because the elderly group has an anatomical and physiological degenerative process so that they are susceptible to disease. Another group is people with comorbidities such as obesity, disease heart disease, hypertension, diabetes, etc. In addition, gender is also a risk factor for COVID-19. Compared to women, men are more at risk, this is related to lifestyle habits in the form of active smokers and a poor immune system in respiration and is also caused by hormonal and chromosomal factors. Women have protection from COVID-19 because they have the hormone progesterone and the X chromosome, which play an important role in innate and adaptive immunity.^{3,4} One of the comorbidities that is a risk factor for COVID-19 is obesity.⁵ Obesity is determined based on the nutritional status of a person who is assessed based on a measuring instrument, namely the body mass index (BMI) based on weight (weight) in kilograms (kg) and divided by TB (height) in meters squared (m2).⁶

Chest radiography has an important role in dealing with COVID-19.^{7,8} presented at corona filtration center, Benazir Bhutto Hospital Rawalpindi, based on CXR classification of British Society of Thoracic Imaging (BSTI Chest radiography can be performed using a chest X-Ray or a computed tomography scan (CT-Scan). Recent COVID-19 radiology literature focuses mainly on CT-Scan findings which are more sensitive and specific than chest X-Ray radiographs.⁹ Chest X-Ray is a first-line method with the advantage of faster results compared to using RT-PCR and reduces patient movement so as to minimize the risk of spreading infection.^{10,11} So Chest X-Ray becomes a cheap, fast, simple and safe examination modality to be used in the examination of COVID-19 patients. The scoring system for COVID-19 Chest X-Ray radiography can use the Modified Chest X-Ray Scoring System of Dr. RSUP. Soetomo.¹²

Methods

This type of research is descriptive observational with consecutive sampling technique. The study took

place from August to November 2021. The research sample was medical record data of hospitalized COVID-19 patients at the Department of Internal Medicine, Dr. Mohammad Hoesin Palembang in 2021. The number of samples taken was 70 samples.

Inclusion criteria included inpatients who were declared positive for COVID-19 through antigen examination and/or PCR swab, chest X-ray radiology examination and anthropometric measurements (weight and height) and recorded in the patient's medical record. Exclusion criteria included patients with missing or incomplete medical record data, chest X-ray results that could not be read or identified and chest X-ray results with the impression of pleural effusion. The data obtained will be processed and analyzed univariately using IBM SPSS Statistics 26 software.

Results

Distribution of respondent characteristics by age and sex of COVID-19 patients

Table 1: Shows the characteristics of respondents by age and gender. The majority of hospitalized COVID-19 patients in the adult age group are 75.7% and 51.4% are male.

Table 1: Distribution of respondent characteristics by age and gender

Characteristics	Frequencies (n)	Percentages (%)
Age		
Young age	4	5,7
Mature age	53	75,7
Elderly	13	18,6
Total	70	100,0
Gender		
Man	36	51,4
Woman	34	48,6
Total	70	100,0

Distribution of respondent characteristics based on clinical symptoms of COVID-19 and co-morbidities of COVID-19 patients

Table 2. shows the characteristics of respondents based on clinical symptoms of COVID-19 and comorbidities. Most of the clinical symptoms of COVID-19 were symptoms of shortness of breath as much as 35.7% followed by symptoms including cough, fever accompanied by shortness of breath as

much as 22.9% and the majority of patients did not have comorbidities, which was 30.0%.

Table 2: Distribution of respondent characteristics based on clinical symptoms of COVID-19 and comorbidities

Characteristics	Frequencies (n)	Percentages (%)
COVID-19		
Clinical Symptoms	5	7,1
Fever	4	5,7
Cough	25	35,7
Out of breath	6	8,6
Fever, cough	8	11,4
Fever, shortness of breath	1	1,4
Cough, shortness of breath	16	22,9
Cough, fever, shortness of breath	2	2,9
No symptoms	3	4,3
Others (weakness, anosmia)	70	100,0
Total		
Co-morbidities	14	20,0
Diabetes mellitus	13	18,6
Hypertension	5	7,1
Chronic disease (CKD)	17	24,3
Other chronic diseases	21	30,0
No Co-morbidity	70	100,0
Total		

Distribution of respondent characteristics based on BMI and chest radiographic severity index of COVID-19 patients

Table 3. shows the characteristics of respondents based on BMI and chest radiography severity index. Most of the patient's body mass index was in the normal group as much as 57.1% followed by the overweight group at 27.1%.

Table 3: Distribution of respondent characteristics based on BMI and chest radiographic severity index of COVID-19 patients

Characteristics	Frequencies (n)	Percentages (%)
BMI		
Underweight	6	8,6
Normal	40	57,1

Characteristics	Frequencies (n)	Percentages (%)
Overweight	19	27,1
Obesity	5	7,1
Total	70	100,0
Radiographic severity index		
Normal	15	21,4
Mild	29	41,4
Moderate	14	20,0
Severe	12	17,1
Total	70	100,0

Discussion

In this study, the tendency of hospitalized COVID-19 patients was in the adult age group (75.7%) and the least age group was the young age group (5.7%) and there were 13 patients (18.6%) aged carry on. COVID-19 can be experienced in all age groups, but the elderly are the age group most at risk and are the group that is very vulnerable to infection with the COVID-19 virus.¹³ last three of them entered our life with a fear of outbreak, pandemic or death. Last human coronavirus which emerged world from Wuhan China, SARS CoV-2 and its clinical expression, Coronavirus disease (COVID-19 The age of COVID-19 patients in Indonesia is mostly in adulthood (38.91%). This supports the results of this study, that the tendency of patients is the adult age group, namely in the age range of 20-60 years.¹⁴

However, it is stated that caution is needed in interpreting the characteristics and distribution of COVID-19 patients based on their age group. The reason is that some patients have not been treated at the hospital or have not been tested for COVID-19 so they are not recorded in the study. In addition, perhaps the proportion of COVID-19 patients in the elderly group is under-represented in Indonesia, considering that cases in Italy for COVID-19 patients in the elderly group reached 55% of cases. Another reason is the difference in understanding and access to information regarding the clinical symptoms of COVID-19 and patient mobility. It will be very easy for the adult age group to get more information about the clinical symptoms of COVID-19, which makes this age group able to identify the clinical symptoms of COVID-19 and then report the case to the hospital.¹⁵ This is thought to be related to mobility and activities that are more frequent and high in the adult age group or commonly known as the productive age in several groups of individuals.¹⁴

Based on the results of this study, it was found that the tendency of hospitalized COVID-19 patients was male patients (51.4%) while female patients (48.6%). There are more male patients (62.5%) than women (37.5%).¹⁶ Maino et al., found results in line with this study, from a total of 468 confirmed patients with COVID-19, the tendency for male patients was 328 patients and female patients were 140 patients.¹⁷

According to research, men have a higher risk of infection with the COVID-19 virus and death from COVID-19. This is related to a lifestyle in the form of active smokers and work factors that make men more likely to experience COVID-19 virus infection than women.¹⁸ Furthermore, viewed from the physiological aspect, women have the hormones estrogen and progesterone which play a role in suppressing COVID-19 virus infection through innate and adaptive immune responses in stronger women. The hormone estrogen is a steroid compound that plays a role in clearing the virus from the tissue and supporting the repair of damaged tissue. While the hormone progesterone plays a role in anti-inflammatory and prevents cytokine storms.^{18,19} It also said there were other behavioral and social differences in favor of women, with previous research reporting that women were more likely to follow hand hygiene practices and seek preventative care than men.²⁰

This study found that the clinical symptoms of COVID-19 that tended to be high were shortness of breath. The clinical symptoms of COVID-19 vary based on the severity of the disease. However, the main clinical symptoms are shortness of breath, fever, cough, diarrhea, nausea, headache, and myalgia. Until now, symptoms of fever, cough, and shortness of breath are clinical symptoms that are often encountered.^{41,42}

In this study, patients were found to be asymptomatic (2.9%) this is contrary to the study in India where almost half (42.9%) patients were asymptomatic. However, in another study, only 23% of cases were asymptomatic.¹⁹ Kermani, 2020 reported in his research that most of the clinical symptoms that appear are asymptomatic (without symptoms) to mild clinical symptoms. In patients who have the concomitant disease, severe clinical symptoms will be found more often.²¹

The most common comorbidities found were diabetes mellitus (20.0%) and other chronic diseases

(24.3%). The other chronic diseases found in this study were lung cancer, anemia, Acute Kidney Injury (AKI), and sarcoma. The co-morbidities of diabetes mellitus are associated with imbalanced ACE-2 regulation associated with pro-inflammatory conditions and are considered to be the cause of the more severe clinical symptoms of COVID-19. The mechanism of diabetes mellitus resulting in high mortality and morbidity of COVID-19 patients has been studied by Pamantow et al., 2021. It has been reported that the relationship between diabetes mellitus in COVID-19 patients is based on the mechanism of chronic systemic inflammation, high coagulation activity, abnormal on the body's immune response, and possibly damage to the pancreas by SARS-CoV-2. In addition, there are changes in ACE-2 receptor expression, dysregulation of immune cell activity and number, endothelial and alveolar dysfunction.²² In addition, patients with co-morbidities with diabetes mellitus have levels of Furin which is a protease that can increase viral invasion into the body. This makes patients with diabetes mellitus have a higher tendency to be infected with SARS-CoV-2.²¹

This study also obtained the discovery of COVID-19 patients with comorbidities CKD (Chronic Kidney Disease). It is known that patients with comorbidities with CKD must routinely perform hemodialysis, which is a dialysis therapy procedure using an artificial kidney or hemodialyzer.²³ Patients undergoing hemodialysis must apply health protocols such as the use of masks.^{23,24} However, in some cases this causes uncomfortable conditions and sometimes makes the patient feel congested, washing hands and not being waited on by the family during hemodialysis therapy has an influence on the patient's quality of life and the emergence of stress and anxiety disorders. This patient is susceptible to contracting COVID-19 due to immune system dysfunction and must receive collective hemodialysis therapy three times a week for four hours causing cross-contamination to occur easily.²⁴

The majority of BMI obtained in this study was in the normal category. This result is not in line with other studies which reveal that the obesity rate in Indonesia is high and increasing.²⁵ However, in this study, the majority of body weight was found to be normal, presumably due to gender, age, and changes in lifestyle and eating patterns which are risk factors for obesity.²⁶⁻²⁸ The majority of the sexes with obesity are women (23.9%) from a total of 6,313

women this is related to the hormone estrogen can increase adrenergic antilipolytic-2A receptors on subcutaneous fat cells.²⁷ This is contrary to the prevalence of COVID-19 patients where COVID-19 patients are male relatives.^{16,21} In addition, based on the distribution of comorbidities obtained in this study, 24.3% of patients had chronic diseases that could affect BMI in individuals. In the study of Kamisah et al., of 95 chronic disease respondents, 52 (54.7%) respondents showed normal BMI calculations, which was in line with the 2014 study, which showed a correlation between BMI and patients with a chronic disease which showed normal values.²⁹

This study is in contrast to the 2013 study, which revealed that the BMI of patients with co-morbidities with diabetes mellitus mostly had an overweight BMI (51.4%). The accumulation of free fat can cause the oxidation of free fatty acids which can inhibit glucose metabolism in muscles.²⁹ However, the obesity BMI category must remain vigilant, because COVID-19 can become more severe with increasing BMI and obese patients admitted to hospital have a higher mortality rate and are more likely to require mechanical ventilation, with most patients with a BMI >35 kg/ m² requires intubation.³⁰

Most of the patients had a mild category score of 29 patients (41.4%). Chest X-Ray images are usually normal in the early or mild phase. Based on the research of Wong et al., of COVID-19 hospitalized patients, 69% had normal features on admission, and 80% had chest X-ray pneumonia during hospitalization. These findings often occur 10 to 12 days after the onset of clinical symptoms.³¹

Conclusion

The majority of COVID-19 patients included in the study sample were in the adult age group and were male. Most of the clinical symptoms of COVID-19 are symptoms of shortness of breath followed by symptoms that include cough, fever accompanied by shortness of breath and the majority of patients do not have comorbidities. Most of the patient's body mass index was in the normal group (57.1%) followed by the overweight group (27.1%). Description of the chest radiography severity index based on the Modified Chest X-Ray Scoring System, Dr. Soetomo were the normal (21.4%), mild (41.4%), moderate (20.0%) and severe (17.1%) groups.

Conflict of Interest: Nil

Source of funding: Universitas Sriwijaya

Ethical clearance: Certificate of Ethical Approval from Universitas Sriwijaya. Protocol number: 130-2021

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Ovarian Cyst Disease in Cattle: Bioanalysis of Associated Hormones

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How to cite this article: Ammar M.K. AL-azzawi, Ali Abbas Ali, Ghassan H. Jameel. Ovarian Cyst Disease in Cattle: Bioanalysis of Associated Hormones. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):69-72.

Abstract

Ovarian cysts are structures that appear on ovaries, their size reaches 2.5 cm in diameter or greater remaining for 10 days or more if they are untreated. They are classified into three major categories includes follicular cysts, luteinized follicular cysts, and cystic corpora luteal result in failure of reproduction in dairy cows. There is insignificant depression in the mean values of FSH and LH in the repeat breeder cows (infected group) when compared with the normal cows (control group). There is a significant elevation in the mean values of the prolactin hormone in the repeat breeder cows. In recommendation, balanced feeding to dairy cows with high milk production would decrease the risk factors associated with cystic ovary syndrome.

Keywords: Ovarian Cyst Disease in Cattle; prolactin; hormones; LH; FSH.

Introduction

Cystic ovarian disease (COD) in cattle leads to a decrease in the reproduction rate, resulting in significant economic losses for the dairy industry. Ovulation of a mature follicle is failed at the appropriate time during the estrous cycle.¹ Ovarian cysts, whose size reaches 20 mm in diameter lead to the disappearance of a corpus luteum, are classified as follicular and luteal. The follicular cyst wall thickness is less than 3 mm and more than 3 mm in the luteal cyst.² In infield conditions, this differentiation is difficult to recognize and is often omitted from the diagnosis.^{3,4} The genetic predisposition for ovarian cysts is clear. Genetic selection would reduce the incidence of the disease from 11% in 1954 to 3% in 1977 in Sweden. Also, for cows with greater milk production.

COD is more prevailing.⁵ Depending upon steroid production the ovarian cysts can be classified as follicular or luteal. The younger follicular cyst has a thick granulosa cell layer that disappears gradually with age. Luteal cyst is a luteinized follicular cyst with time.⁶

Gonadotropin-releasing hormone (GnRH) stimulates the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary gland.⁷ Luteinizing hormone (LH) or lutropin, is a hormone produced by gonadotropic cells found in the anterior pituitary gland.⁸ Follicle-stimulating hormone (FSH) is a gonadotropin and, in other aspect is a glycoprotein polypeptide hormone which is synthesized and secreted in the same location when LH is secreted. FSH regulates the development,

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growth, and pubertal maturation. It works together with luteinizing hormone (LH) in regulating the reproductive processes of the body.⁹ FSH initiates the growth of the ovarian follicles, specifically affecting granulosa cells. Slight a rise in FSH level at the end of the luteal phase that seems to be of importance to start the next ovulatory cycle.¹⁰ In response to eating, mating, estrogen treatment, ovulation, and nursing, prolactin (PRL) or luteotropic hormone is secreted from the pituitary gland.¹¹ Prolactin plays an essential role in metabolism, regulation of the immune system, and pancreatic development. Bole-Feysot *et al.* (1998) referred that prolactin is associated with milk production in mammals.^{11,12}

Materials and Methods

1. Groups of Animals

Forty lactated cows were conducted in this study, twenty were infected with repeat breeder and, twenty were in a normal state as a control group. Cows divided randomly into two groups (20 cows for each group and handled as follows: (I) Control group: Animals of this group fed on basal diet and ordinary tap water. (II) Twenty were infected with repeat breeders. The collected blood samples (5ml) were kept in tubes then, serum was separated by centrifuge at (3000 rpm) after that conserved at (-20 C°) in the freezer until used. The hormonal assay of FSA, LH, and PRL was estimated in the serum samples performed by solid-phase radioimmunoassay using standard kits. Results are expressed as mean ± SE. statistical analysis of data was performed based on two-way analysis of variance ANOVA II. Group differences and within-group differences

were determined using the least significant difference (LSD) test at (P<0.05) (11). iChroma tests (iChroma - Boditech Med Inc.) for LH with REF 13010 iChroma tests (iChroma - Boditech Med Inc.) for PRL with REF 164002.

2. Assay Procedure

By a transfer pipette (150 ul, 150 ul, and 150ul) was transferred these quantities of serum from each cow to a tube containing the detection buffer of the hormones FSH, LH, and prolactin levels respectively. Closure of the lid of the detection buffer tube was done, followed by mixing the sample thoroughly by shaking it about 10 times. Pipette out 75ul of sample mixture and load it into the sample well on the cartridge and left the sample in the loaded cartridge at room temperature for 15 minutes. When the incubation time is over, scanning of the sample immediately to obtain an exact result, then the sample was inserted into the cartridge holder of the instrument for I Chroma tests (I Chroma - Boditech Med Inc.). To start the scanning process would press the select button on the instrument for I Chroma tests. Reading of the result on the display screen of instruments for I Chroma tests must be performed after that.

Results

The results in Table 1 were revealed significant depression in the mean values of FSH and LH in the repeat breeder cows when compared with the normal cows. There is a significant elevation in the mean values of the prolactin hormone in the repeat breeder cow.

Table 1: Revealed the total number of normal and repeat breeder cows associated hormones

Total Number and the character of the cow	FSH level (mlu/ ml) ± Standard	LH level (mlu/ ml) ± Standard error	Prolactin level (ng/ ml) ± Standard error
40 normal cow	1.625 ± 0.311	2. 575 ± 0.744	0.098 ± 0.033
40 repeat breeder cow	0.594 ± 0.431	0.445 ± 0.254	0.287 ± 0.172

(P<0.05)*

Discussion

Cystic ovarian disease is a serious cause to decrease the rate of reproduction in dairy cows. In mature females during a time of the estrus cycle, an acute rise of LH level proceed ovulation and development of the corpus luteum later. luteinizing hormone has a synergistic action with follicle-

stimulating hormone (FSH).^{13,14} Barui *et al.* (2015) were mentioned in their study that the results show significant depression in the level of FSH whereas, the level of LH did not show a significant difference.¹⁵ Our study came in agreement with a previous study was done by Ahammed *et al.* (2018) who found significant depression in the levels of

the FSH and LH in Bangladesh.¹⁶ No, refer to the level of prolactin hormone. A linear decrease in serum LH occurred in pregnant cows via pregnancy procession, whereas serum LH remained unaffected in non-pregnant cows.¹⁷ LH is responsible for causing the rupture of the mature follicle will release the egg. Following ovulation, the LH also stimulates the development of the corpus luteum.¹⁸ We are saying, an adequate quantity of LH or a low level of LH results in the failure of ovulation and proceeding with ovarian cyst later. Regardless of the FSH and its responsibility for the development of the ovarian follicle, FSH shares LH in the secretion of estrogen from this follicle.¹⁹ Our illustration for this case depends upon the failure of a low concentration of LH to rupture mature follicles through the phase of ovulation, resulting in enlargement of ovarian cyst in the following estrus cycle. Prolactin is a protein that is best known for its role in enabling mammals, usually females, to produce milk.¹¹ Our observations and investigations through fieldworks are summarized to the most cows with repeat breeder were with high milk production because they possessed a high level of prolactin and, these observations and investigations came with agree with multiple studies refer to the cystic ovary disease are more common in cows with greater milk production.^{5,20} Sobrinho (2003) was referred to the cows with hyperprolactinemia tend to suppress the secretion of GnRH from the hypothalamus and in turn, decrease the secretion of FSH and LH from the anterior pituitary result in disrupting the ovulatory cycle.²¹

Recommendation

Balanced feeding to dairy cows with high milk production would decrease the risk factors associated with cystic ovary syndrome. Administration of prostaglandin (PGF₂α) after fifteen days of parturition to resolve the corpus luteum and predispose to the formation of the new ovarian follicle. Source of Funding The research was performed independently; there no funding, influence over study design, analyses, manuscript, or scientific publication.

Ethical clearance The project was approved by the local ethical committee (College of Veterinary Medicine/Diyala University).

Conflict of interest: Nil.

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The Investigation of Significant Leptospirosis Hotspots during the Initial COVID-19 Pandemic in the City of Jakarta, Indonesia

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How to cite this article: Andre Yunianto, Dian Perwitasari, Dasuki Dasuki et al. The Investigation of Significant Leptospirosis Hotspots during the Initial COVID-19 Pandemic in the City of Jakarta, Indonesia. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):73-77.

Abstract

Leptospirosis is a common bacterial infection caused by pathogenic *Leptospira* spp. in tropical region, including in Indonesia. During the first year of COVID-19 pandemic, Jakarta reported a significant increase in leptospirosis cases. A study was conducted to analyze the distribution of leptospirosis and to identify hotspots of the leptospirosis. Leptospirosis notifications for the period of January to December 2020 were collected from the online surveillance database provided by the Provincial Health Office of Jakarta. Global and local spatial clustering at the village level across Jakarta was examined using Moran's *I* and local indicators for spatial association (LISA). In 2020, total of 207 people infected by *Leptospira* spp. The highest number was recorded in January (n=142), accounting for 68.5% of the total reports over the period studied. The incidence was geographically dissimilar at village-level with the highest rates was observed in the west of the city. Moran's *I* analysis demonstrates that leptospirosis incidence was significantly clustered ($I = 0.191$, p -value = 0.001). Total of 19 high-risk clusters in 9 sub-districts were identified and approximately 891,202 people were at higher risk of leptospirosis during the year of 2020. The findings suggest needs an improved disease surveillance to support spatially targeted interventions to control leptospirosis transmission.

Keywords: leptospirosis, spatial analysis, clustering, COVID-19, Jakarta

Introduction

Leptospirosis is a bacterial infection caused by pathogenic spirochetes *Leptospira* spp. The disease is commonly found in tropical and sub-tropical countries and can be found in both urban and rural areas.¹ Annually, more than one million people infected and up to 60,000 people died because of leptospirosis across the globe.² People can be infected through direct or indirect exposure to *Leptospira* infected animal tissues or contaminated water and soil. The bacteria enter human body through

injured skin or mucous membranes.¹ Rodents are the most important animal responsible for shedding the bacteria into the environment although some domestic, livestock and wildlife animals can also play in the transmission of leptospirosis.³ Leptospirosis have broad clinical manifestations including fever, chill and headache. and it is often misdiagnosed with other febrile illness such as dengue.⁴

In Indonesia, leptospirosis is also a public health problem, affecting both urban and rural population. Leptospirosis outbreaks have been reported in many

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places including Jakarta, Semarang, Demak and Banyumas. The largest leptospirosis outbreak has been recorded in Jakarta in 2002 after severe flooding. Since then, the number of leptospirosis cases in Indonesia is continuously increasing with significant death rates.^{5,6} Leptospirosis prevention and control is challenged by the low awareness and lack of knowledge on the epidemiology of leptospirosis. Identifying areas where leptospirosis risk is higher is important as this would help understand patterns and potential factors facilitating the transmission. There is a need to improve preparedness and monitoring tools to prevent and control following leptospirosis outbreaks.

Geographic information system (GIS) and spatial statistics approaches have been used in broad fields, including health. The use of such tools in understanding disease transmission from the spatial perspective have been documented in many studies, including in the field of leptospirosis.⁷⁻⁹ Yet, there is no study have used such tools exploring the spatial pattern of leptospirosis in Jakarta. GIS can be used to map pattern of disease, allowing to examine the geographical extent of disease of interest. In addition, the advancement of geospatial statistics tools provide opportunity to better explore the spatial autocorrelation in the data and help to generate and test hypotheses, which can help in shaping knowledge on disease epidemiology.¹⁰

An ecological study using GIS and spatial analysis was conducted, aimed at examining the presence of spatial autocorrelation of leptospirosis incidence and to further locate the high-risk areas for leptospirosis transmission in Jakarta during the first year of COVID-19 pandemic.

Materials and Methods

Study Site

The study was performed in the metropolitan Jakarta, Indonesia in 2020. It has an area of 622.33 km² which is shared into six municipalities, 44 sub-district (*kecamatan*s) and 267 villages (*kelurahan*s). The city is inhabited by at least 9.6 million people. At the *kelurahan* level, the population density is ranging from 1013 persons per km² (Kamal Muara, North Jakarta) to 319,107 persons per km² (Jelambar Baru, West Jakarta). Jakarta has a tropical climate with mean temperature of 28.7°C and annual precipitation ranging from 1459 to 1600 mm with the highest precipitation rate is on February.¹¹

Data Collection

The *kelurahan* level monthly human leptospirosis cases recorded in hospitals for the period of January to December 2020 were obtained from the online surveillance website of Provincial Health Office (PHO) of Jakarta (<https://surveilans-dinkesdki.net/>). In Indonesia, leptospirosis is a notifiable disease which all health facilities are required to report all cases to the District Health Office (DHO) within 24 hours of diagnosis. Data for population at village level were collected from the Bureau of Statistics.¹¹

Data Analysis

Incidence Mapping: The incidence rate per 10,000 people for each *kelurahan* was calculated by dividing number of cases with number people multiplied by 10,000. The data were then linked to village ID polygons and the choropleth map of incidence of leptospirosis was created using ArcMap v.10.5.1 software (ESRI, Redlands, CA, USA).

Spatial clustering and local clusters detection: Moran's I statistics was performed to measure the clustering of the incidence using the formula as follow:

$$I = \frac{\sum_{i=1}^n \sum_{j=1}^n \omega_{ij} (z_i - \bar{z})(z_j - \bar{z})}{\sum_{i=1}^n (z_i - \bar{z})^2}$$

Where n is the number of areas (*kelurahans*), z_i the count or incidence of leptospirosis in *kelurahani*, \bar{z} is the mean count or incidence of leptospirosis in the region of study and ω_{ij} is the spatial contiguity matrix. In this analysis, Queen contiguity style was built, meaning that the *kelurahan* polygon shares a common edge or vertex. Moran's I value ranging from -1 to 1 with a value close to 0 means no spatial autocorrelation (random). A positive value indicates positive autocorrelation and a negative value means negative autocorrelation.¹² The significance of Moran's I coefficient was examined using Monte-Carlo randomization with 999 permutations. Significance ($p < 0.05$) of the test statistic means that incidence is spatially clustered or dispersed.

Once the global spatial autocorrelation was detected, the local index of spatial association (LISA) was estimated by using the equation below:

$$I_i = \frac{z_i \sum_{j=1}^n \omega_{ij} z_j}{\sum_{i=1}^n z_j^2}$$

LISA analysis was used to detect significant local clusters (hotspots), providing evidence of clusters of *kelurahans* with high incidence (High-High, HH), low incidence (Low-Low, LL) and spatial outliers (High-Low, HL and Low-High, LH). The HH clusters mean that a high incidence *kelurahan* is adjacent to other high incidence *kelurahans* and so on.¹³ All these spatial analyses were performed in open source GeoDA 1.18 software.¹⁴

Results

Geographical variation of incidence of leptospirosis

The incidence of leptospirosis was dissimilar at village level across the Jakarta metropolitan, ranged from 0 to 2.70 per 10,000 people. High rate villages were observed in west Jakarta and small number of villages in central and south. The highest rate of leptospirosis was identified in Rawa Barat in South Jakarta (2.70/10,000 people), followed by Karet Tengsin (2.62/10,000 people), Kedoya Utara (2.38/10,000 people), Kampung Melayu (2.32/10,000 people) and Kamal Muara (1.87/10,000 people).

Spatial Clustering and Local Clusters of Leptospirosis

Moran's *I* analysis indicated the rate of leptospirosis was statistically significantly clustered (Moran's *I* coefficient = 0.191; *p*-value = 0.001). LISA analysis identified significant spatial clusters of leptospirosis in Jakarta. Nineteen High-High clusters were identified with population at-risk was approximately 891,202 people (Table 1). These High-High clusters were primarily concentrated in northwest Jakarta. Small set of High-High clusters also detected in central and east Jakarta. The High-High clusters belong to 9 *Kecamatans* including Cengkareng (4 *kelurahans*), Palmerah (4), Kebon Jeruk (3), Tanah Abang (2), Grogol Petamburan (2), Jatinegara (1), Kebayoran Lama (1), Kembangan (1), and Tebet 1.

Thirty-six Low-Low clusters were identified during period studied, which mainly clustered in northeast Jakarta. Additionally, fourteen significant spatial outliers (7 Low-High dan 7 High-Low clusters) were detected.

Table 1: Summary statistics of local spatial clusters of leptospirosis in Jakarta, 2020

Type of Cluster	No. of Cases	Mean Incidence (Per 10,000 Persons)	No. of Villages	Population at risk	Mean Population Density (per Km ²)
H-H	60	0.62	19	891,202	24,447
L-L	0	0	36	1,575,997	16,590
L-H	1	0.03	7	164,796	13,594
H-L	8	0.28	7	302,694	30,313

Discussion

Using the GIS and spatial statistics approach, this study was aimed to explore spatial heterogeneity of the incidence of human leptospirosis in Jakarta during 2020. This study demonstrated the presence of spatial clusters of leptospirosis in west Jakarta. Nineteen significant clusters involving 9 *kecamatan*s were detected. This clustering patterns suggest that this was because factors (environmental or societal) might have influenced the spread of leptospirosis, which need further investigation.

Spatial autocorrelation test has also been used to investigate the spatial pattern in incidence of leptospirosis in different studies elsewhere¹⁵⁻¹⁷, which also result similar finding with this present study. the emergence of leptospirosis is known to be associated with climate and environmental factors.

Studies have shown that extreme climatic events such as heavy rainfall and flooding have been responsible for numbers of leptospirosis outbreak worldwide, including in Jakarta. Poor drainage system and disrupted access to safe drinking water and sanitation during flooding could magnify the probability of contact with bacterial contaminated environment. In addition, poor waste management (i.e., improper garbage disposal) maintains rodent population that could harbor pathogenic *Leptospira* spp. Such environmental conditions are common risk factors for the transmission of leptospirosis in urban setting.

Clustering in incidence of leptospirosis in northwest Jakarta might be partly explained by the variation in population density. *Kecamatan*s in west Jakarta such as Cengkareng, Kebon Jeruk, Grogol Petamburan and Palmerah are areas known with high people density in Jakarta and frequently experience

severe flooding. This may explain why most High-High clusters for leptospirosis was found in west Jakarta. Population density and socioeconomic condition have been important demographical characteristics associated with the occurrence of leptospirosis in other studies^{18,19}. Densely inhabited urban area, in which basic services and environmental health are lacking, could foster rat infestation and increase the transmission of leptospirosis. People living in slums and near the river are known at the highest risk of leptospirosis²⁰. Further exploration is needed to investigate the role of individual and community level factors explaining the distribution of risk of leptospirosis in Jakarta.

The use of GIS and spatial statistics have been helpful in understanding the disease epidemiology including leptospirosis. This approach can be beneficial in assisting health officials or epidemiologist to zooming-in the factors that may have driven the occurrence and spread of the diseases. This study is an ecological study which is known to have several limitations including the inability to draw the causal link between infection and factors at individual or community level. In addition, the leptospirosis data used here were based on passive surveillance which may suffer from underreported number resulting in underestimation of actual number of infections in population. However, this study has provided important evidence about leptospirosis distribution in Jakarta, which has never been studied before.

Conclusions

Using GIS and spatial analysis, the study demonstrated the significant spatial clustering in the incidence of leptospirosis in 2020. More importantly, the study revealed 19 high-risk *kelurahans* for leptospirosis transmission across Jakarta, which mainly concentrated in the northwest of the city, suggesting the needs for further interventions to better control leptospirosis in these identified areas to prevent outbreak in the future.

Acknowledgments

The authors thank to Provincial Health Office of Jakarta for providing access to the leptospirosis data used in this study. This study was supported by National Institute of Health Research and Development, Ministry of Health of Indonesia.

Ethical clearance: Not applicable. The study utilized aggregated notification data from publicly available database (<https://surveilans-dinkesdki.net/>).

Source of Funding: The authors have no support or funding to report. This study did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of Interest: The authors declare that they have no competing interests.

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Evaluation of Post-vaccination Symptoms of COVID 19 Vaccines among People in Basrah- Iraq Society

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How to cite this article: Aseel k. Thamer. Evaluation of Post-vaccination Symptoms of COVID 19 Vaccines among People in Basrah- Iraq Society. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):78-83.

Abstract

Introduction: Vaccines are one of the most effective interventions for destroying COVID-19. Many organizations have worked to establish an efficient and safe preparation in the minimum time possible. Currently, several products that different in form and effectiveness are approved for sale.

Objectives: The goal of this study was to obtain evidence on COVID-19 vaccination adverse effects.

Methodology: A cross-sectional study was made between October and December 2021 to obtain data on the influence of the COVID-19 vaccine among people in the Iraq- Basrah. There were two types of questions listed. The first one covers the subject's background information, such as nationality, gender, age, educational level, and past COVID 19 infection. The second set of questions focused on information about the COVID-19 vaccines and their side effects.

Result: Local site adverse reaction, Hypersensitivity reaction, bone and muscle pain, headache and fever were more common in persons received second dose than in participant received first dose. There was a significant difference in the number of people those under 60 years of age who reported fever, headache, bone and muscle pain compared to those 60 years old and over.

conclusion: The COVID-19 vaccine's side effects are similar to those seen with earlier immunizations, and the most of them are tolerable.

Keywords: COVID 19 vaccine; sinopharm; pfizer BioNTech; astraZeneca vaccines; side effects

Introduction

COVID19 vaccinations have been the most wanted item on the market for the past year. Many organizations have worked to establish an efficient and safe preparation in the minimum time possible. Currently, several products that different in form and effectiveness are approved for sale.

Moderna and Pfizer are two companies that make products based on mRNA technology. Others are vector vaccines include AstraZeneca and Sputnik-V.¹

Sinopharm COVID-19 vaccine is an inactivated vaccine that uses to inject a dead copy of SARS-CoV-2 into the body.²

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Sometimes there is growing public-health issue fueled by misconceptions about vaccine effectiveness and safety and that's called "vaccine hesitation (VH)" which is defined as "a delay in accepting or refusing vaccines despite vaccine services being available. Aversion to vaccines' potential side effects was the most common reason for vaccine hesitancy in all population groups in the United Kingdom. In the case of COVID-19 vaccines, this finding was confirmed by the fact that fear of adverse effects was the most common reason for healthcare workers and students to be less ready.³

Recently, all available data on COVID-19 vaccine adverse effects has been published by manufacturer-funded studies that have been approved by the authorities. COVID-19 vaccines are subjected to post-marketing examination by drug regulators and cooperating academic and clinical institutes, just like any other innovative pharmaceutical products.⁽⁴⁾

Methodology

This cross-sectional survey-based study was conducted out from October 2021 to December 2021 to determine the prevalence of COVID-19 vaccination adverse effects among the people. The Arabic language was used to create a Google Forms questionnaire (the scientific terms for the symptoms were written and explained in the public language) and distributed via social media (mainly Facebook and WhatsApp) to participants. Furthermore, the value of the study in informing the public about the vaccine's negative effects was emphasized.²

After weighing the acceptable side effects against the disease's severity, participation may encourage everyone else to get the vaccine. The setting were adjusted such that each participant may only send one response.

There were two types of questions listed. The first one covers the subject's background information, such as nationality, gender, age, educational level, and past COVID 19 infection. The second set of questions focused on information about the COVID-19 vaccines and the adverse effects that related with the vaccination, as well as when these side effects first appear. The questionnaire asks if the individual received one or two doses of COVID-19 vaccination, as well as what type of vaccine they received.

The participants were asked to choose from a list of symptoms that included injection site adverse

effect, Hypersensitivity Symptoms as "fast heartbeat, difficulty breathing, body allergy", headache, fever, bone or muscle Symptom, chills, Sore throat, GIT Symptoms like nausea, vomiting, as well as any other symptoms they had after receiving the immunization.

Sample size

The sample size for conducting this survey was 650 with a 5% margin of error and a 95% confidence level. We have hypothesized that the prevalence of the side effects in the population is $50\% \pm 5$ (the reported side effects by FDA ranged from 14.2% to 84.1%). In this study, 650 subjects completed the COVID-19 vaccine questionnaire (Figure 1).

Statistical analysis

Descriptive statistics were performed for the collected data. The responses were displayed as (percentage). A chi-squared test was employed for statistical analysis. The data were statistically analyzed using the IBM SPSS Statistics Version 26.0.0.0 software.). The level of significance was set at $p \leq 0.05$.

Results

People have been concerned about the dangers and risks of vaccination administration since the beginning of vaccine manufacture. There is a wide range of people's faith in vaccinations, which is influenced by various reasons including vaccine awareness, potential hazards, personal experiences, religious or political factors, as well as social and economic standing.⁵

Furthermore, it has been discovered that consumers estimate vaccination-related hazards compared to other risks in different way than specialists do.⁶

Some side effects are unlikely to manifest in pre-licensure clinical investigations because of their minimal frequency, the limited numbers of respondent individuals, and other study restrictions, As a result, post-marketing surveillance of side effects after vaccine delivery is critical.⁷

For this study, 650 individuals, all of them of Iraqi descent, participated. Women were the majority of the participants (401, 61.8%), while men constituted the minority of participants (249, 38.2%). It was found that (395, 60.7%) participants were younger than 60 years of age, and (255, 39.3%) were

60 years or older. Most of the study population was made up of people with the university (494, 76%) and postuniversity (55, 8.4%) education levels, while the minority were those with pre-university (101, 15.6%) education. The study population's past medical history revealed that (351, 54%) had previously been infected with the COVID 19 virus, while (299, 46%) participants had never been infected. Participants of (16%, 104) had Sinopharm vaccine, while (484, 74.5%) received Pfizer BioNTech vaccine and (62, 9.5%) received AstraZeneca vaccine. Participants of (399, 61.5%) had received both COVID-19 vaccine doses, while (251, 38.5%) had received only one dose of their vaccines doses. Participants of (518, 79.7%) reported the presence of the side effects after receiving their doses of the vaccines (first and second dose), (132, 20.3%) of participant reported that they had no side effects after received the vaccine, see Table 1.

Table 1: The study participants' demographic characteristics, COVID-19 past infection, type of vaccine, number of vaccine doses, and the onset of side effects

Characteristics	Frequency (n and %)
	All Participants (n= 650)
Gender	
Male	249 (32.2%)
Female	401 (61.8%)
Age (year)	
< 60	395 (60.7%)
≥ 60	255 (39.3%)
Education Level	
Pre-university education	101 (15.6%)
University education	494 (76%)
Post-university education	55 (8.4%)
Previous Infection with Covid 19	
Infected	351(54%)
Not infected	299 (46%)
Type of COVID-19 Vaccine	
Pfizer-BioNTech	484 (74.5%)
AstraZeneca	62 (16%)
Sinopharm	104 (16%)
Inoculated Vaccine Dose	
1st dose	251 (38.5%)
1st and 2nd dose	399 (61.5)
Presence of Symptoms	
Presence of symptoms	518 (79.7%)
Absent of symptoms	132 (20.3%)

Side effects of the COVID-19 vaccine and their correlation to the first and second doses

The data obtained from the participants in this trial revealed that the vaccine's side effects were recognized after the two doses, with the majority coming after the second dose. The most common side effects, which include discomfort at the injection site, headaches, muscle and joint ache, and raised body temperature, could remain for days, according to the fact sheet, and were more common after the second dose than the first. See Table (2).

Chills, sore throat, hypersensitivity symptoms, vomiting, abdominal pain, and diarrhea are less common adverse effects.

There is significant difference ($p < 0.001$) between the number of patients experiencing side effects following the second dose of the vaccination (75%) than those reported side effect after receiving the first dose (40.2%), according to the findings of this study. The number of participants reporting injection site adverse effects following the second dosage of the vaccine (65.1%) which is nearly similar to those reporting side effect after first dose (64.5%) and this show that there is no significant difference between the two groups ($p > 0.1$).

There is significant difference ($p < 0.03$) between The number of people who reported hypersensitivity symptoms like fast heartbeat, shortness of breath and rash all over the body after getting the second dose of the vaccination (15.8%) than the number of people who reported hypersensitivity symptoms after receiving the first dose (5.3%).

Also the number of people who reported bone and muscle pain after receiving the second dose of the vaccine was significantly different) $p < 0.01$) (47.5%), (40.1%) respectively than the number of people who reported bone and muscular pain after receiving the first dose (15%) (10%), according to the findings.

Furthermore, there is significant difference ($p < 0.001$) between the number of people who reported fever after receiving the second dose of the vaccination (49%) than the number of people who reported fever after receiving the first dosage (26%).

There is also significant difference between individuals that develop chills ($p < 0.04$) and sore

throat ($p < 0.05$) after second vaccine dose (15%) (7.5%) respectively as compared to individuals reported chills and sore throat reported after their first vaccine doses (2%) (1.5%).

The number of participants who reported a desire to sleep after getting the second dosage of the vaccine (63.5%) which was significantly different ($p < 0.02$) than the number of participants who reported a desire to sleep after receiving the first dose (40.7%).

Besides, there was a significant difference ($p < 0.002$) in the number of participants who reported headache after receiving the second dose

of the vaccine (45.5%) compared to the number of individuals who reported headache after receiving the first dose (20%).

The results also showed a significant difference in the number of persons who reported GIT symptoms: nausea ($p < 0.01$), vomiting ($p < 0.03$), abdominal pain ($p < 0.04$), and diarrhea ($p < 0.01$) after receiving the second dose of the vaccine (21.9%), (4.1%), (9.1%), (5.4%) respectively compared to the number of persons who reported GIT symptoms after receiving the first dose (5%), (0.5%), (1.5%), and (1.5%).

Table 2: The reported COVID-19 vaccine side effects and their correlation with the vaccine's first, second doses

	1st Dose (%)	2nd Doses (%)	Chi-Square p value
Presence of Symptoms			
Presence	(40.2%)	(75%)	0.001
Absence	(59.8%)	(25%)	
Local Symptoms	(64.5%)	(65.1%)	0.1
Arm pain, Injection site pain, Injection site swelling and redness			
Hypersensitivity Symptoms, A fast heartbeat, Difficulty breathing, body allergy	(5.3%)	(15.8%)	0.03
Bone and Muscle Symptoms	(15%) (10%)	(47.5%) (40.1%)	0.001 0.001
Headache	(20%)	(45%)	0.002
Fever	(12%)	(49%)	0.001
Chills	(2%)	(15%)	0.04
Sore throat	(1.5%)	(7.5%)	0.05
GIT Symptoms			
Nausea	(5%)	(21.9%)	0.01
Vomiting	(0.5%)	(4.1%)	0.03
Diarrhea	(1.5%)	(5.4%)	0.01
abdominal pain	(1.5%)	(9.1%)	0.04
Desire to sleep	(20%)	(63.5%)	0.002

Reported COVID-19 vaccine side effects and their correlation with participants' ages

The results of the study revealed significant differences ($p < 0.05$) between those under the age of 60 who were experiencing COVID-19 vaccination adverse effects and those at the age of 60 and older. See Table 3.

In comparison of individuals under 60 years old with those 60 years and older, noted that there is no significant difference ($p > 0.2$) following the first and second doses of the vaccine in the local symptoms. local symptoms and injection site effect in 60 years old and over is (75.8%) while under 60 years were (80%).

The results of this study revealed that injection site discomfort was reported similarly in people aged 60 and up and in younger people, also the same thing occur following first and second dose of vaccine.

Headache was from the most common symptom after receiving a vaccination injection. Also fever more likely to develop following the second dose in young persons.

There was a significant difference in the number of people who reported fever ($p < 0.01$) and headache ($p < 0.02$) under 60 years of age (75.5%) and (67.2%) respectively compared to those 60 years old and over (60.4%) and (52%).

Besides, there was a significant difference in the number of people who developed bone (p < 0.01) and muscle pain (p < 0.03) over 60 years of age (31.4%) and (6.4%) compared to those under 60 years old (56.2%) and (19.7%).

The study also note that there were no significant differences between the other side effects of the vaccine when comparing their incidence between participants according to their ages.

Table 3: The reported COVID-19 vaccine side effects and their correlation with the participants ages

	Frequency (n and %)		
	Age < 60 (Year)	Age ≥ 60 (Year)	Chi-Square
	(%)	(%)	p value
Local Symptoms, Arm pain, Injection site pain Injection site swelling and redness	(80%)	(75.8%)	0.2
Hypersensitivity Symptoms A fast heartbeat Difficulty breathing body allergy	(5%)	(9%)	0.1
Bone	(56.2%)	(31.4%)	0.01
Muscle Symptoms	(19.7%)	(6.4%)	0.03
Headache	(67.2%)	(52%)	0.002
Fever	(75.5%)	(60.4%)	0.01
chills	(8%)	(5%)	0.1
Sore throat	(4%)	(2%)	0.3
GIT Symptoms			
Nausea	(9%)	(6.4%)	0.2
vomiting	(4%)	(2.5%)	0.1
Desire to sleep	(67%)	(64%)	0.1

Correlation between presence and absence of post COVID-19 vaccine side effects and the participant’s sex

According to the findings, there was a significant difference (p< 0.05) in the number of females who experienced COVID-19 vaccination side effects (80%) as compared to males (60%).

Discussion

The majority of the symptoms are most likely due to an overabundance of a cytokine called type I interferon, which plays an important role in potentiating early stages of the immune response (IFN-I).⁸

The data obtained from the participants in this trial revealed that the vaccine’s side effects were recognized after the two doses, with the majority coming after the second dose. this is due to the fact that the immune system identifies the viral spike protein from the first vaccine dose and produces a stronger response.⁹ The FDA Fact Sheet for Recipients and Caregivers confirmed these findings.

Another theory is based on the immune system’s response. The immune system could release cytokines

that could cause inflammation in the blood vessels, muscles, and other tissues.

Injection site tenderness as a subjectively reported symptom has a variety of confounders that should be taken into account in future vaccination side effect research, including injection method, vaccine temperature, and injection velocity. These variables are difficult to regulate and will have a major impact on one’s experience. Furthermore, injection into a relaxed muscle causes less discomfort than injection into a stiff muscle, so it’s best to lower the patient’s arm that will be injected.³

The results of this study revealed that injection site discomfort was reported similarly in people aged 60 and up and in younger people, also the same thing occur following first and second dose of vaccine. This is agree with the predictions of Polack et al, who found that injection site discomfort was more common in those younger than 55 years old compared to those older.¹⁰

This could also explain why persons under the age of 60 are more likely to have adverse effects than older people, as younger people have stronger and more efficient immune systems. Severe allergic

response would occur within minutes to one hour after getting the vaccine dose.

Our findings are in agreement with those of Polack et al, who found that vaccine-associated systemic adverse effects were more common among younger participants and more common after the second vaccine dose in both trials.¹⁰

In comparison to the intensity of typical immune responses, IFN-I production is significantly higher in females than in males, and in younger people than in older people.⁸

Conclusion

In general, the COVID-19 vaccine's side effects are similar to those seen with earlier immunizations, and the most of them are tolerable.¹¹ Furthermore, the majority of the symptoms have been recorded by the manufacturer and the FDA Facts Sheet.¹¹ However, for certain people, monitoring them for a short time after receiving their vaccine doses is required.

Ethical clearance: Approval of the study design was obtained by the ethics committee at the university of basrah / medical college. The committee was very supportive of the project

Conflict of interest: the authors declare no conflict of interest

Source of funding: self

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Does the Proximity of the Area Affect in Incidence of Stunting? : Study on Densely Populated Provinces in Indonesia

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How to cite this article: Astridya Paramita, Nailul Izza, DwiHapsari Tjandrarini et al. Does the Proximity of the Area Affect in Incidence of Stunting? : Study on Densely Populated Provinces in Indonesia. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):84-91.

Abstract

The prevalence of stunting in Indonesia is still above WHO standards, so it needs to be addressed immediately, given the adverse effects on individuals and countries. This study aims to analyze the incidence of stunting in East Java by paying attention to the proximity of the area as an effort to solve stunting problems. The study was conducted using secondary data from 2017 from official government agency reports. Administrative area (regency/city) analysis unit. Analysis using spatial regression test. The results show that Moran's I test shows a spatial dependency or location autocorrelation ($p < 0.20$). The Lagrange Multiplier SAR spatial regression test can explain that spatial factors can increase 5% greater (75%) of the 5 selected factors that cause toddler stunting, compared to using the OLS classic regression test. It could be concluded that spatial factors, namely geographic areas, can increase the percentage of clarity in the regression modeling for the incidence of stunting in an area. In this study, spatial factors are known to be associated with regional proximity and ethnic similarity, namely the Madurese ethnicity.

Keywords: Stunting; the proximity of the area; child health; community nutrition.

Introduction

One indicator of the success of achieving the Sustainable Development Goals (SDGs) in the health sector is the nutritional status of children under five. A toddler group is a group that is prone to experiencing nutritional problems, one of which is stunting. Stunting is a condition of chronic malnutrition that occurs due to inadequacy of nutrients in the past, as indicated by the z-score of height for age (height/age), which is less than -2 SD.¹

The prevalence of stunting of children under five in the world has decreased for 17 years, from 2000 to 2017, which was 10.4%. Based on the results of the 2013 Indonesia Basic Health Survey, for the national scale, the prevalence of stunting of children under five in Indonesia was 37.2%, while for East Java Province in 2013 the prevalence of stunting was 35.8%.² According to WHO, if the problem of stunting is above 20% it is a public health problem.² Based on Nutritional Status Monitoring data for

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three consecutive years (2015-2017), the prevalence of children under five with stunting in Indonesia is still far above the WHO standard, namely 29%, 27.5%, and 29.6%.³

Stunting in children under five is a consequence of several factors. There are five main factors causing stunting, namely poverty, social and culture, increased exposure to infectious diseases, food insecurity, and community access to health services.^{4,5} The results of a systematic review regarding the risk factors for stunting in developing countries show that it is consistently influenced by family socioeconomic status (family income), mother's education, low birth weight, premature birth, non-exclusive breastfeeding, birth length, and macronutrient and micronutrient deficiencies.⁶

In the long term, stunting conditions will interfere with the health, education, and productivity of children under five in the future. Stunting children

under five tend to experience degenerative diseases in the future, it is difficult to achieve optimal growth and development potential both physically and psychomotor, which of course affects intellectual abilities.^{7,8}

With the high prevalence of stunting nationally, including in the province of East Java, and its impact on individuals and countries in the future, stunting is still one of the priority health problems in Indonesia that must be immediately followed up. Indonesia has a geographical condition with the number of islands reaching 17,499 making it the largest archipelagic country in the world. Indonesian state with social life and culture diversity, and has a unique geographical conditions, raise issues and determinants of stunting are different in each region, and therefore the area is used as a case study in this research is the province of East Java as an area with densely population.⁹

Table 1: Map of stunting prevalence grouping in East Java Province in Indonesia in 2017

Kriteria WHO	Regional
Low (<20.0)	Mojokerto City, Gresik and Sidoarjo Regency, Bojonegoro Regency, Blitar City, Madiun City
Medium (20.0 - 29.9)	Pacitan, Ponorogo, Trenggalek, Tulungagung, Blitar, Jombang, Nganjuk, Madiun, Magetan, Ngawi, Tuban, Lamongan, Malang, Lumajang, Pasuruan, Mojokerto Regency and Malang City, Banyuwangi Regency, Sampang Regency, Kediri City, Surabaya City
High (30.0 - 39.9)	Probolinggo, Situbondo, Bondowoso, and Jember Regency, Kediri Regency, Sumenep regency, Pasuruan City, Batu City
Very High(≥40.0)	Bangkalan and Pamekasan Regencies, Probolinggo City

Source: Handbook of Nutrition Status Monitoring, Directorate of Community Nutrition.

Table 1 is a map of the distribution of stunting prevalence in East Java Province according to the 4 WHO criteria (1997), namely low (<20.0; light pink color), medium (20.0 - 29.9; dark pink color), high (30.0 - 39.9; red color), and very high (≥40.0; dark red color) to determine the percentage distribution of the prevalence of stunting in East Java from a regional aspect.¹⁰ The highest prevalence of stunting in Bangkalan and Pamekasan regencies are in proximity of the area, and 2 other areas in the Madura archipelago are included in high prevalence (Sumenep Regency) and medium (Sampang Regency). 4 Regions with high prevalence and 16 regions with moderate prevalence of stunting tend to cluster together, as well as 2 regions with low prevalence.

The results of the mapping show that there is a tendency for the similarity in the percentage of stunting

categories from nearby locations compared to remote locations so that it becomes the basis for an assessment of the stunting problem in East Java using a spatial approach. This is in line with Tobler's first law of geography in 1970 that everything is related to others, but those that are located close together have a more relationship than those that are located far apart.¹¹

Based on the background description, this study aims to analyze the incidence of stunting in East Java by paying attention to the proximity of the area as an effort to solved stunting problems. From this analysis, it is hoped that a spatial regression model can be obtained and a large spatial dependence on the prevalence of stunting in East Java so that it can be a matter for regional considerations in formulating policies and programs for handling area-based stunting.

Methods

Study design

This type of research was analytic descriptive using secondary data in the form of survey reports and annual reports from several government agencies. The report was obtained from the survey results with a cross-sectional design.

Data sources and variables

The data used in this research was secondary in 2017 obtained from reports from several government agencies, namely from the Directorate of Community

Nutrition in the form of a Pocket Book for Monitoring Nutrition Status³, Health Research and Development Agency in the form of Health Personnel Research Report¹², The Central Bureau of Statistics is in the form of a Social Economic Survey Statistical Report¹³, and the Ministry of Finance in the form of Regulation of the Minister of Finance of the Republic of Indonesia number 119/PMK.07/2017 concerning the Regional Fiscal Capacity Map.¹⁴ The unit of analysis of this research is the geographical area at the district level as many as 38 districts/cities in East Java Province. The variables used in this study could be seen in Table 2.

Table 2: Independent variable and dependent variable

Variable	Description
X1	Percentageofbabieswho are exclusivelybreastfedfrombirth, aged 0-5 monthsbyregencies/cities, 2017*
X2	Percentageofchildrenaged 6-59 monthsreceiving Vitamin A capsulesbyregencies/cities, 2017*
X3	Per capitadailycalorieconsumption per capitabyregencies/cities, 2017**
X4	Per capitadaily protein consumption per capitabyregencies/cities, 2017**
X5	Percentageofpopulationwithanaveragemonthlyexpenditure per capitabelowthepovertylinebyregencies/cities, 2017**
X6	PercentageofFiscalCapacity Index thatreflectsthefinancialcapacityofeach region byregencies/cities, 2017***
X7	Percentageofhealthworkerswith a background as a generalpractitioner per 10,000 inhabitantsbyregencies/cities, 2017****
X8	Percentageofhealthworkerswhohavecompletednursingeducationprograms per 10,000 populationbyregencies/cities, 2017****
X9	Percentageofhealthworkerswhohavecompletedthemidwiferyeducation program per 10,000 populationbyregencies/city, 2017****
X10	Percentageofhealthworkerswhohavecompleted a publicealtheducation program per 10,000 residentsbyregencies/cities, 2017****
X11	Percentageofhealthworkerswhohavecompletednutritioneducation per 10,000 populationbyregencies/cities, 2017****
X12	PercentageofHealthCenters per 10,000 populationbyregencies/cities, 2017****
X13	Percentageofactive Posyandu (Integrated Service Posts) per 10,000 populationbyregencies/cities, 2017****
Y	Prevalenceofchildrenunderfive (0-59 months) withheight per agelessthan -2 SD byregencies/cities, 2017*

Source: *Directorate of Community Nutrition;

**Central Bureau of Statistics;

***Ministry of Finance;

****Health Research and Development Agency

Data analysis

The statistical test used was the spatial regression test. The spatial regression test is a method to obtain a mathematical model of the relationship between the dependent variable (Y) and the independent variable

(X) concerning spatial elements.^{15,16} The spatial lag parameter (ρ) shows the level of correlation of the spatial influence of an area to other areas around it.¹⁷ Spatial dependence occurs due to dependencies in spatial/region/area data. Tobler's first law of geography says that everything is related to other things but that something closer has great influence.¹¹

The test used to determine the spatial dependency in the error of a model was by using Moran's I test

and the Lagrange Multiplier (LM) test to identify specifically the right type of spatial regression test.¹⁶ Moran’s I test is a diagram to see the relationship between the value of observations at a location (standardized) with the average value of observations from locations adjacent to the locations concerned.¹⁷

Results and discussion

Outlier Data Test

The results of the univariate outlier test showed that 3 regencies/cities had Z scores that deviated from the average, namely Mojokerto Regency, Madiun City, and Surabaya City. Besides, 4 variables have outlier values, namely the fiscal capacity index (X6), medical workers (X7), public health workers (X10), and Posyandu (X13). Likewise, the results of the multivariate outlier test using the Mahalanobis

Distance statistical test show that the maximum Mahalanobis Distance value was 16.802, while the $X^2_{(0.001; 9)}$ was 27.87, and the maximum Cook Distance value was 0.297, which means it was smaller than the multivariate rule of thumbs ($0.297 < 1$), so it could be concluded that there was no outlier data.

Classic Assumption Test

Before the spatial regression test was carried out, the classical assumption test was carried out on the variables studied, to assess the feasibility of the next statistical test, namely the spatial regression test. The classic assumption test includes a normality test, multicollinearity test, heteroscedasticity test, and autocorrelation test. The results of the classical assumption test can be seen in Table 3.

Table 3: The results of the classical assumption test

No	ClassicAssumptionTest	Hypothesis	P-value	Results
1	Normality Test (Jarque Fallow Test)	H0: Residuals are normally distributed (p value> 0.05) H1: Residuals are not normally distributed	0.494	p = 0.494 > α then H0 is accepted, or the residualis normally distributed.
2	Multicollinearity test (Farrar-Glaubery test)	H0: Multicollinearity was not occur H1: There was multicollinearity (CI > 30)	114.807	Condition Index (CI) = 114.807 > 30) then Ho is rejected or multicollinearity occurs.
3	Heteroscedasticity Test (Breusch Pagan Test)	H0: There was no heteroscedasticity H1: Heteroscedasticity occurs	0.616	p-value = 0.616 > 0.05 then H0 is accepted or the error variance is homoscedasticity.
4	Autocorrelation Test (Durbin-Watson Test)	H0: There was no autocorrelation H1: There was autocorrelation	d = 2.224 N = 35 k = 9 dL = 0.908 dU = 2.114 4-dL = 3.092 4-dU = 1.856 4-d = 1.776	The Durbin Watson test results show: dL < d < dU d < 4-dL d > 4-dU dL < dU < d dL < (4-d) < d Thus the classic terms of auto correlation through the Durbin Watson test cannot be concluded.

Spatial Dependency Test

The results of the spatial dependency test using Moran’s I test obtained a p-value of 0.026. The p-value smaller than α = 0.20 indicates a dependency or autocorrelation between locations (H₀ is rejected).

Based on Table 1, the very high category is in Bangkalan Regency (43%), Pamekasan Regency (42.5%), and the high category is in Bondowoso Regency, Situbondo Regency, Probolinggo Regency, and Jember Regency. If the prevalence is mapped

based on the value of the local indicator from spatial association (LISA), there are 8 significant areas, namely the High-high and Low-low clusters. Cluster High-high is a neighbor located around regencies/cities that has a very high prevalence of stunting, which is occupied by Sampang Regency, Sumenep Regency, Probolinggo Regency, and Jember Regency. Low-low clusters are neighbors around regencies/cities that have a low prevalence of stunting.

Table 1 shows the grouping or similarity of stunting prevalence categories for adjacent areas. The meaning of this mapping is stronger because it is in line with the results of spatial regression analysis which shows the influence of the location or surrounding area with the occurrence of stunting in an area. This is indicated by the moran's I index value of 0.216 with a p-value = 0.026 ($\alpha < 0.20$) and the cluster mapping results of moran's I stunting prevalence using LISA. The results of LISA mapping identify that areas with a very high risk of stunting prevalence Sampang districts, Sumenep districts, Probolinggo Regency, and Jember Regency), tend to

have occurred in the province of East Java, where the majority of the population is ethnic Madura.

Comparison of Regression Models

The accuracy of the regression model in approaching the truth can be seen from the R-square value, the Akaike Info Criterion (AIC) value, or the Schwarz Criterion (SC) value. The greater the R-square value or the smaller the AIC value and the SC value, the better the model.

Table 4 informs that the results of the Lagrange Multiplier SAR test for spatial lag (**p<0.05) are more precise than the spatial error, so it can be concluded that there is an effect of spatial correlation in the model of stunting. The SAR model has an R-square value of 0.75, which means that the variables used in this analysis can explain the causes of stunting by 75% after including spatial factors, while the other 25% are caused by other factors outside the variables used in the research analysis.

Table 4: Comparison of Classical Regression Model (OLS) with spatial regression

No.	Model	p-value	R-square	Akaike Info Criterion (AIC)	Schwarz Criterion (SC)
1	ClassicalRegression (OLS)	0.000014*	0.709695	253.895	270.271
2	Lag Model SpatialRegression	0.022** (Rho = 0.328407)	0.752382	251.077	269.09
3	SpatialRegressionError Model	0.114 (Lambda = 0.409734)	0.750618	250.088	266.464

The following was a model of the resulting spatial regression equation:

$$\hat{y} = -7,275 + 0,328 \sum_{j=1, j \neq i}^n w_{ij}y_j - 0,048X_{1i} - 0,453X_{2i} + 0,721X_{3i} - 0,035X_{4i} + 3,716X_{5i} + 0,062X_{8i} - 0,278X_{10i} - 13,706X_{11i} - 2,685X_{12i}$$

Intercept -7,275 means that if the percentage of not exclusively breastfed = 0, vitamin A is not given = 0, lack of energy and protein = 0, poverty = 0, lack of nurses = 0, lack of midwives = 0, lack of nutrition workers = 0, and Health Center is not available = 0, it is estimated that it will reduce the prevalence rate of stunting by 7 cases. Based on the model formed, it was found that the variables that affected the level of 0.2 were the variable giving Vitamin A, under-energy children under five, nutritionists, and the existence of the Health Center.

Results and Discussion

The results of the analysis found that the spatial factor in the prevalence of stunting under five in East Java was related to the proximity of the region and the similarity of ethnicity, namely Madurese ethnicity. This situation can be explained based on several previous studies. Previous studies conducted in Bangkalan-Madura informed that the Madurese ethnic community has socio-cultural practices related to nutrition that make stunting possible.

Socio-cultural practices related to stunting include food taboo for pregnant women. Pregnant women were found to abstain from eating squid and stingrays, citing concerns about difficulties in delivering babies (babies coming in and out like squid) and concerns about the birth shape of babies such as stingrays. This practice carries the risk of protein deficiency, while the protein needs of pregnant women increase compared to before⁽¹⁸⁾. A previous longitudinal study reported a significant effect on maternal protein intake during pregnancy on stunting nutritional status. Babies born to mothers with protein consumption less than the average (<58% RDA) in the second trimester, babies born less than 48 cm, and lack of protein intake at birth have a risk of stunting in infants aged 12 months.¹⁹

Another previous study on taboo food among the Madurese, Javanese, and Pentalungan ethnic communities in Jember Regency informed that in the health aspect, the practice of taboo food has both negative and positive impacts. Taboo food practices have a negative impact which results in nutrition from taboo foods that cannot be consumed or nutritional needs are not fulfilled optimally and have a positive impact as an effort to prevent adverse health impacts in the pregnancy and breastfeeding (lactation) phase.²⁰

Fetal growth restriction is a major risk factor for stunting worldwide (95% CI 9.1 million - 12.6 million of 44.1 million) which is characterized as linear growth retardation. Furthermore, poor sanitation (95% CI 6.3 million - 8.2 million) and diarrhea (95% CI 2.4 million - 9.2 million) are estimated as environmental factors as the second cause of stunting globally and in the Asian region. South, sub-Saharan Africa, and East Asia and the Pacific, while child nutrition and infection are the second group of risk factors in other regions based on the results of reviews conducted in developing countries.^{21,22}

The spatial regression results of the SAR model inform that involving spatial factors (areas) has increased the percentage of the ability to explain the effect of predictor variables on the incidence of stunting by 5%. This means that spatial factors need to be considered in preparing an intervention for handling stunting. The results of this spatial regression are in line with the results of research in Burkina Faso, sub-Saharan Africa that statistical spatial modeling is very useful in identifying or developing intervention programs and strategies for intervention target areas in managing malnutrition.²³

The factors that influence the occurrence of stunting in East Java Province are toddlers receiving Vitamin A, lack of energy in under five, the availability of nutritionists, and the existence of an ineffective Public Health Center. This factor is only able to explain its effect on the occurrence of stunting by 75%, and the other 25% is caused by other factors not used in the analysis of this study. Previous studies that analyzed the determinant incidence of stunting in East Java Province found that places, where toddlers live in rural areas, have a 0.855 times greater risk of stunting than those living in cities, age under five, age of mothers under five and education of mothers under five at SD level 2.206 times more risk of stunting than with a college-educated mother.²⁴

The results of previous studies revealed that a child's birth weight <2500 grams has a 2.5 times greater chance of stunting. Likewise, households with three or more children under five, maternal antenatal care less than 4 times, household ability to access proper sanitation, exclusive breastfeeding, low socioeconomic status, preterm birth, short birth length, and low maternal education are the determining factors for stunting.²⁵⁻²⁸ The ability of the economy also affect the occurrence of stunting population in Iran with the poorest cluster socioeconomic 3.04 times greater risk of the occurrence of stunting.²⁹

The occurrence of stunting in an area is not only due to a single cause but also due to differences in the social construction of the community that was built, communication patterns, and meaning between health workers and the community. A study in Jember-Indonesia revealed that the social construction of stunting according to theory is a phenomenon produced by health providers based on the existing reality, while the community understands short toddlers as something normal as long as the child is still active, playing, and still has an appetite, and also heredity from short parents.³⁰

Madurese culture views the role of the father as merely the head of the family and breadwinner in the family. The results of the study using the Binary Logistic Regression test showed that the role of the father had a significant effect on the prevention of stunting with significant results ($0.001 < \alpha < 0.05$). Parenting culture has a significant effect on stunting prevention in children under five with significant results ($0.019 < \alpha < 0.05$). Parenting culture is beneficial for fathers in increasing their role in preventing stunting in toddlers and nurses need to understand

their culture before understanding transcultural nursing that is applied to society.³¹

Stunting is often not recognized in the community and short stature is considered normal. Stunting is the result of a complex set of contextual causative factors, requiring a multifaceted and transdisciplinary approach.^{32,33} The results of the spatial regression analysis and the description of the geographic area that are interrelated can increase the risk factors for the prevalence of stunting in the surrounding area. Therefore, synergy is needed in overcoming the occurrence of stunting, namely by collaborating with local governments between surrounding regencies/cities, as well as collaboration from communities that care about public health with community leaders and religious leaders regarding educational innovation efforts to form correct social constructions related to nutrition and problems. Stunting as a form of approach to reduce trust in food taboo so that it can be accepted by the community. This collaboration needs to be continuously fostered in carrying out any activities related to efforts to increase nutrition or reduce the prevalence of stunting.

Conclusion

Based on the results of the study, it can be concluded that proximity areas can increase the percentage of clarity in the regression modeling of stunting incidents in an area. Based on these conclusions, handling the stunting problem in an area needs to consider spatial factors, namely the proximity to the surrounding area which is often neglected. In this study, the proximity of the region is known to be related to ethnic similarities, namely the Madurese ethnicity.

It is recommended that the synergy/cooperation of local governments between regencies/cities concerning spatial and ethnicity, a collaboration of community health care with community leaders and religious leaders concerning educational innovation efforts to form the correct social construction related to "food taboo" and the problem of stunting, and organizing other activities.

Additional Informations

Conflict of interest: The authors declare that they have no conflict of interest.

Ethics approval: Not applicable, because this article does not contain any studies with human or animal subjects.

Source of funding: National Institute of Health Research and Development.

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Clinical Profiles of Patients with Optic Neuritis and Papillitis

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How to cite this article: Atul A. Modesara, Bhavna Dhanji Galgal. Clinical Profiles of Patients with Optic Neuritis and Papillitis. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):92-95.

Abstract

Background and Aim: Optic neuritis causes substantial visual impairment and potential long-term visual defects in addition to serving as an important prognostic indicator for future development of demyelinating diseases such as multiple sclerosis. Hence this study was done with the objective to collect data of the clinical profile in patients of optic neuritis.

Materials and Methods: A prospective analysis was done for the patients clinically diagnosed with optic neuritis. A total of 60 patients were included in the study. Patients admitted with optic neuritis after taking valid consent detailed history was taken, with documentation of onset of visual loss, duration of visual loss, pain and history of any other ophthalmic and neurological symptoms.

Results: Of the total 120 eyes of 60 patients were inspected, the left eye was more commonly diagnosed with neuritis and papillitis. DOV was seen as the chief complain in majority of patients with optic neuritis, 21 patients complained of pain with eye movements and twelve patients complained of non specific pain in an around the eye. Four patients had uthoffs phenomenon.

Conclusion: All patients treated were treated with ONTT trial and also investigated for cause and follow up done. The results revealed that the prevalence of papillitis were almost equal in males and females. Patients responded quickly to ONTT regimen while papillitis recovered late. VA improvement after ONTT was statistically significant. Colour vision and contrast also improved. Our study showed that ONTT regimen has benefit in optic neuritis with fast Visual recovery.

Keywords: Clinical Profile; optic Neuritis; ONTT; Papillitis.

Introduction

The optic nerve develops in the substance of the optic stalk. This stalk, which becomes apparent at the fourth week of gestation, has a wide circular lumen that is continuous with the cavity of the forebrain at one end and the optic vesicle at the other.¹

Optic papillitis is a specific type of optic neuritis. Inflammation of the optic nerve head is

called "papillitis" or "intraocular optic neuritis"; inflammation of the orbital portion of the nerve is called "retrobulbar optic neuritis" or "orbital optic neuritis". It is often associated with substantial losses in visual fields, pain on moving the globe, and sensitivity to light pressure on the globe.²

Optic neuritis causes substantial visual impairment and potential long-term visual

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defects in addition to serving as an important prognostic indicator for future development of demyelinating diseases such as multiple sclerosis.³ So ophthalmologist has a very significant role to aid in prevention of full blown MS. Fortunately, in most cases, optic neuritis recovers either spontaneously or with treatment. Recovery can be partial or absolute, depending largely upon severity and co-existing conditions.^{4, 5}

Walsh and Hoyt (1969) has described optic neuritis as the general term used to describe involvement of the optic nerve as the result of any inflammation, demyelination or degeneration.⁶ The term optic neuritis according to Chamlin (1953) is technically incorrect in as much as cases what we refer to as optic neuritis is not always inflammation of the optic nerve as the term implies, but also degeneration or demyelination as caused by plaques of multiple sclerosis.⁷

In developing countries like India the clinical profile of optic neuritis is somewhat different. Not many studies have been done on optic neuritis. A few studies clarify that the scenario in Indian subcontinent is different as infectious diseases play a important role in causation of optic neuritis and prognosis is not so good. Hence this study was done with the objective to collect data of the clinical profile in patients of optic neuritis.

Materials and Method

The present study was done in the department of ophthalmology and neurology in the medical college and associated hospital. A prospective analysis was done for the patients clinically diagnosed with optic neuritis. The ethical committee of the institute was informed about the study and the ethical clearance certificate was obtained prior to the start of the study. The patients were informed about the study and the consent was signed before their inclusion in the study. A total of 60 patients were included in the study.

Patients admitted with optic neuritis after taking valid consent detailed history was taken, with documentation of onset of visual loss, duration of visual loss, pain and history of any other ophthalmic and neurological symptoms. Clinical examination included Snellen's visual acuity testing, evaluation of pupils. Parainfectious and postvaccinated optic neuritis were excluded from the study. Other causes of disc edema like ischemic optic neuropathy,

traumatic neuropathy were thoroughly ruled out. Cases thought to have other neurological deficits were referred to neurologist for evaluation.

Haemogram, total and differential white blood count, erythrocyte sedimentation rate, chest X ray, mantoux test, and serology for syphilis, toxoplasmosis, HIV were obtained in all cases. Magnetic resonance imaging (MRI) of the brain and orbit with contrast were done in patients who were affordable and suspected to have demyelinating disease and retroorbital mass. Patients with contraindications to systemic steroids like active systemic infection, uncontrolled diabetes etc were excluded from study. All patients were treated as per treatment guidelines i.e ONTT regimen which consisted of Injection Methylprednisolone 1g for 3days followed by oral prednisolone 1g/kg/body wt for 11 days and then tapered. Patients suspected of infectious cause of optic neuritis were supplemented with systemic antibiotics. Data was recorded in a specially designed proforma which was transferred to master sheet. The data was subjected to statistical analysis by the biostatistician of our institution.

Results

A total of 60 patients who meet the inclusion criteria were included in the study. The study was conducted over the duration of one year. The included patient's age ranged from 15 years to 55 years. Maximum numbers of patients were in the age group of 41 to 50 years. Minimum numbers of patients were in age under 20 years and also above 55 years. Out of the total sixty patients there were 38 females and 22 males. The complete history was recorded and the risk factors that are associated with optic neuritis were smoking, alcohol, tobacco, pregnancy and diabetes.

Of the total 120 eyes of 60 patients were inspected, the left eye was more commonly diagnosed with neuritis and papillitis. DOV was seen as the chief complain in majority of patients with optic neuritis, 21 patients complained of pain with eye movements and twelve patients complained of non specific pain in an around the eye. Four patients had uthoffs phenomenon.

All patients with optic neuritis had pupil abnormality at presentation. 79.68% had RAPD and 20.32% had sluggishly reacting pupil. After treatment i.e at one week RAPD was detected in only 17.43%

of cases and at end of one month RAPD was noted in 8.25% of cases. In case of bilateral disease after treatment pupil was sluggishly reacting in 12.07% cases. At end of 3 months 86% had normal pupillary reflex.

At presentation denoted by blue line initially there were no cases with normal pupil reaction but after treatment the number of cases with normal pupil spiked at 1month. Also the number of cases with RAPD steeped downward than cases with sluggish reacting pupils in about 1 week duration.

Table 1: Chief complain of the patients recorded

Complaints	Optic Neuritis
DOV	27
Non specific pain	21
Pain with eye movements	12
Uthoffs Phenomenon	4

Table 2: Showing Papillary Reaction Comparison Before and After Treatment

	Clinical Presentation	One week	One month
Normal	0	68.29%	79.68%
Rapd	79.68%	17.43	8.25
Sluggish	20.32%	14.28	12.07

Discussion

Western data suggest that at least 50% of patients with ON will eventually develop MS, but studies from Asia and Africa present a contrasting scenario.⁸ An Indian study conducted by Rohit Saxena et al.⁶ before the commencement of the ONTT had indicated that the clinical profile of ON in our country may be different from that presented in the Western literature. Apart from the above studies no other study is available that clarifies the status of ON in India. The present study has been conducted with the aim of understanding the clinical picture of ON in India.

In our study 70% of the patients suffering from optic neuritis were in the age group of 3rd 4th and 5th decades of life. The age of presentation and female preponderance noted in the present study was similar to that reported by the ONTT and other studies conducted by Wakakura M et al.⁹ and Wang JC.^{10,11} Bilateral presentation was seen in 23.33% of the patients in the present study and compares to

16%-35% reported in other studies from this region conducted by Woung LC et al.¹² and Lim SA et al.¹³, whereas an African study conducted by Pokroy R et al.¹⁴ has reported it to be as high as 80%.

All patients with optic neuritis had pupil abnormality at presentation. 79.68% had RAPD and 20.32% had sluggishly reacting pupil. After treatment i.e at one week RAPD was detected in only 17.43% of cases and at end of one month RAPD was noted in 8.25% of cases. In case of bilateral disease after treatment pupil was sluggishly reacting in 12.07% cases. At end of 3 months 86% had normal pupillary reflex.

Although it was not possible to do MRI in all patients, intracranial de-myelination changes consistent with MS were not seen in any patients. We acknowledge that there is a possibility of underestimation of MS in our study given the fact that MRI was not performed in all cases; however, other reports from the south eastern region of India also show low incidence of MS in the population from this part of the world.

The limitations of our study include not doing automated perimetry and not obtaining MRI in all cases. Despite that we found that ON in the Asian region is different from that reported in the Western population. Whether environmental factors, ethnicity, and genetic composition could play a role in the discrepancy in clinical profile in this region remains to be studied.

Conclusion

All patients treated were treated with ONTT trial and also investigated for cause and follow up done. The results revealed that the prevalence of papillitis were almost equal in males and females. Patients responded quickly to ONTT regimen while papillitis recovered late. VA improvement after ONTT was statistically significant. Colour vision and contrast also improved. Our study showed that ONTT regimen has benefit in optic neuritis with fast Visual recovery.

Ethical approval was taken from the institutional ethical committee and written Informed consent was taken from all the participants.

Source of funding: Nil

Conflict of Interest: None declared

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Study of in Vitro Antimicrobial Activity and in Vivo Wound Healing Potentiality of Leaves of *Callistemon Viminalis*

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How to cite this article: Avijit Mazumder, Utpal Anand, Saumya Das. Study of in Vitro Antimicrobial Activity and in Vivo Wound Healing Potentiality of Leaves of *Callistemon Viminalis*. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):96-101

Abstract

Callistemon viminalis, often known as the stream bottlebrush, is a tropical plant in the Myrtaceae family and is renowned for its ability to grow rapidly in riparian zones. It is exclusively found in New South Wales, Queensland, and Western Australia. The roots of this species are entangled, which helps stabilize the soil, which reduces the danger of erosion. This plant has antibacterial, antifungal, antioxidant, and other pharmacological and insecticidal abilities that are also related to other medicinal properties. This research investigates the benefits, applications, and features of several extracts obtained from components of *C. viminalis* (branches, flowers, fruits, bark, and leaves). Bioactive compounds were characterized in detail by noting their chemical structures. The use of *C. viminalis* in folk medicine was fully supported by the results. Collecting and compounding plant materials, including processing, drying, and grinding, and then doing research to screen pharmaceutical effects, are all part of making the cure. Rats had their wound healing ability tested using a method known as in vitro experimentation. This method relies on excision wounds. The capacity of the extracted substance to close wounds when applied as an ointment was shown to be greater than in the control. The epithelization time in the standard and treated groups was significantly shorter than in the basic ointment base treatment group. The group treated with 5% w/w extract ointment had greater traction intensity than the control group (1.96 gm/mm²), with a significant difference of (1.12) gm/mm². The tensile strength of the (3.86) gm/mm² of the 5% w/w treated group. A substantial increase in tensile strength was seen on the tenth day for both the extract level and the normal medicine.

Keywords: *Callistemon viminalis*; incision Wound model; healing potentiality; antibacterial activity

Introduction

Herbal medicine use dates back millennia, and in recent times, it has gained a more enthusiastic following, seeing an extraordinary expansion in its applications, especially for medicinal purposes. Many poor nations followed by indigenous communities were stuck with plant medicine as their only option. Excessive usage of artificial substances,

resulting in bio-amplification, has been shown to boost agricultural output (Kavitha and Satish, 2013).¹ Herbal medications have complicated chemical compositions, which may lead to both minor and severe side effects. As a result, herbal medicines should be subject to well-documented scientific toxicity tests to show they are safe. Of the 3800 species of shrubs and 5800 species of trees found virtually everywhere in the tropics and subtropics

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of the world, the most common family is Myrtaceae, which contains 121 genera and many species.² Bottlebrushes is one of the 34 species of *Callistemon* in the Myrtaceae family and is commonly called *Callistemon*. There are three species with a presence in the Sierra Madre Occidental Mountain range: *Metrosideros viminalis* Sol. ex Gaertn., *C. viminalis* (Sol. ex Gaertn.) G. Don, and *Melaleuca viminalis* (Sol. ex Gaertn.) Byrnes (Salem *et al.*, 2017).³ *C. viminalis* (the widespread aromatic wood tree found mostly in the wet tropics) is often referred to as *C. viminalis* in tropical Asia, South America, and Australia. Though most often used for decoration, it also serves as a weed eliminator (Brophy *et al.*, 1997).⁴ Plants suffer daily damage because of constant exposure to stimuli. When plant tissue is injured, it's a wound. There are many plant extracts and phytoconstituents available for use in wound healing treatments. Their varied active components, minimal side effects, and ease of access make them promising wound healing treatments. To prevent viruses and germs from assaulting the human body, the skin is critical. Damaged skin may cause physical and mental health issues, and perhaps injuries as well. Wound healing has been an essential topic in the medical field, and this causes problems and higher expenses (Chi *et al.*, 2020).⁵ One of the most physically and mentally distressing injuries is when you lose skin since it is both psychologically and physically difficult to recover from. Post-traumatic and chronic wounds affect about 100 million people throughout the world. People with diabetes and vascular disease, together with metabolic syndrome, are predisposed to wound healing problems (Shefa *et al.*, 2020).⁶ When a puncture develops, skin's protective function is disturbed, and this unequal loss of connective tissue causes injuries to be called puncture wounds (Hamilton, 2008).⁷ Such treatments may leave patients with long-term problems, such as physical trauma and the installation of medical devices like catheters. Damaged cellular integrity may be repaired through the skin's capacity to mend the problem. Most explanations of the healing process include three steps: inflammation, proliferation, and tissue remodeling. The loss of blood from a torn vessel in the wound has resulted in the loss of blood components, such as platelets. Platelets adhere to the clotting factors, which makes clotting possible. In addition to filtering out polymorphonuclear leukocytes and monocytes (macrophages), which are known to provide

chemotactic and growth factors for fibroblasts and endothelial cells, WBCs can function as regulators of fibroblast and endothelial cell production. Healthy epidermal cells go to the wound while fibroblasts and keratinocytes make a robust barrier by assisting in the creation of new epidermis. Surface damage and short epithelialization are caused by epidermal cell migration, which is influenced by the water content of the wound bed (Deodhar and Rana, 1997).⁸ The creation of collagen, which connects back together to make new connective tissue, happens because of the action of fibroblasts. The remodeling will go on for many months since the reepithelialization process has concluded. Depending on how the body moves, the individual may experience it throughout their whole life.

Material and Methods

Plant materials- Specimen of *Callistemon viminalis* were collected from college campus NIET, Greater Noida, Uttar Pradesh. Plant identification was made by experts from the National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi-110012 (AC-13/2020).

Preparation of extract

The leaves were shade dried in a well-ventilated area. To extract fat and chlorophyll, the dried leaves were separated, powdered, weighed (about 270 g), and extracted with petroleum ether. After this, the sample was solvent-extracted using eight different solvents, which included water, ethanol, chloroform, ethyl acetate, and isopropyl alcohol. The yield of each extract was determined as % w/w about the total weight of dried leaves that were used in the extraction process.⁸

After cleaning with water that had been properly sterilized, *Callistemon viminalis* leaves (30 g) were used in the experiment. The dried leaves were placed in the shade to dry. A mixer grinder was used to grind the leaves. To make 32% ethanol, the pulverized leaves were put in a blender. Whatman filter paper was used to run the mixture through a Whatman filter paper (No 1). To ensure sterility, the suspension was filtered (membrane pore size 0.22 mm). The crude dry extract was obtained by freeze-drying the filtrate (4.56 g). The last step was to preserve the crude extract at 200°C. In every experiment, the crude dry extract was used to make fresh stock solutions.⁹

Preliminary phytochemical test

Phytochemical analysis was done for the presence of phytoconstituents using various reagents including Mayer's, Molash's, Fehling's, Borntrager's, Legal's, Foam, Salkowaski, Ferric chloride, Gelatin, Alkaline reagent, Lead acetate, Copper acetate etc (Abdullahi, 2013; Ismail *et al.*, 2016).⁹

Animals:

The rats were 150-200 g Wistar albino rats for the experiment. The animals were maintained in ideal circumstances (22 ± 2 degrees Celsius, 55 ± 5 percent humidity, and a 12-hour light/dark cycle) in CPCSEA Approved Animal House of NIET Pharmacy Institute.

Acute dermal toxicity

The test on the polyherbal extract's acute cutaneous toxicity was performed following OECD guideline no. 402 [OECD guideline]. Rats were selected at random and had both genders. Nine animals were split into three groups, with three individuals in each. The test animals were depilated from the dorsal region of their back 24 hours before the test using a depilatory preparation (Veet). Control animals were those that did not receive PNG; Group II animals got topical treatment with 2000 mg/kg of PHG (5% w/w). For 14 days, researchers kept track of changes in appearance, behavior, and hazardous responses that may occur after applying new products.

In vitro antimicrobial activity

For testing antibacterial properties, 2000 $\mu\text{g}/\text{mL}$ concentrations of the extracts were tested against the Gram-positive bacteria (*B. subtilis*, *B. cereus*, *M. luteus*, *S. lutea*, and *S. aureus*) and the Gram-negative bacteria (*E. coli*, *S. marcescens*, *S. typhi*, *P. vulgaris*, and *P. aeruginosa*).

Disc diffusion method

To find out how well the *Callistemon viminalis* combats microbes, the agar disc diffusion technique was used. On solid medium plates, 0.1 mL of 1×10^8 cells/mL was loaded with a suspension of the tested microorganisms. A twenty-microliter extract of the specimen was put on a filter paper disc with a diameter of five millimeters, and it was placed on an infected plate. After two hours in the fridge, the plates were put into the incubator at 37°C for 24 hours. In millimeters, the widths of the inhibition zones (IZs) were measured. The control substance was produced

by preparing it insolvent. The positive control (with a concentration of 20 $\mu\text{g}/\text{disc}$) was tetracycline, which was tested on the tested microorganisms.

Determination of minimum inhibitory concentrations (MICs)

The experiments were conducted using 96-well microplates (32-34). To make a 250, 500, 1000, 1500, 2000, 3000, 4000, and 5000 $\mu\text{g}/\text{mL}$ dilution of *Callistemon viminalis* in EtOAc, a serial dilution was performed. All wells were filled with sterile Mueller Hinton broth at a concentration of 50 μL . We used the INT (0.2 mg/mL) (p-iodo-nitrotetrazolium violet, Sigma-Aldrich) to test bacteria after overnight incubation of the 50 μL bacterial culture at 37°C at 100% humidity, then applied INT to each well in the micro-plate. Every extract was dissolved in 10% DMSO (which is from Sigma-Aldrich) and diluted in distilled water to a stock solution of 5,000 $\mu\text{g}/\text{MI}^{(10)}$.

Wound healing activity

The wound healing activity of leaves of *Callistemon viminalis* was investigated using both excision and incision wound healing models. Animals were randomly assigned to groups of six ($n=6$) each, and there were three distinct categories:

Group I: control group was given DMSO.

Group II: a standard group that was suspended in the vehicle and was then treated with Vitamin E (100 mg/kg body weight) (0.5 percent sodium carboxymethyl cellulose suspension in distilled water).

Callistemon viminalis treated test group (Group III).

Incision wound model

There was an incision, following the technique that has previously been described. To knock out the rats, we gave them ketamine hydrochloride (100 mg/kg, i.p., body weight) and then separated them by the groupings outlined above. Using a sharp scalpel, the rats had an incision of 4 cm in length on their shaved skin (an incision was performed on both sides of the spinal column). After the incisions had completely healed, they were sewn with black silk surgical thread (000), and each stitch was put 1.5 cm apart (number 11). To deal with the wound, the surgeon left it exposed and administered the doctor's standard medication. This treatment consisted of a few different medications, but the doctor's staple treatment included using the natural antiseptic

leaves and applying the liquid to the wound three times a day. All rats were put under anesthesia on the 10th day when sutures were removed and wound breaking strength (WBS) was measured.⁸

Wound-breaking strength: A wound was drawn on the rats at least 3 mm away from the wound. Wounded, sedated rats were fastened on the operating table, and then a line was drawn on both sides of the wound. The two ends of the line were opposite each other when forceps grasped it.¹¹ The light polypropylene container was hung by a pulley with a string that linked to weight, and one end of the forceps was attached to the container. When wound edges were freed from the constraint of the fixed end by raising the water level, it was found that the water's weight was just enough to move the wound edges away. Three independent measurements of incision wound's breaking strength had their results averaged into one reading. The ability to stretch was also quantified using the formula:

The amount of force required to break a material (e.g., an elastic band) is its tensile strength (mm²)

Excision wound model

In addition to using the standard technique, changes were used when the excision wound was formed. In the experiment, depilatory cream (Veet) was used to remove hair from the animals' backs, and ketamine hydrochloride (100 mg/kg, i.p., body weight) was used to give them general anesthesia. The surgical location was demarcated before the doctor began removing hair from the incision site.¹²

A 500 mm² circular region was made by employing surgical blades, scissors, and forceps with a total thickness excision wound. The rats were taken out into the open aseptic environment completely unclothed. After the surgery, the patient received 500 mg of the simple gel base, a designed extract gel, and standard medication administered daily for healing. This treatment started on the day of the operation and continued until full healing. To determine the size of the wound, the measurement was taken right away using a translucent paper placed over the wound and

traced out; then, the resulting size was estimated by graphing. The wound contraction % was calculated daily using the same procedure. Wound size was measured as 100% when it was first incurred. The number of days needed for the formation of a scab before the presence of any lesion had to be counted to discover the length of epidermal regeneration.

Results

Phytochemical Evaluation of leaves:

The % yield of each extract of each of the *Callistemon viminalis* leaves is given in Table 1. Water extract of *Callistemon viminalis* was obtained with maximum yield (13.54 %) whereas chloroform extract was obtained minimum amount.

Extract	Yield (% w/w dried plant material)
Petroleum ether	5.32
Ethyl acetate	8.61
Chloroform	0.10
Ethanol	9.21
Water	13.54

The leaves reveal the presence of alkaloids, tannins, flavanoida and saponins on phytochemical screening.

In vitro antimicrobial activity

The research focused on ethanolic extract of *C. viminalis* at 2000 µg/mL to explore its in vitro antibacterial activity. *E. coli* exhibited the greatest response in the ethanolic extract with zone of inhibition of (19±1.6) mm, while *S. marcescens* had the smallest response at (10±1.0) mm. The MIC for both bacteria was 250 µg/mL. The ethanolic extract of *C. viminalis* leaves was very effective against the other tested bacteria, all of which had zone of inhibitions between 13 and 18 mm.

It was concluded from the evidence that ethanol had significant antibacterial against the chosen microorganisms. Its antibacterial action was also notable, similar to the standard antibiotic tetracycline with 20 µg/disc.

Bacterial strain	Negative control	Positive control	Ethanolic extract of <i>Callistemon viminalis</i>
Gram-positive			
<i>B. subtilis</i>	R	18	16±1.4 ^C
<i>B. cereus</i>	R	17	15±1.1 ^B

<i>M. luteus</i>	R	19	13±1.2 ^D
<i>S. lutea</i>	R	20	18±1.8 ^A
<i>S. aureus</i>	R	23	14±1.7 ^C
Gram-negative			
<i>E. coli</i>	R	18	19±1.6 ^A
<i>S. marcescens</i>	R	20	16±1.0 ^D
<i>S. typhii</i>	R	20	11±1.7 ^A
<i>P. vulgaris</i>	R	23	13±1.4 ^D
<i>P. aeruginosa</i>	R	22	18±1.6 ^C

For 2000 µg/mL, the diameter of the inhibitory zone, with a disc diameter of 5 mm, is shown as mean ± SD (three repetitions). In superscripts, the extracts are shown to have MICs against the tested strains. MICs <250, 250, 500, 1000, 1500, and >5000 µg/mL have values A, B, C, D, and E, respectively. R: 2000 µg/mL is its breaking point. Tetracycline (20 micrograms per disc). The measurements were done four times, and the kind of precipitate's color was used to categorize it.

Wound healing activity

An enormous spike in healing was seen in *C. viminalis* experimental groups, as opposed to the control group, where the decrease in wound area of each group was noted after 21 days. The region of the injury was measured on the days after the surgery: 1, 4, 7, 10, 13, 16, 19, and 21 in all of the groups. Significant wound closure was seen from the fourth day to the tenth day. *C. viminalis* wounds in the DMSO group had a mean wound area of 200 mm² on the 16th-day post-surgery, whereas the wound area of the *C. viminalis* group treated with DMSO was 30 mm². Leaf extract was shown to be effective in wound healing ($p < 0.05$), whereas in the normal vitamin E group, wounds had started to close, but were not fully closed by the 19th-day post-surgery. It was discovered that the 19th day after the administration of *C. viminalis* was the period that it took for the epithelium to regenerate. The top wound was removed surgically and histological tests were done on it. Histological investigation of rats' wounds treated with *C. viminalis* and Vitamin revealed that scar formation was reduced, while the process of fibroblast proliferation, angiogenesis, keratinization, and epithelialization was improved over the control or the treatment with vehicle.

Post wound day	Wound area (mm ²) (Mean±S.E.)		
	Control	Standard	Leaf extract
1	525±2.1	515±2.5	522±3.8
4	439±2.2	415±14.1	408±2.2
7	317±4.2	270±2.8	247±2.8
10	306±3.9	189±1.6	117±5.2
13	266±2.7	108±2.2	65±1.8
16	200±2.4	73±1.8	30±2.2b
19	35± 2.2b	8±0.2	00± 2.1
21	00±00b	00±00	00±00b

P<0.05 statically significant difference in comparison with control group

Post wound day	Percentage of wound contraction (%)		
	Control	Standard	Leaf extract
4	16.01	18.94	21.53
7	35.2	48.29	52.9
10	41.49	63.08	77.50
13	49.5	78.9	87.30
16	61.75	85.96	93.16
19	93.16	100	98.43
21	100	100	100

P<0.05 statically significant difference in comparison with the control group

Conclusion

The *Callistemon* species of the Myrtaceae family are often found in forestry and horticulture. The hydro-distillation process produced an essential oil that was analyzed using GC/MS. To compare

the extracts, their active components have been thoroughly examined, with special attention paid to the total phenolic and flavonoid content, as well as the number of active chemicals produced in a DPPH test and a growth inhibition assay. Among the essential oil's fourteen identified components, 98.94% of the entire oil was made up of them. The two key elements were pineal (34.3%) and 1,8-cineole (64.53%). (9.69 percent). In addition, the significant inhibition seen compared to the traditional antibiotic was identified for the researched bacterial strains (tetracycline). It was shown that both the crude methanol extract and ethyl acetate fraction were very effective in killing various types of bacteria. *C. viminalis* may serve as a major source of antibacterial chemicals and antioxidants that will be useful in developing novel antimicrobial medicines derived from natural sources. *C. viminalis* possesses antibacterial properties when extracted in different ways. Most *Callistemon* species are known for their antipathogenic actions, which have also been used as an anti-inflammatory, antimicrobial, and antiseptic essential oils. *C. viminalis* has natural antibiotic and antioxidant capabilities which may be used to produce new medical treatments with antimicrobial properties. The ethyl acetate fraction of *C. viminalis* leaves was very potent in anti-bacterial activity against bacterial strains as of the current research. The current research discovered that the essential oil and methanol extracts, as well as the *C. viminalis*, leaves ethyl acetate fraction were all extremely potent in anti-bacterial activity.

Ethical clearance: Taken from NIET committee, Greater Noida, Uttar Pradesh committee NO-02151

Source of funding: The authors thank the management of Noida Institute of Engineering and Technology (Pharmacy Institute) for supporting this work.

Conflict of Interest: There is no conflict of interest amongst the authors for the work.

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A Development of Health Massage Establishments in Phetchabun Province Thailand using Appreciative Inquiry Application

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How to cite this article: Boadsaporn Anusornpanichakul, Nusaraporn Kessomboon. A Development of Health Massage Establishments in Phetchabun Province Thailand using Appreciative Inquiry Application. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):102-107.

Abstract

Thai government sets a policy in order to develop Thailand as Global Medical Hub, so the Health Establishment Act BE 2559 was provided to regulate related business reaching the required standards. The researcher carried out this action study by applying appreciative inquiry to develop health massage establishments in Phetchabun Province, Thailand. The appreciative inquiry is a positive and collaborative approach to change all sizes of organizations that techniques employed by successful entrepreneurs were used to improve unachievable ones. 73 volunteer participants were included then a surveillance was conducted by finding out "superior models" which were passed all of three categories and each passed >60% as well as were passed all important drawbacks and not found any illegal performances. The five superior models employed good techniques with 8 lists: 6 lists in category 1 location and surroundings, 1 list in category 2 safety, and 1 list in category 3 service followed by assessment form of the health service standard for health massage establishment. The remaining 68 intervention cohorts implemented those techniques for 30 days. The results were passed 91.18% (62 of 68 sites) comparing to 69.12% (47 of 68 sites) before implementation. Classified by each category, it was increasingly passed all of three categories. Moreover, there are some fresh techniques were explored and used for improvement. It is assumed that the appreciative inquiry is based on their experiences, skills, and individual exploration of entrepreneurs and massagers to enhance their establishments reaching achievement.

Keywords: Appreciative inquiry; risk management; health massage establishments; health massage establishments; surveillance.

Introduction

According to health tourism potential in Thailand, the government sets a policy aiming to develop Thailand as Global Medical Hub in order to enhance service quality of establishments related to

medical and health.¹ Health service business becomes popular among Thais and foreigners. Market value expansion of health service business was continually expanded to 4.75 billion in 2012, comparing to 3.24 billion in 2011 which the growth rate was 15% per year. Health massage establishment is a kind of

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health service business that causes national income was highly increased.^{2,3} According to the apparent enlargement, the Health Establishment Act BE 2559 was provided to protect consumers for health service. The Act is to regulate three types of business refers to Section 3 including spa, health massage or beauty massage, and others identified in the ministerial regulations. Department of Health Service Support under Ministry of Public Health is a principal department driving the policy to be practical. A provincial and district sector were authorized to approve licenses and audit the establishments.⁴⁻⁶ A person who required to run a business was asked to submit an application attached identified documents. Then a surveillance was conducted followed by assessment form of the health service standard for health massage establishment including three categories: location and surroundings, safety, and service. The assessment form was designed by Health Establishment Division under Ministry of Public Health. Before a five-year license was approved and a receipt of business annual fee was issued, the establishment could be achieved the required criteria.⁵

Nowadays, there are 8,795 health massage establishments in Thailand certified by Ministry of Public Health.⁷ However, there are some obstacles for health establishments such as preparation, service providers, service quality, customer's safety, and hidden affairs that influence consumer protection about health service.^{8,9} Some health massage establishments performed some hidden illegal affairs such as prostitution that directly affected to reliability and image for Thais service users as well as foreigners towards health massage establishments.⁴ Furthermore, some are not standardized including place or surroundings, safety, and service^{4,10} that physical and mental effects were come out towards the users. Also, some entrepreneurs are inexperienced and have insufficient knowledge to handle the business followed by the required standards.¹¹ Some massagers are unskilled, not frequently trained and not improved themselves. According to the stated problems above, improvement studies of health massage establishments using collaborative approach were not found after reviewing literatures. Therefore, appreciative inquiry application was used in the action research in order to deal with the obstacles of health massage establishments that lead to meet the achievement.

Appreciative inquiry is an approach to organizational learning and organizational change and it has been globally used by public and private organizations. The approach emphasizes inquiry into strengths rather than focusing on fixing weaknesses. The people were asked positive affirmatives to explore on success and strengths that already exist then the answers can be used as a model to build future direction for improvement. The appreciative inquiry is comprised of four phrases: discovery, dream, design, and destiny. Discovery phrase: in order to explore good techniques, positive questions were asked to identify what successful techniques and strategies. Dream phase: in order to encourage and stimulate creating energy and motivation, achievable goals were set to apply those techniques and strategies. Design phrase: an activity plan was created to implement the techniques and strategies. Destiny phrase: in order to accomplish, the implementation was performed.

The study which was used application of appreciative inquiry was to develop health massage establishments followed by assessment form of the health service standard for health massage establishment. Firstly, it is to explore new and useful techniques and strategies of accomplished establishments. Then implement these techniques and strategies to improve unachieved establishments.

Materials and Methods

Participants

In the 2020 fiscal year, there were 81 health massage establishments in Phetchabun Province, Thailand. Seventy-three of these are volunteers participated in the study. Firstly, results of a surveillance were sorted out. Next, the five establishments with the highest scores >60% were "superior model" which passed all important drawbacks and not found any illegal performances. The remaining 68 establishments were classified into the "intervention cohort" which implemented the identified techniques and strategies.

Study design

In the discovery phrase, firstly the five entrepreneurs of the superior models were interviewed by the researcher aiming to find out what made them success through positive affirmatives. The three questions were "What are your good stories and good practices in your site?" "What are

your inspirations or what stimulates you to carry out these good things?" and "What made you achieve the inspection?" Later, interviewee answers were considered along with audit results referring to assessment form of the health service standard for health massage establishment. The good practices were identified in order to further implement.

In the dream phrase, 6 representative of the entrepreneurs which were specifically selected from three zones of Phetchabun Province, 2 for each zone, 6 district public health officers and 2 from the provincial level, and 73 volunteer entrepreneurs had a meeting purposing to set goals and to plan involved processes and performances. The questions asked were "How do you want to improve your business?" "What push you to achieve?" and "Who involves the establishment improvement?"

In the design phrase, the 14 engaged participants from the dream phrase had a discussion about how best to apply the identified techniques in the discovery phrase to meet the goals set in the dream phrase. They developed a plan about how to introduce the successful methods to the intervention cohort. The plan were specified subjects, objectives, advantages, performance timing, monitoring, and outcome reports.

In the destiny phrase, those public health officers visited all 68 establishments in the intervention cohort and explained about the activity plan and how to implement. Each establishment was given 30 days to perform. The researcher continuously followed and monitored during the implementation aiming to identify obstacles through interview with the question "How do you deal with any problems?" To verify effectiveness of the intervention, the officers returned to the establishments for examination after 30-day implementation.

Results

Followed by assessment form of the health service standard for health massage establishment

including three categories, the five superior models of health massage entrepreneurs employed good techniques and strategies with 8 lists: list no. 1-6 of category 1 location and surroundings, list no. 7 of category 2 safety, and list no 8 of service.

Category 1: location and surroundings

1. Establishment name board which was made from available hardwood was beautifully handwritten and it was obviously displayed in front area.
2. Interior divisions were affordable cabinets, interweaved bamboo, and sewn rag or unused cloth which were used as partitions to divide service unit.
3. Lockable doorknobs were removed and rag or unused cloth was replaced in order to protect inner locking. (In cases of rented house, the owners did not allow to remove any doors or doorknobs).
4. Trash lid was made from available materials such as cardboards or scraps of wood.
5. Washbasin was made from holed plastic basin and waste pipe was inleted.
6. Changing unit was designed by cornered cabinet with curtain, bended sticks attached with sewn cloth as curtain, two modified hula hoops on the top and at the bottom attached cloth in the middle looking like a cylinder, and interweaved bamboo making like a threshing basket with cloth covered.

Category 2: safety

7. An assistant equipment of service user was a squeezing toy.

Category 3: service

8. Service lists and service charge board were made from wood board or cardboard with beautiful handwriting and clearly shown.

Table 1: Identified good techniques for development of health massage establishments

Techniques	Parlor 1	Parlor 2	Parlor 3	Parlor 4	Parlor 5
1. Establishment name on board with handwriting	√	√	×	×	×
2. Interior partition - affordable Cabinet	×	√	×	×	×
- interweaved bamboo	√	×	×	√	×

Contd... Table 1: Identified good techniques for development of health massage establishments

- sewn rag as a curtain	×	×	√	×	√
3. rag or unused cloth instead of removed doorknob	×	√	√	×	×
4. Trash bin					
- cardboards or scraps of wood as trash lid	×	√	×	×	√
- lid of plastic bin	√	×	×	√	×
5. Washbasin: holed plastic basin with inleted waste pipe	×	×	√	×	√
6. Changing unit					
- cornered cabinet with curtain	×	×	√	×	×
- sticks attached with sewn cloth as curtain	√	×	×	×	×
- two hula hoops with cloth covered	×	√	×	×	×
- interweaved bamboo like a threshing basket with cloth covered	×	×	×	√	√
7. A squeezing toy as assistant equipment	×	√	√	√	×
8. service list and charge on board with hand-writting	√	×	√	√	×

Remarks: √ employed the techniques, × not employed the techniques

Before the 68 entrepreneurs classified as “intervention cohort” employed the good techniques, their establishment surveillance was passed 69.12% (47 of 68 sites). In the other hands, their inspection after implementation was passed 91.18% (62 of 68

sites). Prior to the intervention, classified by each category: it was 76.47% in location and surroundings, 72.06% in safety, and 75.00% in service. After the implementation, it was obviously increased to 95.59%, 91.18%, 94.12% respectively.

Table 2: Surveillance results of health massage establishment of the intervention cohort

Inspection	Before		After	
	Passed	Failed	Passed	Failed
Passed parlors all categories	47(69.12%)	21(30.88%)	62(91.18%)	6(8.82%)
Passed parlors classified by each category				
Category 1 location & surroundings	52(76.47%)	16(23.53%)	65(95.59%)	3(4.41%)
Category 2 Safety	49(72.06%)	19(27.94%)	62(91.18%)	6 (8.82%)
Category 3 Service	51(75.00%)	17(25.00%)	64(94.12%)	4(5.88%)

Remarks: Passed parlors mean they were passed all of three categories and each passed >60% with passed all important drawbacks and not found any illegal performances

Discussion

In this study the researcher applied the appreciative inquiry to improve health massage establishment in Phetchabun Province, Thailand followed by assessment form of the health service standard for health massage establishment, and it became successful. After implementing good

techniques identified by the five superior models, inspection results were obviously increased, so it was assumed that the strategies were practical and productive. The appreciative inquiry approach required collaboration of all participants including entrepreneurs, masseurs, government officials as well working together in order to reach the standards which was consistent with Barrett’s study¹² that

organizations engaging in appreciative inquiry were reported to have increased system-wide collaborative competence. Besides, some fresh techniques and strategies used by successful entrepreneurs were found such as service user report which were unexplored potential and a key component of AI. Also, the participants engaged in a meaningful process that acknowledge their experiences, skill and enthusiasm. They have great enthusiasm to provide positive ways shifting form problems to solutions offering a new practice to improve themselves and to promote the establishment reaching achievement.^{13,14,15} For example, entrepreneurs and masseurs were dressed with unique uniform attached with individual name tags. While further studies are needed to explore appreciative inquiry process in various healthcare contexts that consistent with¹⁶ appreciative inquiry is recommended for sustainable development through exploration, preparation, and intervention, there was an observation about the assessment form of the health service standard for health massage establishment. It was divided into three categories including location and surroundings, safety with many related lists, and service with an involved list. The assessment form seems to focus on physical features, but it was not covered service quality features consistent with Kruthakul 2012 mentioned the service is a vital component creating customers' impression, reliability as well as good image.¹⁰

Acknowledgements

The researcher would like to sincerely thank department of consumer protection and public health pharmacy under Phetchabun Provincial Public Health Office, Faculty of Pharmaceutical Science at Khon Kaen University, and the entrepreneurs of health massage establishments for their participation.

Ethics Approval and Consent to Participate

This current study was approved by Ethical Review Committee for Human Research, Phetchabun Provincial Public Health Office. (reference no.1/20 - 06 -24/02/20)

Human and Animal Rights: Not applicable

Consent For Publication: All the participants were given an information letter explaining the purpose and the nature of the study, confidentiality, voluntary participation at any time with no consequences.

Source of Funding: None

Conflict of Interest: The authors declare no conflict interest, financial or otherwise.

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Hematological Parameters Alteration in Thai Garlic Farmers Exposed to Mixed Pesticides

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How to cite this article: Chepatee Srilesin, Jirapak Ruttanapattanakul, Yutti Amornlertwatana et al. Hematological Parameters Alteration in Thai Garlic Farmers Exposed to Mixed Pesticides. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):108-111.

Abstract

Fungicides, herbicides, and insecticides are commonly agrochemicals used to eliminate pests during garlic cultivation. Occupational exposure to mixed pesticides can cause hematological abnormalities. The aim of this study was to assess the adverse effects of chronic mixed pesticide exposure in the garlic farmers by using the hematological parameters. The blood sample was collected from 137 Thai farmers who chronically exposed to mixed pesticide and 59 control group who were not a history of exposure to mixed pesticide. Complete Blood Count (CBC) analysis was performed to investigate the hematological parameters alteration pattern of Thai farmers. Statistical analysis also performed by using Mann-Whitney U test. Our investigations revealed that the mean values of white blood cell (WBC) count, lymphocyte, basophil, platelet distribution width, mean platelet volume and platelet large cell ratio significantly decreased in the chronic exposed group but did not affect when compared with the normal reference ranges. The results clearly demonstrated that chronic exposure to the mixed pesticides during garlic cultivation alters the hematological parameters. The farmers should be aware and check their health to prevent the adverse effects from pesticide intoxication.

Keywords: Hematological change; Pesticides; Public Health; Toxicology; Agrochemicals.

Introduction

Pesticides are group of synthetic chemicals that have been used for control weeds, insect infestation and diseases. Herbicides, insecticides and fungicides are major type of pesticides and have been highly

imported for agriculture in Thailand⁽¹⁾. Inhalation during spraying, skin absorption during preparation of pesticide solutions and cleaning of agriculture instruments are major causes of occupational exposure. The adverse effects can be enhanced by various factors, including type of pesticides, lack

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of personal protective equipment, duration and frequency of exposure.² The high intensity uses of pesticides contribute to morbidity and mortality in agriculture farms and their families through acute and chronic toxicity.²⁻⁴ Chronic low-level exposure to pesticides may be a cause of serious health problems, including metabolism dysfunction, neurotoxicity, carcinogenesis, reproductive and immunological effects which need to be investigated.^{4,5}

In Northern Thailand, fungicides (particularly mancozeb, azoxystrobin, difenoconazole), herbicides (2,4-D, glyphosate, atrazine and paraquat) and insecticides (chlorpyrifos, carbaryl, cypermethrin, abamectin) have been used during garlic cultivations. It is critical to evaluate health problems, especially metabolism dysfunction and hematological toxicity after mixed pesticides exposure. The aim of this study was to assess the adverse effects of chronic mixed pesticide exposure in the garlic farmers by using the hematological parameters.

Materials and Methods

In this cross-sectional study, One hundred and thirty seven individuals (64 male and 73 female) who were occupationally exposed to mixed pesticides including fungicides, insecticides and herbicides at least 15 years (Chronic exposure group) and fifthly nine individuals (24 male and 35 female) who were not a history of occupational exposure to mixed pesticides or any other industrial chemicals (Control group) were recruited from Lee district, Lamphun province, Thailand with age group ranging from 30 to 70 years. The participants who had hematological diseases, autoimmune, infectious diseases, hypertension, diabetes, thyroid disorders, parkinson, alzheimer, cancer, asthma, chronic obstructive pulmonary disease, alcoholic and heavy smoking were excluded from this study. The Ethics Committee of the Faculty of Medicine, Chiang Mai University, Thailand approved this study (study code: FOR-2562-06349). All participants were informed about the protocol of this study and signed the consent before study.

Complete blood count was determined on the fresh EDTA blood samples by using an automatic analyzer (Sysmex XS-800i hematological analyzer). Hematological parameters including WBC differential, white blood cells (WBC) count, red blood cells (RBC) count, platelet count (PLT), hemoglobin (HGB), hematocrit (HCT) and plateletcrit (PCT) were performed. In addition, mean corpuscular volume

(MCV), mean corpuscular hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), red blood cell distribution width (RDW), platelet distribution width (PDW), mean platelet volume (MPV) and platelet large cell ratio (P-LCR) were also calculated from the hematological data.

Data were analyzed using statistical package for social science (SPSS) version 22. The descriptive analyzes were done through mean \pm standard deviation (S.D). Significant differences between mean values of chronic exposure and control group were statistically analyzed using the Mann Whitney U test. Results were considered significant when p -value is less than 0.05 ($p < 0.05$).

Results and Discussion

A total of 137 farmers and 59 controls were recruited in October 2020. They lived in the same environmental conditions including rural town, similar nutritional habits and lifestyle. The garlic farmers had highly contacted to pesticides and averaged approximately 6 hours per week. The fungicides, especially mancozeb, azoxystrobin, fluopyram and trifloxystrobin had been used at least once a week continuously four months per year. Herbicides (glyphosate, oxyfluorfen and paraquat) had been sprayed for two to three times per year to prepare the planting area. Insecticides had been used once a month. The types of pesticides are classified in Table 1. The types of personal protective equipment reported by farmers included boots, gloves, hoods, long sleeve shirts, and trousers.

Table 2. demonstrates the hematological parametersthat were evaluated in the chronic exposure group and the control group. The values of each parameter in both the control and the exposure group appeared to be normal when compared with the reference range. The WBC count, lymphocyte, basophil, PDW, MPV and P-LCR significantly decreased in the chronic exposed group when compared with the control group ($p = 0.039, 0.010, 0.014, 0.042, 0.016$ and 0.016 , respectively). Arafa⁶, Ismail⁷ and Jazayerri⁸ and their colleagues revealed that WBC count significantly decreased ($p < 0.01$) in the farmers who exposed to pesticides. Similarly, it significant differences decrease in WBC count between farmers and control groups.⁹⁻¹¹ However, mean values of monocytes, HCT and Hb presented lower but mean values of granulocytes, lymphocytes, and platelets presented higher levels in the male

pesticides applicators.¹² The study of Ayiet *al.* showed that MCH and MCHC in the pesticide-exposed group were similarly values compared with the control group.¹³ Some studies found a significant decrease in hematological parameters, especially HGB, HCT, MVC and RBC in the sprayers.^{14,15}

Table 1: The types of pesticides used by garlic farmers.

Insecticides	Herbicides	Fungicides
Organophosphate	Glyphosate	Azoxystrobin
Benzamide	Paraquat	Difenoconazole
Chlorpyrifos	Atrazine	Mancozeb
Phosphonate	Glufosinate	Fluopyram
Sulfotep	Paraquat	Trifloxystrobin
Carbamate	Oxyfluorfen	Iprodione
Benfuracarb		Carbendazim
Carbaryl		Procymidone

Insecticides	Herbicides	Fungicides
Carbosulfan		Fosetyl-aluminum
Methomyl		
Organochlorine		
Endosulfan		
Pyrethroid		
Abamectin		
Cyhalothrin		
Cypermethrin		
Emamectin		

Divergences of hematological response to the pesticides exposure might attributed to types of pesticides, duration and frequency of use. The progression of hematotoxicity is induced by pesticides have been established in animal models for evaluating to human toxicity but its mechanism of action in hematopoiesis still need to understand.¹⁶⁻¹⁹

Table 2: Hematological parameters of blood samples from the study groups.

Parameters	Reference range	Control (n=59)	Chronic exposure (n=137)	p-value
		Mean ± SD	Mean ± SD	
WBC (x 10 ³ cells/μL)	4 - 10	7.85 ± 1.93	7.28 ± 2.04	0.039*
RBC (x 10 ⁶ cells/μL)	4.10-5.60	5.07 ± 0.65	5.03 ± 0.66	0.595
HGB (g/dL)	12.5-17.0	13.51 ± 1.35	13.48 ± 1.52	0.812
HCT (%)	36.0-50.0	41.50 ± 3.70	41.28 ± 4.04	0.961
MCV (fL)	83-97	82.85 ± 10.49	83.02 ± 10.35	0.880
MCH (pg)	27 -33	27.00 ± 3.50	27.10 ± 3.66	0.650
MCHC (g/dL)	31 -35	32.54± 1.08	32.61 ± 1.15	0.564
PLT (x 10 ³ cells/μL)	150 - 450	266.32 ± 65.01	273.19 ± 62.56	0.475
RDW(%)	11.7-15.0	14.32 ± 2.03	14.22 ± 2.28	0.555
PCT(%)	0.15-0.62	0.29 ± 0.07	0.29 ± 0.06	0.800
MPV (fL)	8.6-15.5	10.87 ± 0.76	10.60 ± 0.73	0.016*
PDW (fL)	8.3-25.0	12.34 ± 1.97	11.70 ± 1.52	0.042*
P-LCR (%)	11.9-66.9	30.73 ± 6.02	28.51 ± 5.76	0.016*
Neu (cells/μL)	1800-7800	4489 ± 1398	4291 ± 1644	0.171
Lym (cells/μL)	700-4500	2512 ± 646	2258 ± 735	0.010*
Mono (cells/μL)	100-1000	482 ± 196	431 ± 145	0.113
Eos (cells/μL)	0-400	325 ± 297	267 ± 264	0.271
Baso (cells/μL)	0-200	43 ± 25	33 ± 15	0.014*
%Neu (%)	40 - 70	56.82 ± 7.18	57.98 ± 9.14	0.506
%Lym (%)	20 - 50	32.48 ± 6.08	31.82 ± 8.41	0.600
%Mono (%)	2- 10	6.09 ± 1.55	6.03 ± 1.57	0.607
%Eos (%)	1-6	4.07 ± 3.58	3.69 ± 3.32	0.693
%Baso (%)	0-1	0.55 ± 0.31	0.48 ± 0.23	0.344

* Statistically significant difference from the control group at *p* < 0.05.

Conclusion

This study discovered that chronic exposure to mixed pesticides during garlic cultivation affected to complete blood count of farmers. Hematological parameters were not differ comparing with the normal range. However, WBC, MPV, PDW, P-LCR, lymphocytes and basophils were lower in the chronic exposure group. The hematological parameters should be annually evaluated for prevalence of the side effect of chronic mixed pesticides application.

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

Acknowledgements

This work was supported by the Faculty of Medicine, Chiang Mai University (Grant number 062-2564). Additionally, Cheepatee Srilesin. was supported by the Faculty of Medicine Graduate Student Scholarship, Chiang Mai University. CheepateeSrilesin was supported in part by grants from the Teaching Assistant and Research Assistant (TA/RA) scholarships, Graduate School, Chiang Mai University, Chiang Mai, Thailand. Additionally, we are grateful for Mae Tuen Health Promoting Hospital and Mr. NachaNoijinda for their kind cooperation.

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Measuring Success of Meditation, Curcumin Supplementation and Sunlight Exposure among Office Workers with Mild Depression

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How to cite this article: Chirra Taworntawat, Karl J. Neeser, Ratana Somrongthong et al. Measuring Success of Meditation, Curcumin Supplementation and Sunlight Exposure among Office Workers with Mild Depression. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):112-121.

Abstract

Background: Globally, major depression is the primary cause of disability, where a large part of cases is reported among the working-class.

Methods: We conducted comparative cross-sectional study with 8-week observation includes mindfulness meditation, curcumin supplementation and sunlight exposure (MCS program) among mildly depressed office workers of both sexes - 34 in observed group (OG) and 34 in control group (CT). At baseline, day 30, and day 60, participants were compared in terms of (a) vitamin D, (b) brain-derived neurotrophic factor (BDNF), (c) interleukin-6 (IL-6), and (d) depression scores using PHQ-9. ELISA were performed for all serum samples.

Results: The rates of increase of vitamin D in both day 0, 30 and day 30, 60 intervals were significantly higher in OG ($p < 0.05$). BDNF levels varied extensively between the groups. IL-6 means were significantly lower in OG at day 30 than in CG ($p < 0.05$). Depression scores rate of change demonstrated appreciable fall in day 0, 30 interval and stayed down to day 60 in OG. Significantly, lower depression scores were observed for OG at both day 30 and day 60 ($p < 0.05$).

Conclusion: MCS program resulted in significant decreases in depression scores and some of its related biomarkers, thus can be a sustainable and cost-effective approach to alleviating psychological depression among the working-class.

Keywords: 25-hydroxyvitamin D; brain-derived neurotrophic factor; interleukin-6; major depression; working-class; mindfulness meditation; curcumin; sunlight exposure.

Introduction

By 2030, major depression will become the number one leading cause of disability globally, according to WHO.¹ Today, it is the second leading

cause of disability and of global burden of disease, troubling more than 150 million individuals.^{2,3} Without treatment, depression has the potential to take a chronic course, be recurring, and be linked with increasing disability over time.^{4,5} This critical public-

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health problem often occurs alongside other long-lasting diseases and can exacerbate health outcomes especially in people who are in the workforce.^{6,7} As working is a substantial part of a person's life, the environment of the workplace is where psychological burden can accumulate. Among all medical illnesses, depression may have the greatest negative impact on time management and productivity and is one of the major causes of absenteeism and presenteeism.^{8,9} Both medicinal treatments and psychotherapy treatments have been found to be the most economical choices for organizations.¹⁰ Ever-increasing numbers of organizations are creatively addressing the issue of depression in their places of business.

Department of Mental Health found that the prevalence of depression among all Thais was 2.4%, signifying that 1.5 million individuals had depression. 58.5% of the depressed individuals were at risk of committing suicide.¹¹ Between all regions of Thailand, Bangkok scored the greatest prevalence of individuals with depressive occurrence at 4.1% where 28.8% was found in female employees, notably greater than the national rate.^{11,12}

Workplaces have been recommended as a supreme location for psychotherapy interventions in several regards. Firstly, 60% of the global population is employed in some industries, and the majority of those people's waking hours are spent at the workplace – ideal for influencing a large number of individuals in a reliable and controllable approach.¹⁰ Secondly, an unfavourable psychosocial work atmosphere is well-known as a risk factor for mental disorders, suggesting that work-based programs can be multi-modal and at the same time reducing risk factors while improving employees' coping skills and resilience.¹⁰ Thirdly, the expenditure of interventions could be shared by both the private and health sectors.¹⁰ A meta-analytic review shows that prevention or treatment programs focused on individuals with subthreshold depression have real potential.¹³

Major depression, a multi-factorial disorder, has been intensively linked to the increase of a pro-inflammatory cytokine, interleukin-6 (IL-6). During depression episodes, increased serum IL-6 levels are observed, indicating a worsening of a person's depression.¹⁴ The cytokine is involved in the management of numerous physiological pathways, predominantly in the immune system, metabolism, sleep regulation, and mood

disorders.¹⁵⁻¹⁷ As depression symptoms intensify, inverse correlation has been shown with brain-derived neurotrophic factor (BDNF) as well as vitamin D levels. Moreover, IL-6 is an independent predictor of BDNF.¹⁴

25-hydroxy vitamin D (25-(OH)D) elevation is naturally achieved by sunlight exposure (SE), it is theorized to repress cellular inflammation.¹⁸ 25-(OH)D is inversely and independently connected with serum IL-6.^{18,19} On its own, 25-(OH)D may have the action of suppressing the production of IL-6.²⁰ In addition, natural light exposure has been shown to improve depressive symptoms which could be the result of the increase in 25-(OH)D production.¹⁸

Only a handful of depression intervention programs, however, have been performed on the working-class. Thus, our study consisted of 3 components: mindfulness meditation + curcumin supplementation + sunlight exposure (MCS) given to mildly depressed office workers conducted with a control group (CG) and an observed group (OG). In past studies, the 3 attributes have all been shown to alleviate scores, symptoms, and/or biomarkers attached to depression. The primary outcome of this study is the difference of change of value in depression scores over time represented by the difference of slopes between groups. We also evaluated the slope's changes in 25-(OH)D, BDNF, and IL-6 level as secondary outcomes.

Materials and Methods

Research Design

Our study was an 8-week comparative cross-sectional study with a control group (CG) carried out amongst two real-estate companies nine kilometers apart from each other in the middle of Bangkok. Employees of both companies had similar job tasks and daily routines. For logistical and contamination prevention purposes, one company comprised OG and the other CG.

At the preliminary phase, all consenting employees from the two companies were screened using standard tests, Thai version of Patient Health Questionnaire 2 and 9 (PHQ-2 and PHQ-9). Upon completion, we observed a total of 61 employees (54.46%) in company A and 42 employees (48.28%) in company B with mild depression (PHQ-9 score of 7-12).²³ PHQ-2 and PHQ-9 are professionally translated into Thai and standard in depression

evaluation. In the screening process, voluntary participation was achieved via posters posted on bulletin boards and a meeting where information sheets, purpose of study, methodology, and consent forms were clarified for each company at a time. Consented participants were screened once more for exclusion and inclusion criteria by an expert.

This experiment included office workers between 25-59 years of age working full-time in 2 real-estate companies with mild depression. Individuals with cancer, cardiovascular disease, hypertension, liver disease, history of psychiatric illnesses, and taking psychiatric medications were excluded. Per protocol, we also excluded people who had been taking vitamin D and/or curcumin supplementation (CS) prior the study for more than 2 weeks, who were suffering from alcohol addiction, who were pregnant or breast feeding, who just lost a loved one in the past 6 months. The suitable sample size of participants was confirmed by calculation result of G*Power 3 software version 3.1.9.2 where the input effect size of Cohen's *d* was 1.70.²²

Ethical approval was obtained from the Chulalongkorn University Ethics Committee (ID number: 176.2/62). The methodology was explained. Signed informed consent were voluntarily obtained from every participant. Resignation from the program was permissible at any time.

Intervention

Participants in OG underwent an 8-week program involving curcumin supplementation (CS) of 2 capsules after breakfast and dinner (1000 mg of curcumin), sunlight exposure (SE) in sky garden at 4:30 pm for 10-15 minutes 4 times a week, and 20 minutes of sitting mindfulness meditation (MM) in a meeting hall on same days as the sunbathing. Men and women were asked to wear short sleeves tops and shorts for being in the sun. During MM sessions, participants were asked to sit comfortably on floor with or without a provided cushion or on chair and to close eyes and focus on their breathing. Whenever other thoughts enter their minds, participants were asked to lightly and non-judgmentally refocus their minds on the breathing.²¹ A 5-minute session of question and answer was available for participants to ask about uncertain things that should have happened during each session to the licensed meditation instructor.

For every session, sunlight intensities were measured by a standard calibrated digital lux meter

version LX-1010B.²² There was a total of 24 meeting sessions, each participant was allowed to miss 4 sessions. The fifth time a participant missed a session, he/she had to compensate by doing SE and MM in their own time. CG did not participate in the treatment and lived normal daily lives. Retention rate was 96%.

Data Collection Procedures

For both the OG and CG groups, blood was drawn, general questionnaires were administered, and PHQ-9 were self-administered on day 0, day 30 and day 60 of the MCS program at the two companies meeting halls.

Blood tests

Day 0, day 30 and day 60 of follow-up serum blood tests were measured for BDNF ELISA, IL-6 ELISA, and 25-OH (D). Two licensed medical technicians drew blood from participants at both locations before the intervention began.

Blood (6 ml) was drawn and transferred into lithium heparin tubes, centrifuged at 3000 rpm for 10 min to obtain serum, and stored at -20 degree Celsius. 1 ml each of which was used for BDNF, IL-6, and 25-OH (D) immune fluorescent analysis. The medical technician collected blood samples in an air-tight, temperature-controlled container and transported to Chiang Mai University Biochemistry laboratory for analysis. A sandwich enzyme-linked immunosorbent assay (sandwich ELISA) was applied for quantitation of BDNF (Catalogue No. SEA011Hu) and IL-6 (Catalogue No. SEA079Hu). Competitive inhibition enzyme-linked immunosorbent assay (cELISA) was applied for 25-OH (D) (Catalogue No. CEA920Ge) according to the manufacturer's guidelines (Cloud-Clone Corp., Uscn Life Science Inc., Wuhan, P.R. China).²⁶

Depression evaluation

Thai PHQ-2 and PHQ-9 are standard self-evaluated questionnaires which were used to screen and follow-up on people with high risk of depressive symptoms, translated into Thai version by Lotrakul²³.

Curcumin Acquisition

The curcumin supplementation given to OG were Curcuma Longa L. extract obtained from The Government Pharmaceutical Organization - the same one of a study done by Chuengsamarn et al in 2012.²⁴ Each capsule contained 250 mg of curcuminoids with

product number of 110211750411. The supplement received Good Manufacturing Product and Certification of Analysis number of 040000012169. It passed appearance test and identification test. The curcumin capsules consisted of 103.40% curcuminoids concentration, water content of 1.08%, and disintegration time of 8.0 minutes (Thai FDA number of 1A 1/60(H)).

Statistical analysis

A descriptive statistic was made to assess the similarity of participants characteristic at the baseline. Analysis of several factors were done to reveal confounding effects, including sociodemographic factors using bivariate analysis. Differences in vitamin D, BDNF, IL-6 and depression scores between study times for each group were conducted by using fixed effects regression methods in SAS version 9.2 (SAS

Institute, Cary, NC). Comparison of demographic data between OG and CG were analyzed by Mann-Whitney U-test in SPSS version 17. P-values of < 0.05 were deemed significant.

Results

Sociodemographic Characteristics

There was 50% male and 50% female in CG, and 77% female and 23% male in OG. Weight mean of CG was 65.20 ± 10.98 SD slightly higher than OG (61.03 ± 9.22 SD), but without statistical significance ($p > 0.05$). The average height was higher in CG (163.40 ± 9.17 SD) than in OG (158.97 ± 6.82 SD) ($p < 0.05$). There were no significant differences of sleep quality (De0_SQ), physical activity (De0_PAL), income satisfaction (De0_IS), alcohol drinking (De0_AD), and sunlight exposure (De0_SEL).

Table 1: Sociodemographic characteristics

Variable	Abbreviation	Treated group	Control group	p-value
Sex				
Male	—	N=23 (77%)	N=15 (50%)	
Female	—	N=7 (23%)	N=15 (50%)	
Weight	—	61.03 ± 9.22	65.20 ± 10.98	0.12
Height	—	158.97 ± 6.82	163.40 ± 9.17	0.03 *
Day0: Sleep quality	De0_SQ	30.13 ± 8.22	26.73 ± 5.98	0.07
Day0: Physical activity	De0_PAL			0.83
Score 1 (No exercise)		40	26.7	
Score 2 (Exercise 1-4 times/week)		46.7	70	
Score 3 (Exercise 5-7 times/week)		13.3	3.3	
Day0: Income Satisfaction	De0_IS			0.052
Score 1 (Not satisfied)		3.3	20	
Score 2 (Satisfied)		96.7	80	
Day0: Alcohol drinking	De0_AD			0.19
Score 1 (Do not drink)		30	46.7	
Score 2 (Drink)		70	53.3	
Day0: Sunlight exposure level	De0_SEL			0.84
Score 1 (No exposure)		3.3	0	
Score 2 (5 mins or less per day)		36.7	40	
Score 3 (6-30 mins per day)		53.3	53.3	
Score 4 (31 mins or more per day)		3.3	6.7	

Vitamin D

Data shows no difference in vitamin D at baseline (day 0). From SAS output, the slope (rate of increase) from day 0, day 30 in OG is higher (by 0.1633) than in

CG. Specifically, in [day 0, day 30] interval, the slope is $0.2297 + 0.1633 = 0.3930$ for OG, and 0.2297 for CG.

Similar difference in slopes hold true. [day 30, day 60] slope of OG also exceeds CG [day 30, day

60] slope of CG by the same amount (0.16). The slope for OG is 0.90 and for CG is 0.74 in [day 30, day 60]. We conclude that the rate of increase in vitamin D is consistently higher for OG versus CG over the range of study (Figure 1).

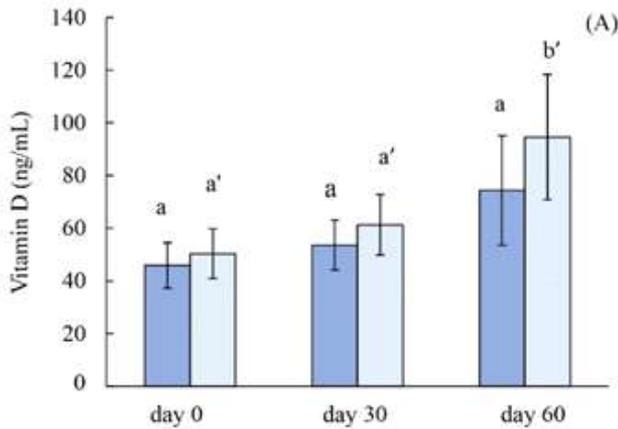


Figure 1: Means (\pm SD) vitamin D of CG (dark blue) and of OG (pale blue) at each study time. Significant values are indicated with difference in letters ($p < 0.05$). Asterisks indicate significant difference between CG and OG ($p < 0.05$).

BDNF

Time profiles are different between OG and CG. First, the means of BDNF differs at baseline: 3876 for CG and $3876 + 1395 = 5271$ for OG. Second, the slopes from [day 0, day 30] differ: 86.59 for CG and $86.59 - 157.18 = -70.59$ for OG. Finally, the slopes from [day 30, day 60] differ as well: $86.59 - 253.28 = -166.69$ for CG and $86.59 - 253.28 - 157.18 + 282.64 = -41.23$ for OG. Therefore, the profiles for the BDNF data vary extensively between the two groups (Figure 2).

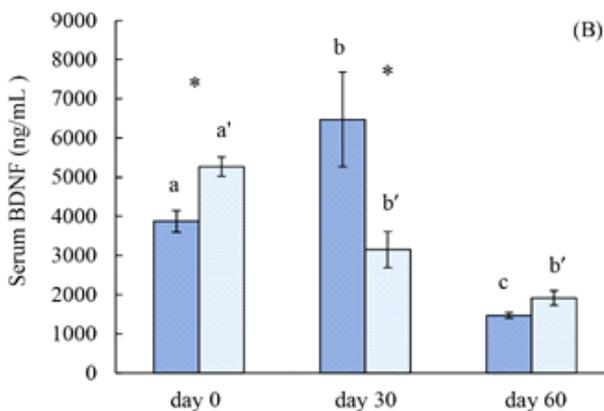


Figure 2: Means (\pm SD) BDNF of CG (dark blue) and of OG (pale blue).

IL-6

IL-6 data shows variability, especially for CG at day 30; suggesting that IL-6 values vary widely in CG (Figure 3). Separate analysis shows very marginal evidence of a difference in means at day 30, but because of the two outliers, this means test is not considered here due to violation of assumptions. The result of this noise is that there is no significant difference in the CG and OG slopes at [day 0, day 30] and no difference in the CG and OG slopes at [day 30, day 60] ($p = 0.1126$ and $p = 0.1247$, respectively) with no difference in means at baseline, as well. The output determines that for both groups, the slope in [day 0, day 30] is 0.2015 and the slope [day 30, day 60] is $0.2015 - 0.3953 = -0.1938$. However, there is a significant difference at day 30 means between the groups.

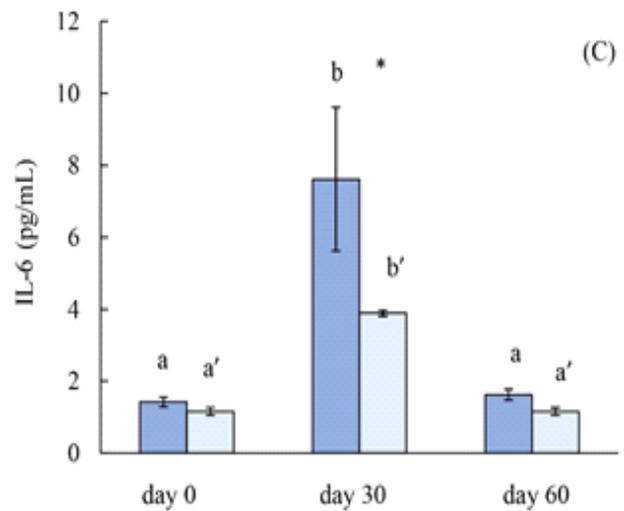


Figure 3: Means (\pm SD) IL-6 of CG (dark blue) and of OG (pale blue).

Depression Scores

Depression scores do not differ at baseline. For CG, the average line segments from [day 0, day 30] and [day 30, day 60] are flat with non-significant slopes ($p = 0.8714$ and $p = 0.5444$, respectively). For OG, the slope of [day 0, day 30] is -0.1151 and of [day 30, day 60] is $-0.1151 + 0.09263 = -0.0225$, and these changes differ significantly from the (flat) control line segments. Depression scores significantly drop in OG at day 30 and stay significantly below CG until day 60 (Figure 4).

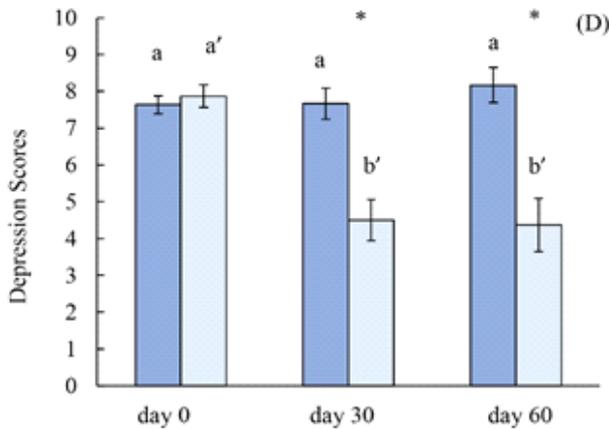


Figure 4: Average (±SD) depression scores of CG (dark blue) and of OG (pale blue).

Discussion

Our results are divided into four outcomes:

Vitamin D

The cause of steady increase of 25-(OH)D in both groups could be that the intervention period was toward the end of the year with more days-off than previous months. Participants usually stayed and worked indoors, but with longer holidays they had more outdoors, thus receiving more SE. Being

consistent with literature, the more sunlight skin exposure one receives the more chance for him/her to produce more vitamin D.²⁵ However, the rate of increase between OG and CG from [day 30, day 60] had a significant difference. The results indicate additional 3 times a week in the sun resulted in greater 25-(OH)D production. Consequently, more 25-(OH)D circulating in their blood could have facilitated with the decrease in depression scores that participants received.

Several hospitals also introduced programs with SE on psychiatric patients which were successful as they lessened their depressive symptoms.²⁶ Studies show that 25-(OH)D levels change seasonally—higher in summers than in winters. SE can stimulate serotonin production helping to brighten mood, reducing anxiety while lacking serotonin can cause stress.²⁷ Less exposure to sun during thick cloud is found to reduce about 50% of ultraviolet (UV). Toxic haze can also reduce UV levels by about 60%.²⁸ Moreover, UVB cannot go through glass; therefore, shaded SE might not provide enough vitamin D.²⁹ Skin tones with more melanin has been shown to absorb more UVB and reduce vitamin D synthesis.³⁰ Participants in MCS were all Thais which helped in terms of having less variations in skin tone.

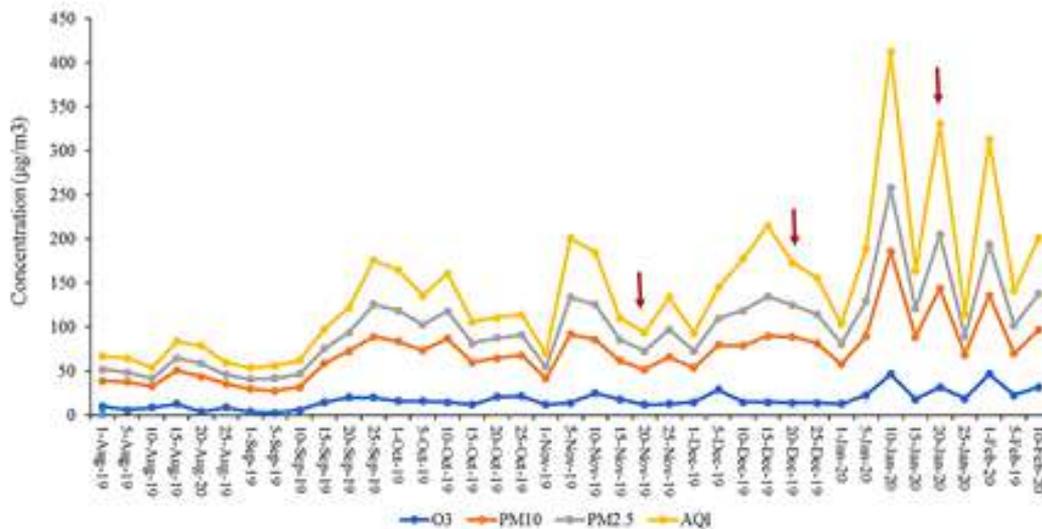


Figure 5: Air Quality Index data, www.pcd.go.th 2019. Red arrows are the three times of blood sample collection.

IL-6

IL-6 values were higher at day 30 compared to day 60 and day 0 in both OG and CG. The perplexing outcome could be the result of air quality index (AQI) which disturbed all participants equally.

Thai government reported in 2019 that there was a haze event towards the end of the year which raised the particulate matters (PM) in Bangkok air (Figure 5). PM10, PM2.5, and AQI have been extensively reported to be linked with increased expression of many pro-inflammatory interleukin

genes, including IL-6. PM 10 was reported to have an IL-6 increase effect on human dermal fibroblasts, peripheral blood mononuclear cells in vitro, and the pulmonary epithelial barrier.³³⁻³⁵ PM2.5 was also reported to facilitate an increase of IL-6 in human osteoarthritis synovial fibroblasts through ASK1 activation.³⁶ During 2013 haze events in China, biomarkers of pro-inflammatory oxidative potential, IL-6, IFN- γ and TNF- α levels were also found to be altered in a dose-dependent manner.³⁷

Nevertheless, day 30 IL-6 of OG was noticeably higher than day 30 IL-6 of CG. The finding could have been the result of the program taking effect on withstanding the increase of inflammatory cytokines including IL-6. Curcumin has been reported extensively that it has IL-6 lowering effects with both dose and duration of treatment.³⁸ The high IL-6 levels found in major depressive disorder (MDD) patients in clinical studies are more obvious in treatment-resistant patients. Thus, to continue with clinical recovery for depression, IL-6 activity suppression is necessary.³⁹ Clinical studies also showed IL-6 antibodies' potential anti-depressive effect.⁴⁰⁻⁴² When the body is inflamed or stressed, it adjusts to produce more inflammatory cytokines, resulting in destruction of molecules in the nervous system causing depression.⁴² Studies revealed that exercise, natural products, light therapy, electroconvulsive therapy, and psychological interventions may normalize IL-6 levels.

BDNF

In both OG and CG, the level of the BDNF steadily decreased from day 0 to day 60. The undesirable finding, could well be the impact of the continual increase in AQI, PM2.5, and PM10 in Bangkok atmosphere from beginning to the end of the program (Figure 5). Not only there were not any increase but also a decrease in BDNF, indicating that air pollution could have been the uncontrollable factor that impacted all members in both groups biologically. In 2014, Bos et al. showed that BDNF of cyclists did not increase as a result of increased physical activity in the city near a busy road where PM10 and PM2.5 were found high, concluding that traffic-related air pollution exposure during exercise may well be the major factor hindering positive influences of exercise on cognition via BDNF inhibition⁴³. All participants either lived in the middle of the city or had to commute to their companies using mass transit exposing themselves

to the increase in PM10 and PM2.5. The surge in the pollution could have been the answer for the decline of BDNF in both groups from day 0 to day 60. Still, day 30 BDNF of CG spiked up which may be the result of unmanageable factors like physical activities which have been reported to increase serum BDNF levels through the action of the ketone body β -hydroxybutyrate.⁴⁴⁻⁴⁶

Depression Scores

Day 30 of OG had a significant decrease in their depression scores while CG had none which could be due to the success use of meditation which helped reduce ruminative thinking and increase simultaneously attentional control as demonstrated by past studies.⁴⁷ MM might cause neuroplastic changes in the structure and function of brain areas responsible in attention management, emotion and self-awareness.⁴⁷ Generally, the content of the mind includes dysfunctional attitudes and negative self-referent ideas.⁴⁸ When depression prone individuals engage in rumination, they ponder in the 'recycles' process of the content of the negative thought, amplifying the opportunities of relapse and the intensity of the depressive episode.⁴⁸ Meditation could have worked by increasing present moment awareness and by welcoming emotions nonjudgmentally.⁴⁸⁻⁵⁰ OG depression score stayed down to the end, while CG score did not change. Corresponding with trials, where mindfulness therapies lessened depressive signs and prevented relapses.^{47,51} Majority of participants verbally expressed lasting improvements. The study results are consistent to past findings where large-scale mindfulness approaches by organizations have been found to be even more effective than small-scale approaches.^{51,52}

Leaders could use mindfulness interventions to improve well-beings of their workforce, where they may have more potential to improve health than individual lifestyle modifications.⁵⁴ Future research should extend to a larger population of workers in a variety of occupational settings, delve deeper into reasons for drop out, and explore if results correlate to healthcare utilization costs or health status which can add value to both the individual and the organization and may be an especially practical reason for organizational sponsorship of the intervention itself.

Limitations and Strengths

The small sample size, the assignment of companies to OG or CG, and the uncontrollable

change in air pollution particulate matters were limitations. Potential strengths of this study include measuring biological confirmation for 3 time points, well compliance of participants, and high retention rate (96%).

Conclusions

This study provides evidence-based program for the increase in vitamin D and the reduction of depression scores and its related biomarkers. MCS could be developed and implemented in clinical practice. It is recommended that future studies include comparisons between active placebo and CG as well as consider time of year especially in cities prone to air pollution when inflammatory markers are involved. When applied together, MM, CS and SE, MCS program could conceivably be an effective tool to help alleviate depressive symptoms in the most venerable and most economically structural group of people, the working class.

Funding: This research was self-funded and received no external funding.

Institutional Review Board Statement: Ethical approval was obtained from the Chulalongkorn University Ethics Committee, Bangkok (ID number: 176.2/62). The study was conducted according to the guidelines of the committee.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the participants to publish this paper. The nature and methodology of the intervention were explained clearly. Resignation from the program was permissible at any time.

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

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Role of Antioxidant Supplements in Idiopathic Male Infertility in Erbil City

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How to cite this article: Chro Yaseen Qader, Tarq Aziz Toma, Sizar Sherzad Rasool et al. Role of Antioxidant Supplements in Idiopathic Male Infertility in Erbil City. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):122-129.

Abstract

Background: Fertil Aid for Men is a non-prescription dietary supplement, it's a mixture of multiple antioxidants, vitamins, a blend of herbal ingredients, and amino acid, as L-Carnitine. This research aims to appraise the effectiveness of a mixture of vitamins and antioxidants on seminal fluid parameters in males with idiopathic infertility.

Method: Thirty-three men with idiopathic infertility were involved in this prospective clinical study. The baseline semen specimens were taken from the subjects following three to seven days' abstinence. Semen parameters including (volume, pH, concentration, and motility) were measured. Abnormal sperm sample subjects were given a FertilAid capsule three times daily for 90 days then final semen analysis was estimated.

The outcomes of this study demonstrated consumption of fertilAid has significantly improved sperm count/ml ($p < 0.05$), also significant amelioration in the sperm motility including progressive and non-progressive motility ($p < 0.05$). The rate of total sperm motility was significantly improved ($p < 0.05$). The concentration of immotile sperm has considerably reduced ($p < 0.05$).

Conclusion: The administration of a combination of L-Carnitine, Zinc, Maca Root, Asian Ginseng, Vitamin C, E, Beta Carotene, and Selenium PLUS CoQ10 in idiopathic male infertility has notably improved sperm parameters.

Keywords: Antioxidants; FertilAid; Seminal fluid analysis; Sperm motility; Progressive motility.

Introduction

The worldwide incidence of infertility is about 15% (1) and is described as the failure of a couple to conceive after one year of regular, unprotected sex.^{2,3}

Semen interpretation determines and describes the following deteriorations in males:

oligozoospermia (reduced sperm concentration), asthenospermia (decreased sperm motility), teratozoospermia (sperms with atypical morphology), and the combination of all of them OAT (oligoasthenoteratozoospermia).⁴ Almost 30% of OAT cases are recognized as Idiopathic Oligoasthenoteratozoospermia (IOAT).⁵

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Seminal vesicles and prostate glands secrete plasma consisting of some elements that provide care for spermatozoa during ejaculation and enhance sperm capacitation.⁶

Its constituents are proteins including many enzymes, prostaglandins, sodium (Na⁺), potassium (K⁺), magnesium (Mg²⁺), calcium (Ca²⁺), inorganic phosphorus (P), and chloride (Cl) which are macro element and microelements such as copper (Cu), iron (Fe) and zinc (Zn).⁷ Some articles established that utilization of these elements in natural foods can boost germinal cell reproduction.⁸

Many randomized investigations have explored the consequence of antioxidant supplementation for the management of male infertility,^{9,10} several of them establish a reasonable effect on seminal fluid quantity and quality including seminal fluid volume, sperm concentration, total sperm motility, progressive motility, and obvious reduction in the percentage of immotile sperm.¹¹ Among all accessible antioxidants, the most remarkably prescribed compounds include vitamins E and C, N-acetyl cysteine (NAC), carnitine, zinc (Zn), and selenium (Se).¹²

The healthy epididymis contains Carnitines in both free and acetylated forms. L-carnitine (LC) plays a main role in cellular energetic metabolism, acting as a shuttle of the activated long-chain fatty acids (acyl-CoA) into the mitochondria.¹³

Among all available antioxidants, the most frequently prescribed compounds include carnitine, vitamins C and E, N-acetyl cysteine (NAC), (Se), zinc, and selenium.¹²

FertilAid for Men is a non-prescription dietary complement that mixes multiple vitamins, amino acids, antioxidants, L-Carnitine, and a mixture of herbal constituents.¹⁴

FertileAid available under the brand name fertilAid manufactured in the United State. FertilAid is classified as a dietary supplement and doesn't need FDA approval.

The ambition of the research is to appraise the effect of a mixture of vitamins and antioxidants on seminal fluid parameters in males with idiopathic infertility of unknown cause.

Study design

This study was conducted in Erbil Iraq at the YadIVF Center, this center was established in 2014 and

is one of the referral centers for infertility treatment. For this retrospective clinical study, data collection initiated from March 1st, 2018 to December 1st, 20.

The study involved thirty-three subjects their age ranged from 21 to 44 years (33.2±5.9). A signed consent form, medical record reviewed, and patients' medical history obtained from the patient.

The baseline semen sample was taken from the patients following 3 to 7 days abstinence, it put inside an incubator at 37°C for 30 min after liquefaction, semen parameters include (volume, pH, concentration, and motility) were measured. Repeated seminal fluid analysis has been performed 3 to 7 days following the first sample.

Abnormal sperm sample subjects were given a FertilAid capsule three times daily for 90 days then final semen analysis was taken following 3 to 7 days' abstinence.

The subjects were selected with abnormal sperm parameters, as delineated by the W.H.O. guideline is defined as one of the following: abnormal sperm count < 15000000/ml, a low percentage of motility, progressive motility < 32%, low percentage of normal morphology < 4%.¹⁵ Composition of fertilAid formulation capsule see Table 1.

Table 1: Content and dosage of antioxidants

Tablet content (average)	Amount (dose)
Vitamin B6 (as pyridoxal 5-phosphate)	2 mg
Folate	500 mg
Vitamin B12 (methylcobalamin)	25 mcg
Vitamin A (as beta carotene)	5000 IU
Vitamin C (as ascorbic acid)	250 mg
Vitamin D (as cholecalciferol)	400 IU
Vitamin E (as d-alpha tocopheryl succinate)	150 mg
Vitamin K (as phytonadione)	80 mg
Thiamin	1.5 mg
Niacin	20 mg
Riboflavin	1.7 mg
Pantothenic Acid (d-calcium pantothenate)	10 mg
Iodin	150 mg
Magnesium (magnesium oxide)	120 mg
Manganese (manganese sulfate)	2 mg

Contd... Table 1: Content and dosage of antioxidants	
Copper (copper gluconate)	2 mg
Selenium (selenomethionine)	100 mg
Zinc (zinc gluconate)	30 mg
Chromium (chromium picolinate)	120 mcg
Proprietary mixture Asian Ginseng extract (root), Grape Seed Extract, CoQ10, L-carnitine (as L-carnitine L-tartrate).Maca (root).	890 mg

Inclusion criteria

1. Age of subjects between 21 to 40 years.
2. Unknown cause of infertility (idiopathic infertility).

Exclusion criteria:

1. Ingestion of antioxidants vitamin formulation within 30 days before enrollment.
2. Azoospermia.
3. Varicocele.
4. Urogenital infection.
5. Diabetes mellitus.

Data analysis

The following parameters were analyzed:

1. Volume of ejaculate.
2. Total ejaculate sperm count.
3. Sperm count per milliliter.
4. Percentage of progressive forward motile sperm.
5. Percentage of non-progressive motility.
6. Percentage of non-motile sperm.

Statistical analysis

Computations were performed using the statistical software package SPSS® Statistics, for Windows, Version 26. The Shapiro-Wilk test was used to determine the normal distribution of the data. A student t-test was used to compare seminal fluid `distribution, the Mann-Whitney U test was used. Changes were considered statistically significant when the p-value was 0.05 or less $p < 0.05$.

Results

The demographic findings of all patients in pretreatment and post-treatment were matched, The consequence of interference in this research showed that the three times daily administration of fertilAid for three months improved sperm count/ml from with a p-value of 0.035 while the volume of ejaculate did not show any significant change. (Table 2).

Table 2: Comparison between sperm count/ml and volume of ejaculate before and after treatment of patients with fertilAid

	Before treatment	After treatment	P value
sperm count/ml	38.44 ± 22.41	47.14 ± 29.55	0.035
volume	3.34 ± 1.09	3.6 ± 0.82	0.234
	Before treatment	After treatment	P value
sperm count/ml	38.44 ± 22.41	47.14 ± 29.55	0.035
volume	3.34 ± 1.09	3.6 ± 0.82	0.234

The observation of sperm parameters before and after treatment with fertilAid showed, significant enhancement in the sperm motility including

progressive and non-progressive motility p-value 0.003 and 0.013 respectively see Figure 1 and Figure 2.

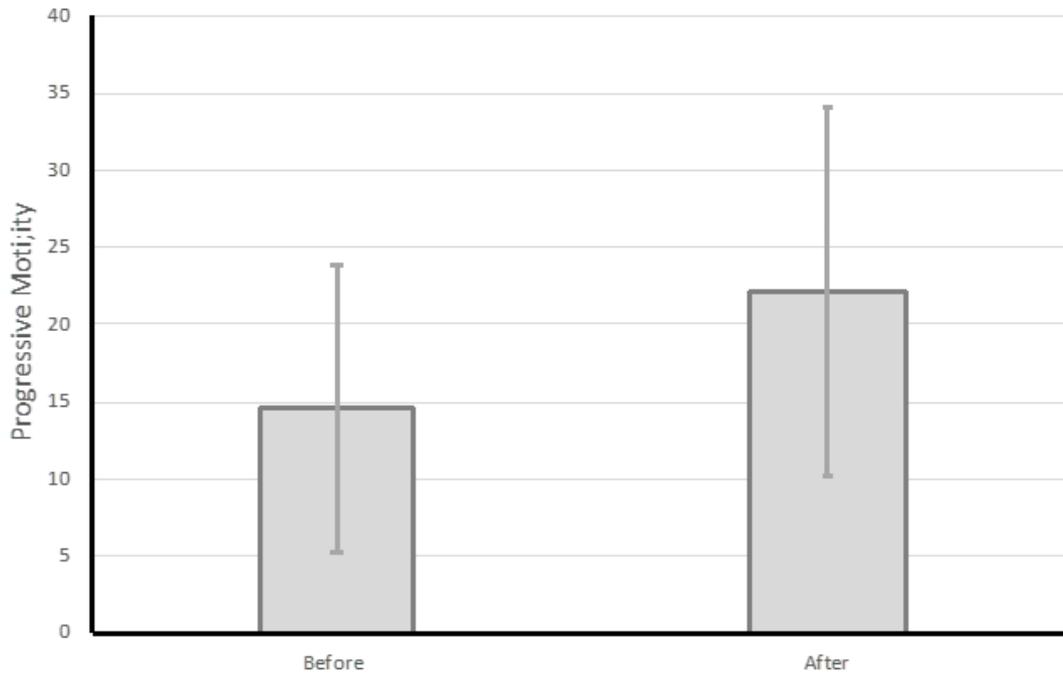


Figure 1: The percentage of progressive sperm motility before and after treatment with FertilAid.

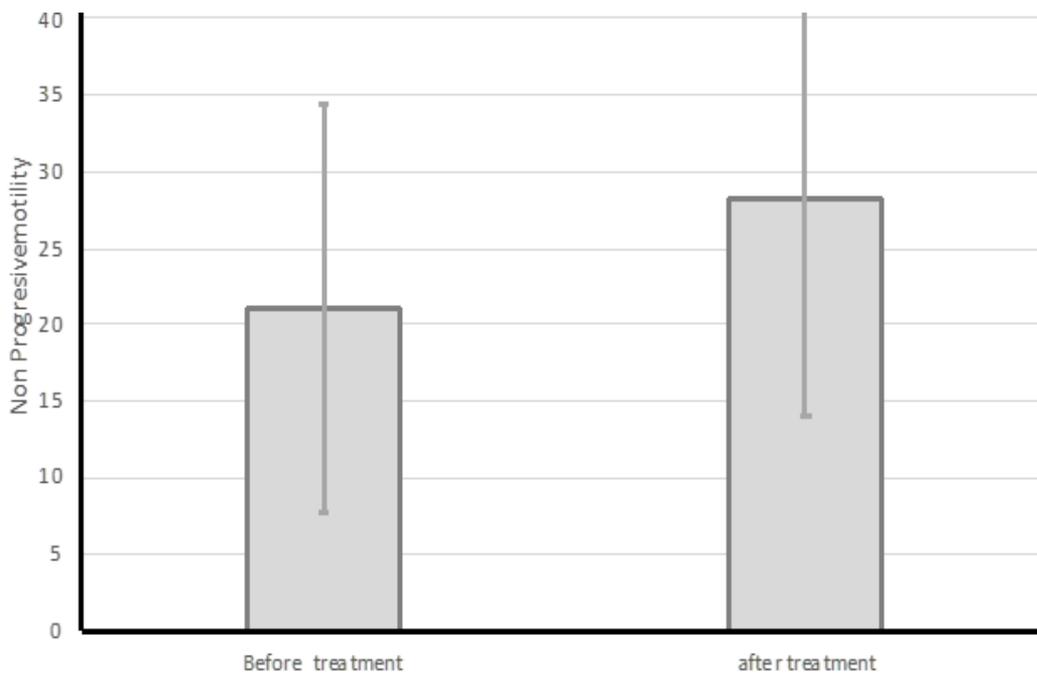


Figure 2: The percentage of non-progressive sperm motility before and after treatment with FertilAid.

The percentage of total sperm motility significantly improved with a p-value of 0.001 (Figure 3).

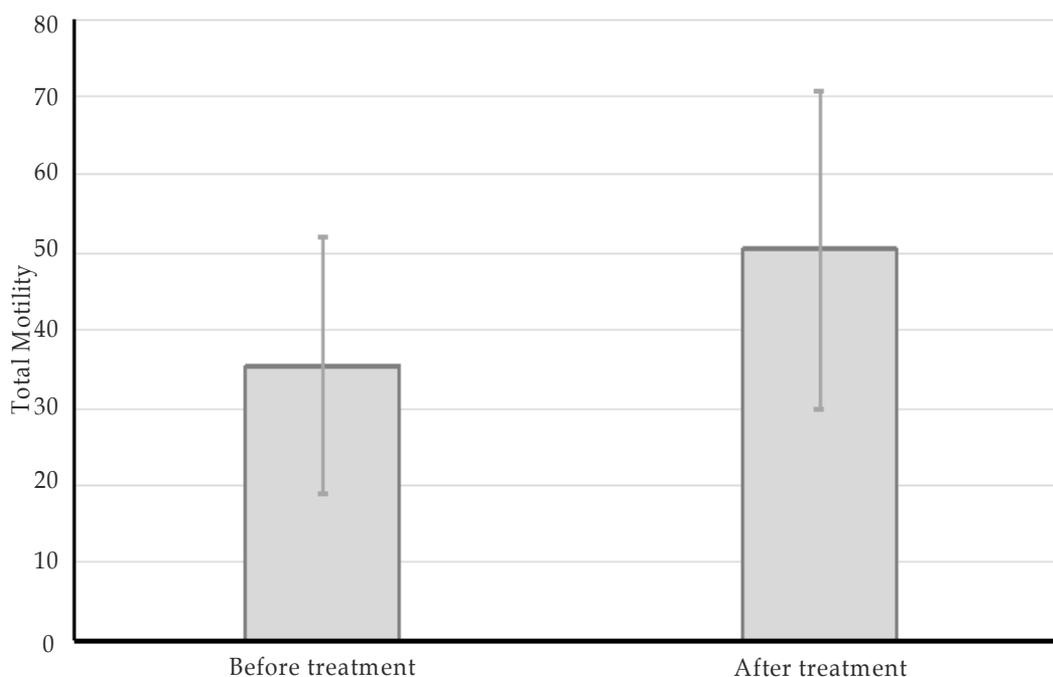


Figure 3: The percentage of total sperm motility before and after treatment with FertilAid.

On the other hand, the concentration of immotile sperm markedly reduced after three months of treatment with p-value 0.002 as shown in Figure 4.

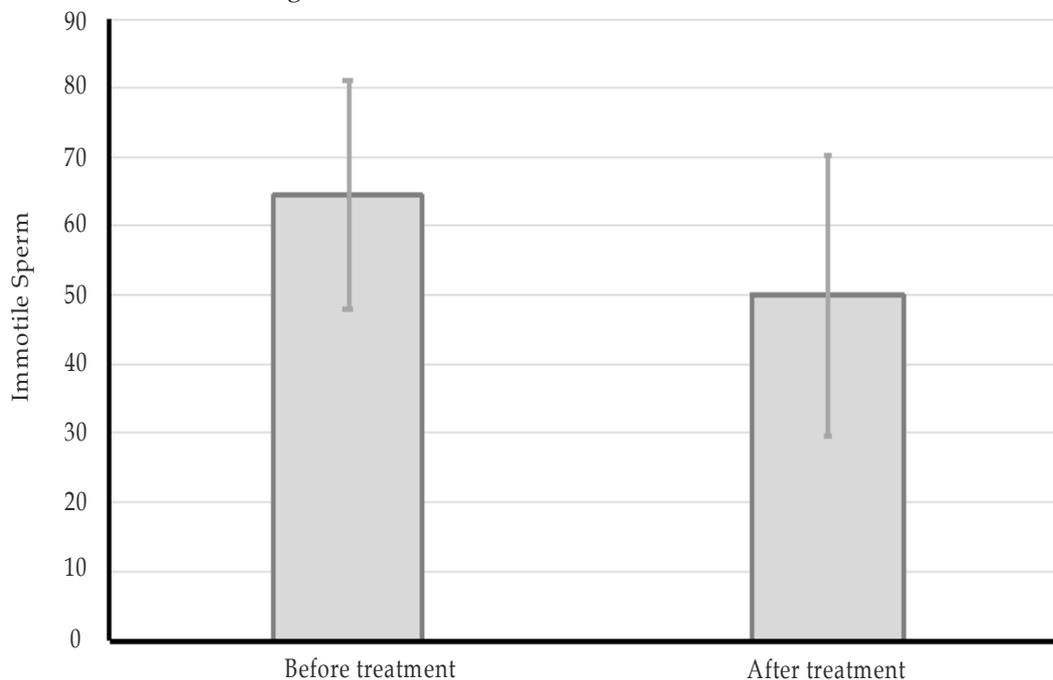


Figure 4: The percentage of immotile sperm motility before and after treatment with FertilAid.

Discussion

IOAT is described as abnormal spermatogenesis and is a status of relatively low sperm count, motility, and a high count dysmorphism of

spermatozoa in the ejaculate. Almost 30% of Oligoasthenoteratozoospermic (OAT) patients are diagnosed as idiopathic.^{5,16}

Commonly, there are two different approaches to

the management of IOAT: pharmacological treatment and/or assisted reproductive technology (ART). The purpose of pharmacological treatment is to stimulate spermatogenesis and to boost sperm maturity, mobility, and diminish sperm dysmorphism.

Harmless consequences of oral antioxidant at or below the recommended daily doses makes antioxidant supplementation a reasonable treatment regimen before continuing with more invasive and expensive managements such as in vitro fertilization or Intracytoplasmic sperm injection.¹⁷

In this research, we have inspected the effect of a combination of L-Carnitine tartrate, Zinc, Maca Root, Asian Ginseng Lots of Antioxidants Vitamin C, E, Beta Carotene, and Selenium plus CoQ10, B12 as pharmacological treatment boosting semen parameters of cases with IOAT.

They were used in various clinical trials, either singly or in combination with the agents, to correct sperm parameters especially sperm motility and concentration.^{7,8,18,19, 20}

Considering sperm concentration, there was a considerable increase in sperm concentration in treated patients. This is in accord with other studies that declared a significant increase in concentration, due to vitamin E content and Beta carotene which Neutralizes free radicals and cushions the cellular membrane against oxygen free radicals, and increase in sperm concentration and motility reduction in oxidative stress measures and SDF (sperm DNA fragmentation).^{9,21,22,23,24}

Maca (*Lepidium meyenii*) improves sperm formation increased stages of mitosis of germ cells Neurobiological activity of antioxidant protection increase sperm concentration, total sperm count, and sperm motility.^{25,26,27,28,29}

Also, the Korean ginseng extract content of the product Induces spermatogenesis via CREM (cAMP-responsive element modulator) activation, increase LH, and FSH, total and free testosterone levels, decrease in PRL (prolactin) in turn increase motility and a total number of sperm.^{30,31,32}

The target of the research was the enhancement of sperm motility, considering overall motility (progressive and non-progressive motility). Statistically considerable enhancement in the sperm motility including the percent of overall sperm

motility and a significant decrease in the percent of immotile sperm. According to the results of previous studies on the amino acid, L-Carnitine shows to boost sperm metabolism and increase the capability of sperm to convert complex molecules into energy, significant effect on sperm motility of men with IOAT.^{12,33,34} In some studies, a dose of 3 gr carnitine administer daily for four months' duration has a considerable improvement of sperm motility in comparison to pretreatment levels. However other studies concluded that a higher dose of LC around 4 grams administer daily over a more shortened period of treatment (two months) could considerably improve the progressive sperm motility.^{16,35}

In vitro semen activation by fertilAid exhibited a considerable increase in the total semen parameters as compared to the activation by L. Carnitine.³⁶ Vitamin B12 at 2.50 mg/mL addition to bovine seminal fluid in vitro enhanced sperm motility³⁷. An oral antioxidant medication containing vitamin B12 found to enhance sperm motility, vitality, and DNA integrity.³⁸

Conclusion

The administration of a combination of L-Carnitine tartrate, Zinc, Maca Root, Asian Ginseng Lots of Antioxidants Beta Carotene, Selenium and vitamin C, E, plus CoQ10 in IOAT patient notably increase the sperm count per milliliter. It also significantly improves overall sperm motility including progressive motility and non-progressive motility and a decrease in the concentration of immotile sperm.

Abbreviations

OAT: oligoasthenoteratozoospermia. IOAT: Idiopathic Oligoasthenoteratozoospermia. ART: assisted reproductive technology. LC: L-carnitine. NAC: N-acetyl cysteine. FDA: food and drug administration. IVF: in vitro fertilization. ART: assisted reproductive technology. SDF: sperm DNA fragmentation. CREM: cAMP-responsive element modulator. PRL: prolactin.

Declarations

Ethics approval:

This work has been approved by the ethics committee in the College of Medicine/Hawler Medical University with approval number meeting code: 3 paper code: 3.

Availability of data and material

The material and data used and analyzed for the study are available from corresponding authors on sensiblerequest.

Conflict of interest: none

Funding: The authors didn't have funding resource.

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Relationship Between Disease History and Fear Level During COVID-19 Pandemic in Bogor City Area

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How to cite this article: Devi Irawan, Nining Fitrianiingsih, Magdalena A. Yosali et al. Relationship Between Disease History and Fear Level During COVID-19 Pandemic in Bogor City Area. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):130-135.

Abstract

Background: COVID-19 pandemic is a major concern in almost all countries in the world, including in Indonesia. The number of COVID-19 confirmed cases in the worldwide as of July 20, 2021 was 192 million cases with 4.13 million deaths. West Java province of Indonesia became one of the highest COVID-19 contributors with 6,928 cases in one day. This very rapid increase has caused psychological impact in society. Some people became more afraid to get infected with COVID-19 while some others started to get careless in performing health protocols. COVID-19 imposes more threat to people with previous diseases and could also raise fear and anxiety during the outbreak.

Objective: The purpose of this study was to determine the relationship between disease history and fear level of COVID-19 in Bogor City area.

Methodology: The research method used was analytical survey with a cross-sectional approach. The sampling technique was snowball sampling with total of 515 participants aged more than 18 years old. The instrument in the study was an online questionnaire using a Google Form. Data analysis performed was univariate and bivariate analysis with chi square test.

Result: Out of 515 participants, 431 respondents (83.7%) had no disease history and 411 respondents (79.8%) had moderate fear level of COVID-19. The results of the chi square test show the p-value = 0.000.

Conclusion: There was a relationship between disease history and fear level of COVID-19 in in Bogor City area.

Keywords: COVID-19; Disease history; fear level.

Introduction

Coronavirus disease 2019 (COVID-19), an infectious respiratory syndrome caused by the novel

coronavirus SARS-CoV-2, has spread across many countries since January 2020, including Indonesia. Corona virus is a virus that causes disease in humans

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and animals. In humans, it usually causes respiratory tract infections, ranging from fever, cold, cough to flu. The COVID-19 is a virus that related to SARS (severe acute respiratory syndrome) which occurred in China in 2002, and MERS (Middle-East respiratory syndrome) which occurred in Middle East in 2012.¹

COVID-19 is a major concern in almost all countries in the world, including Indonesia. The number of COVID-19 cases in the world as of July 20, 2021 was 192 million with 4.13 million deaths. In Indonesia, the first COVID-19 case was found in March 2020 and has been increasing ever since. As of 20 July 2021, the detected positive COVID-19 cases have increased from 38,325 cases to 2,950,058 cases. Death cases increased by 1,280 to 76,200 patients, while recovered cases also increased by 29,791 to 2,323,666 cases. Meanwhile, West Java Province was one of the regions with high cases report.²

The rapid COVID-19 cases increase caused unrest in the community with psychological shocks began to be present in the community. Fear is an emotional symptom that gives feelings of discomfort, anxiety, and worry about something that will happen that is felt to be threatening, which can be caused by an environment that can prevent a person from getting the desired goal.³

In a survey conducted by the American Psychiatric Association (APA) in more than 1000 adults, it was found that 48% of respondents were worried that they would contract the COVID-19.⁴ Around 40% are worried that they will become seriously ill or die from COVID-19, and 6% are worried that their family or loved ones would be infected, 59% of the community said the effects of COVID-19 were quite severe for their daily lives.⁴

Various conditions that occurred during the COVID-19 pandemic had psychological effect on the community.⁵ This is because the COVID-19 pandemic has become a heavy stressor. Anxiety is a common response that occurs during times of crisis. Anxiety is a general condition of fear or discomfort characterized by a variety of symptoms, which include physical, behavioral and cognitive symptoms. Physical symptoms involve shivering and tightness in the abdomen or chest, heavy sweating, sweaty palms, headache, dry mouth, shortness of breath, rapid heartbeat, cold fingers or limbs and nausea. Behavioral symptoms include avoidance

behavior, dependent behavior and restless behavior.⁶ Cognitive symptoms include worry, feeling afraid or anxious about the future, thinking too much or being very alert to sensations that arise in the body, and fear of losing control.⁶

Fear level of COVID-19 varies in every person. Various factors including demographic factors, perceptions of the risk of exposure may also affect a person's level of fear.⁷ According to Saptaputra et al. (2020), a history of comorbidities will underlie a person to be more susceptible to contract COVID-19 and develop severe and clinically worsen condition compared to those who do not have comorbidities.⁸

This study is aimed to determine the relationship between disease history and fear level during COVID-19 pandemic in Bogor City area.

Materials and Methods

Study Design

This research was an analytical survey research with a cross-sectional approach. The study was conducted in Bogor City area. The sampling technique used snowball sampling.⁹ The online questionnaire was developed using Google Forms. In the questionnaire there was an approval section, so that only participants who agreed to the informed consent and those aged more than 18 years could fill out the questionnaire. The questionnaire link was sent via WhatsApp application. Participants were also encouraged to share the link with as many other people as possible, including their friends and relatives. There were 39 questions in the questionnaire and it took approximately 5 to 10 minutes to complete all the questions. The questionnaire section which involved the disease history such as hypertension, hipercholesterol, diabetes, fatty liver, stroke, heart disease, hyperglycemia, insomnia, eczema, parkinson's disease, autoimmune disease, epilepsy, hearing disorder, asthma, and neurological disease.

There were 11 questions asked regarding the level of fear, which included fear of contracting COVID-19, cancer, stroke, heart attack, no social interaction, animals/insects, traffic jam, loss of family members, natural disaster, loss of cellphone, and loss of job. The answers of fear level were in Likert scale, ranging from scale 0 to 10 with 0 as the lowest fear scale and 10 as the highest fear scale.

Data Sources and Settings

The questionnaire has gone through the validity and reliability test stages of 20 participants in June to July 2020 with valid and reliable categories. All participants who were adults (more than 18 years old) and have access to the internet can participate in this study. The questionnaire was prepared in Bahasa Indonesia. Data collection process started from August 1, 2020 to December 31, 2020 with total of 515 participants. The collected data were checked for completeness of the answers. If there was an ambiguous response or no answer, then the data was eliminated.

Statistical Analysis

Data were analyzed using SPSS version 23. The univariate data was analyzed using frequency distribution and bivariate data using chi square test. The *p-value* < 0.05.

Result

Table 1: Characteristics of participants based on age, education, and occupation

Characteristics	Amount	Percentage (%)
Age		
18-24 years old	463	89.9
25-29 years old	25	4.9
30-34 years old	1	0.2
30-34 years old	10	1.9
35-39 years old	7	1.4
40-44 years old	2	0.4
45-49 years old	1	0.2
50-54 years old	3	0.6
55-59 years old	2	0.4
65 years old or older	1	0.2
Education		
Low education level	99	19.2
High education level	416	80.8
Profession		

Contd... Table 1: Characteristics of participants based on age, education, and occupation

Student	448	87.0
Looking for a job	5	1.0
Employee	48	9.3
Self-employed	12	2.3
Housewife	2	0.4
Total	515	100

Based on the table above, out of 515 participants, 463 participants (89.9%) were in the age range 18-24 years old (89.9%), 416 participants (80.8%) had high education level, and 448 participants (87%) were students.

Table 2: Results of univariate analysis

Variable	Amount	Percentage (%)
Diseases History		
No disease	431	83.7
Depression	3	0.6
Stomach ulcer/ gastritis	56	10.9
Hypertension	2	0.4
Chronic obstructive pulmonary disease	2	0.4
High cholesterol	7	1.4
Fatty liver disease	2	0.4
Insomnia/ Sleep Disorders	7	1.4
High Blood Sugar Level	3	0.6
Auto immune disease	2	0.4
Fear Level		
High	16	3.1
Medium	411	79.8
Low	88	17.1
Amount	515	100

The table above showed that of the 515 respondents, 83.7% (431 respondents) had no history of diseases, and 79.8% (411 respondents) had a medium level of fear toward COVID-19.

Table 3: Relationship between Disease History and Fear Level of COVID-19

Variable	Fear Level								P-value	
	Low		Medium		High					
	f	%	f	%	f	%	f	%		
Disease History	No disease	69	13.4	349	67.8	13	2.5	431	83.7	0.000
	Depression	1	0.2	2	0.4	0	0	3	0.6	
	Gastritis	7	1.4	47	9.1	2	0.4	56	10.9	
	Hypertension	0	0	2	0.4	0	0	2	0.4	
	Chronic obstructive pulmonary disease	0	0	2	0.4	0	0	2	0.4	
	High cholesterol	6	1.2	1	0.2	0	0	7	1.4	
	Fatty liver disease	2	0.4	0	0	0	0	2	0.4	
	Insomnia/ Sleep Disorders	0	0	6	1.2	1	0.2	7	1.4	
	High Blood Sugar Level	2	0.4	1	0.2	0	0	3	0.6	
	Auto immune disease	1	0.2	1	0.2	0	0	2	0.4	
Total	88	17.1	411	79.8	16	3.1	515	100		

Based on the table 3, out of 515 participants, 349 participants (67.8%) had no history of disease and moderate level of fear toward COVID-19. The results of the chi square statistical test obtained *p-value* = 0.000, this means there was a relationship between history of disease and the fear level of COVID-19 in the Bogor City area.

Discussion

1. Disease History

Based on table 2, out of 515 participants, 431 participants (83.7%) had no history of disease.

According to Saptaputra et al. (2020), comorbidities could cause a person to be more susceptible to contracting COVID-19 and develop severe symptoms compared to those who do not have co-morbidities.⁸ This finding is in line with another research article which states that people with chronic diseases not only have a higher risk of being infected with COVID-19 but also have higher death risk and multi organ failures when contracting COVID-19.¹⁰ According to reports, the prevalence of Chronic Obstructive Pulmonary Disease (COPD) is higher in patients with more severe presentations than patients without COPD or without a history of comorbidities.¹¹

Chronic disease history or known as comorbidity is a condition where there is more than one disease that occur simultaneously in a

patient. The number of comorbidities increases with age. The most common comorbidities found in COVID-19 patients include: hypertension, diabetes mellitus, heart disease, chronic obstructive pulmonary disease, kidney disease, other respiratory disorders, pregnancy, asthma, liver disease, Tuberculosis, cancer, and immunodeficiency disorders.¹²

This finding is in line with Hadiyanto's research which states that the population at risk of being infected with COVID-19 is those who have comorbidities or chronic diseases, pregnant women and people with history of respiratory diseases such as asthma and the elderly aged more than 60 years old.¹³ Based on the research of Satria et al., COVID-19 patients who have comorbidities are on average more than 45 years old with the number of patients dying reaching (26.08%) who are infected with COVID-19 and have comorbidities.¹⁴

The results from this study showed that 89.9% participants (463 participants) had no history of comorbidities were those aged 18-24 years old.

2. Fear Level

Table 2 showed that of the 515 participants, 79.8% (411 participants) had moderate level of fear of COVID-19.

Research conducted by a psychiatric expert at Gajah Mada University stated that women are

vulnerable group affected by mental health such as excessive fear and stress during COVID-19 pandemic.¹⁵

Fear is a basic emotional state in which individuals identify external dangers emanating from specific objects that can make a person feel attacked by his defenses.¹⁶ Fear is a basic human emotion that can change from a normal state to a very strong fear. The three components of fear include cognitive, physiological, and behavioral, all of which can be present at the same time.¹⁶

Fear of COVID-19 is caused by new habits that are faced every day without any preparation which causes an increase in alertness. This statement is similar to the finding by Hawari (2011) which disclosed that fear can arise in individuals due to increased self-awareness of dangers that threaten to occur.¹⁶

The fear experienced by participants was dominated by the moderate category which can be influenced by age and education level. The majority of participants in this study were aged 18-24 years old and 80.8% had higher education level.

3. The Relationship between Disease History and Fear Level of COVID-19

The results showed that out of 515 participants, 349 (67.8%) participants had no history of disease and moderate fear level of COVID-19. The result of the Chi Square statistical test obtained $p\text{-value} = 0.000$ and there was a relationship between disease history with the fear level of COVID-19 in Bogor City area.

According to Maryam (2017), fear can be expressed directly through physiological and behavioral changes, including cardiovascular palpitations and a sense of fainting, breathing (shortness of breath, pressure in the chest, and choking sensations), neuromuscular (insomnia, pacing, and choking), tense face, gastrointestinal (loss of appetite, nausea, and diarrhea), urinary tract (unable to hold urine), and skin (sweating, flushed face, and a cold feeling on the skin). Behavioral fear symptoms also include impaired attention, poor concentration, forgetfulness, misjudgment, thinking barriers, loss of objectivity, confusion, fear, nightmares, impatience, restlessness, tension, nervousness,

horror, worry, guilt, and shame.¹⁶ The factor that causes high level of fear is the weight of the burden that is faced by an individual. The burden in this epidemic situation for the community is the fear of contracting the COVID-19 and spreading the virus to loved ones.¹⁷

The level of fear regarding a pandemic can vary in each person, depending on the certain factors. Various factors including demographic factors and perceptions of the risk of exposure may also affect a person's level of fear. According to Saptaputra (2020), a history of comorbidities will underlie a person to be more susceptible to contracting COVID-19 and develop severe and clinically worsening compared to those who do not have comorbidities.⁸

Based on the results of the research and theory above, the researchers concluded that there was a relationship between disease history and the fear level during COVID-19 pandemic. In this study, the majority of participants showed no history of disease and the fear level was in the moderate category.

Conclusion

There was a significant relationship between disease history and the level of fear during COVID-19 pandemic in Bogor City area. Health workers can help reduce the fear of COVID-19 in the community by increasing knowledge and providing up-to-date information regarding COVID-19 pandemic.

Conflict of Interest: Nil.

Funding: This research was funded by Wijaya Husada Health Institute.

Ethical Clearance: Not required because data was collected from Google Form during epidemic time but took consent from every participant.

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Suicides During the Pandemic: A Retrospective Study

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How to cite this article: YN Singha, Arpan Mazumder, G Das. Suicides During the Pandemic: A Retrospective Study. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):136-139.

Abstract

Suicide is defined as a fatal self-injurious act with some evidence of intent to die.¹ Suicide occurs more often in older than in younger people, but is still one of the leading causes of death in the late childhood and adolescence. Every year, more than 1,00,000 people commit suicide in our country.¹ Suicide accounts for 1.4% of all deaths, and is the 15th leading cause of death globally.^{2,3} Suicide is associated with an impulsive nature. Several risk factors concerning family structure and interactions have been linked to a suicidal behaviour. Direct conflicts with parents and siblings, Occupational status and social acceptance have a great impact, but so do the absence of communication and a lack of empathy.¹² Interpersonal losses are also strongly associated with suicide cases. But in developing countries one of the major reason always remains unemployment and poverty. There are different rates of suicides and suicidal behaviour between males and females (among both adults and adolescents). While females more often have suicidal thoughts, males die by suicide more frequently.⁵ Hence, this study was planned with a purpose to know the magnitude and the socio-cultural factors of the problem of suicides, so that a sound prevention program could be suggested, planned and implemented for reducing the incidence of suicides.

Keywords: Suicide; pandemic; unemployment.

Introduction

Suicide is defined as a fatal self-injurious act with some evidence of intent to die.⁶ Suicide is a fatal act which represents the individuals wish to die.⁶ The latin word suicide denotes "self-murder". Most of the victims plans it for days, weeks, months even years; but for some it is an impulsive act.³ Suicide occurs more often in older than in younger people, but is still one of the leading causes of death in the late childhood and adolescence.⁴ Every year, more than 1,00,000 people commit suicide in our country.¹ Several risk factors have been linked to a suicidal

behaviour. In developing countries one of the major reason is unemployment and poverty. Majority (71%) of suicide in India are by persons below the age of 44 years which imposes a huge social, emotional and economic burden.¹⁰ Poisoning, hanging and self-immolation (particularly women) were the methods to commit suicide.⁴ Physical and mental illness, disturbed interpersonal relationships and economic difficulties were the major reasons for suicide.¹⁰ The vulnerable population was found to be women, students, farmers etc.¹⁰ There are different rates of suicides and suicidal behavior between males and females (among both adults and adolescents).^{5,10}

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While females more often have suicidal thoughts, males die by suicide more frequently.⁵ Two large epidemiological verbal autopsy studies in rural Tamil Nadu reveal that the annual suicide rate is six to nine times the official rates.¹² It includes both, those who have attempted suicide and those who have been affected by the suicide of a close family or friend.¹² Thus, suicide is a major public and mental health problem which demands urgent action.⁴ It is believed that the most dramatic increase in suicide mortality will be observed in third world countries because of socioeconomic and behavioral factors⁽¹¹⁾. According to the National Crime Records Bureau (NCRB); state of Tamil Nadu, West Bengal, Andhra Pradesh, Maharashtra and Karnataka have registered a consistently higher number of suicidal deaths during the last few years and together accounted for 56.2% of the total suicides reported in the country⁽¹¹⁾. The approach to understanding suicide must be multidisciplinary, involving psychologists, psychiatrists, toxicologists, physiologists and physicians, because suicide is a multi-faceted and complex event.⁸ Hence, this study was planned with a purpose to know the magnitude and the socio-cultural factors of the problem of suicides, so that a sound prevention program could be suggested, planned and implemented for reducing the incidence of suicides.

Material and Methods

This retrospective study was conducted in the department of Forensic Medicine, Silchar Medical

College and Hospital during the period from 1st July 2020 to 30th June 2021, amidst the prevailing Covid Pandemic situation. During the period a total number of 730 cases were brought for Autopsy, out of which 290 number of cases were found to be of suicidal intent.

The accompanying persons and attendants of all such cases were interviewed for relevant information that can be gathered regarding the motive of suicide, any previous medical & Psychiatric History or previous attempts at committing suicide. RTPCR test for COVID-19 suspected cases was done on a routine basis as and when needed.

Aims and Objectives

1. To find the total number of suicide cases during the period.
2. To find the sex ratio.
3. To find out different methods adopted for suicide.
4. To find out the cause of death.
5. To find out the underlying cause of such step (If found relevant).

Observations and Results

In the present study it was found that out of 730 cases, 290 cases were deaths due to suicide.

Out of 290 cases 152 cases were male. Which is similar to the findings of other studies on suicide.

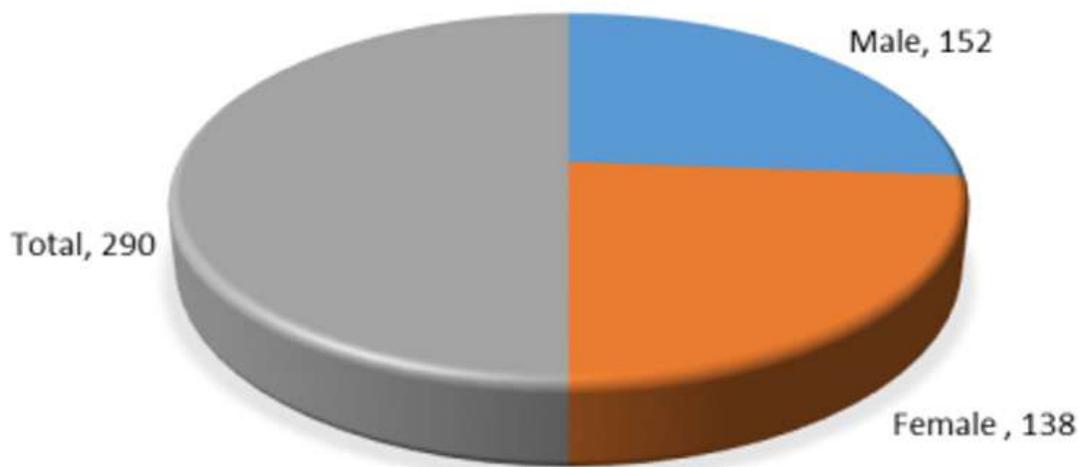


Figure 1: Showing the Sex Ratio of the cases.

Hanging was the most common method adopted by most of the victims, followed by poisoning.

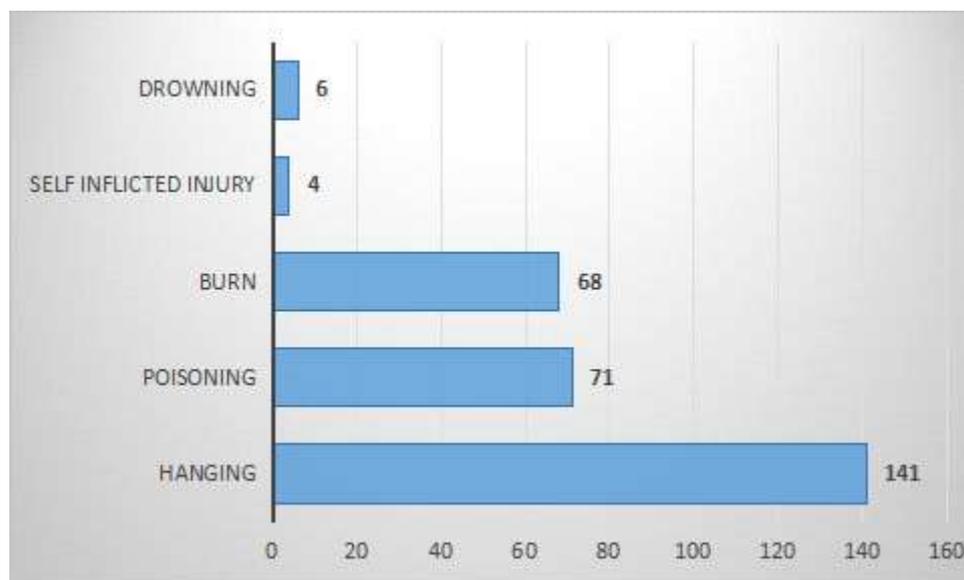


Figure 2: Showing the various methods adopted for suicide.

Asphyxia was the most common cause of death found in the suicide cases.

Most of the victims were self-employed. Some of the victims were house wives and some were daily labours.

Out of 290 cases, attendants of 94 cases gave history of covid 19 like symptoms, but none of them were found to be positive on RAT test and RTPCR test.

A history of depression was common in most narratives by the respective attendants. Poor economic condition, pre-existing and also influenced by the prevailing situation of unemployment and the Pandemic were also mentioned by a few.

Discussion

The present study was conducted to find any possible effects of pandemic as a stress factor on the suicide victim cases. The family members, relatives and friends were interviewed to find the mental state of the victims during that period. In case there was any history of Covid 19 like symptoms RTPCR test was performed in such cases. Nearly 1/3rd cases were found to be death due to suicide.

Males accounted for more than 60% of the cases, possibly due to the traditional role of being the bread winner of the family. This finding is similar to the other studies conducted on suicide earlier. The

finding is different from the finding of Patel V et al. who found that women are commonest victim.⁹

Most common age group involved were between 30 to 45 Years, which is different from the findings of Dasgupta SM, who found 11 to 30 Years as the common age group involved¹² and Gad ElHak S. et al. who found the 20-30 yaers as the commonest age group involved.⁷ Patel V et al found the age group involved to be 15-29 Years.⁹

Hanging was the most common method adopted followed by poisoning. This finding is different from the finding of Bhagabati D, Dasgupta SM and Gad ElHak S. et al who found poisoning to the commonest mode adopted.^{11,7}

Most of the victims were self-employed (157 cases out of 290 cases), which is different from the findings of Bhagabati D.

A history of Depression, Failed ventures and Social embarrassment was found associated, in most of the victims.

Conclusion

In a Developing country like ours, a substantial percentage of the population is still under the poverty line and they earn their livelihood on a daily wage basis. A possible scenario during the Pandemic was the loss of sources of income and livelihood which may have contributed towards a depressed mental

state. The percentage of people committing suicide showed an increase in the numbers as compared to the previous year, although the general numbers also showed a substantial increase. Government agencies and NGOs have been very forthcoming and pro-active in providing relief and minimizing the losses and have been instrumental in bringing back the lives of the common man on track, but the scars of the Pandemic, when nothing was known about the prognosis, fate and outcome of the disease, that positively contributed in a major way towards development of a negative mind-set and a sense of hopelessness in such individuals.

A sense of social obligation and empathy towards understanding the mental set up of such individuals, specially in those who are known to be suffering from such a condition by the family, close contacts and society as a whole would help in bringing down the numbers of such unfortunate deaths and shall be a great leap towards a healthy society.

Conflict of Interest: Nil.

Source of Funding: Self.

Ethical Clearance: Taken from Institutional Ethical Committee.

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The effectiveness of Diabetes Self-Management Education (DSME) with self-acceptance and self-care for diabetes mellitus patients

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How to cite this article: Elis Anggeria, Rahmaini Fitri Harahap, Kristina L Silalahi et al. The effectiveness of Diabetes Self-Management Education (DSME) with self-acceptance and self-care for diabetes mellitus patients. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):140-144.

Abstract

Background: Diabetes mellitus is a complex group of chronic diseases associated with hyperglycemia, resulting in impaired insulin secretion and other organs. Patients with type 2 diabetes mellitus will experience changes in themselves. Each individual responds and has a different perception of dealing with these changes. Providing education aims to increase knowledge and improve the patient's ability in self-care so that health problems are resolved.

Objectives: The study wants to know the effectiveness of Diabetes Self-Management Education (DSME) on self-acceptance and self-care of Type II Diabetes Mellitus patients.

Patient and Methods: A quasi-experimental design was used through one group pre-test and post-test design. The total population is 25 people, with a saturated sampling technique, and a sample of 25 people. Data analysis used a non-parametric statistical test, namely the Wilcoxon Signed Rank Test.

Results: The majority of patients with Diabetes Mellitus had good self-acceptance, and the majority of patients' self-care was found to be good ($p=0.000$).

Conclusions: The resulting study is the effectiveness of DSME on patient self-acceptance and self-care.

Recommendations: The DSME program requires involvement in glucose monitoring by health professionals. Providing education to patients effectively provides psychological support and patient self-care.

Keywords: DSME; self-acceptance; self-care; diabetes mellitus.

Introduction

Diabetes mellitus is a complex group of chronic diseases associated with hyperglycemia, resulting in impaired insulin secretion, causing disturbances in different organs and systems.¹ Diabetes not only

causes premature death worldwide. This disease is also a cause of blindness, heart disease, and kidney failure.²

The Diabetes Federation Organization (IDF) estimates that at least 463 million people suffer from

diabetes at the age of 20-79 in 2019, equivalent to a prevalence rate of 9.3% of the total population at the same age. By gender, the IDF estimates the prevalence of diabetes at 9% in women and 9.65% in men.³ The prevalence of diabetes is estimated to increase as the population ages to 19.9% or 111.2 million people aged 65-79 years. The number is predicted to continue to increase to reach 578 million in 2030 and 700 million in 2045.²

Patients with type 2 diabetes mellitus will experience changes in themselves. Each individual responds and has a different perception of dealing with these changes. Psychosocial and emotional factors related to diabetes, life stress, anxiety, and depression. These factors lead to worse diabetes.⁴ Low self-acceptance in diabetic patients is also associated with impaired self-care and glycemic control.⁵

According to the American Diabetes Association, one form of effort to overcome or reduce stress is through health education and family support. The form of health education provided is Diabetes Self-Management Education and Support (DSME/S).⁶ The results of another study show that 83% of diabetics experience moderate levels of stress.⁷

Diabetes self-management education (DSME) is an ongoing, patient-centered process that helps impart knowledge, skills, and abilities for self-care. Pirkle et al. (2019) showed that DSME was most effective when strengthened by community resources.⁸ Diet education can also be done by promoting a more positive attitude towards the disease. This can be achieved by individual counseling, respecting the patient's needs, and focusing on regular blood glucose testing.⁹

Patients could accept illness and carry out self-care through a learning process by providing knowledge and practice. Good self-care behavior can be adapted through health professionals. Based on the initial survey found at the research site, some respondents felt emotional, felt anxious, lacked confidence in their disease condition, and had feelings of burden because their diabetic wounds did not heal immediately. The purpose of this study was to determine the effectiveness of Diabetes Self-Management Education (DSME) on self-acceptance and self-care of Type II Diabetes Mellitus patients.

Materials and Methods

This study uses quantitative research methods with correlational studies through a Cohort design

approach.¹⁰ A quasi-experimental design was used through one group pre-test and post-test design. This research was conducted at Wound Care Center Medan. The population in this study was 25 patients who underwent wound care. The sampling technique used was saturated sampling, and sample was 25 people.

The research began after obtaining permission from the Faculty of Nursing and Midwifery, Universitas Prima Indonesia in 2020, the researchers submitted a research letter to the Wound Care Center Medan. After the relevant parties allow the research, the researcher explains in advance the purpose of the study, and the respondent signs the consent form.

This research has been approved by the Ethics Commission of Universitas Prima Indonesia No: 038/KEPK/UNPRI/V/2020. Permission was also obtained from a Wound Care Centre to obtain approval for the research site. The researcher had previously visited the unit to obtain information and establish relationships with nurses and respondents.

Then the researcher explained the instruments used before the Diabetes Self-Management Education (DSME) intervention. Researchers provide information about the benefits of education in the self-acceptance and treatment of the diabetes mellitus. The researcher gave a pretest questionnaire, and after the intervention, the researcher distributed a posttest questionnaire. Self-acceptance questionnaire with Diabetes Acceptance Scale (DAS)⁽¹¹⁾, and self-care with the Diabetes Self-Management Questionnaire (DSMQ).¹²

After the questionnaire was filled in, the instrument was collected again by the researcher and checked for completeness. If the instrument is not filled out completely, it will be completed on the spot. Respondents filled out the questionnaire sheet again after giving Diabetes Self-Management Education (DSME) to determine self-care after the intervention.

Data analysis includes univariate and bivariate. Univariate data analysis based on self-acceptance and self-care then analyzed in the frequency distribution table. Bivariate data analysis based on wound care with self-acceptance and self-care. This study uses a nonparametric statistical test, namely the Wilcoxon Signed Rank Test. Nonparametric statistical tests are used when the resulting distribution is not normal or the sample is small using a significance level of 5%.

The reliability of the study findings is ensured by the researcher about the analysis. Initial ideas about the research were discussed with the research team. The study used a questionnaire that had been modified according to research needs. The research findings are derived from data collected from the initial survey and respondents' experiences of self-acceptance and self-care.

Results

The results of this study obtained univariate and bivariate analysis data. Based on self-acceptance

Table 1: The Effectiveness of Diabetes Self-Management Education (DSME) on Self-Acceptance of Diabetes Mellitus Patients

Self-acceptance	N	Mean	Std. Deviation	Z	p-value
Before	25	1.08	0.277	-4,472	0.000
After	25	1.88	0.332		

Based on the results of the study, of p-value = 0.000 (p-value <0.05). This means that there is a

before DSME in Diabetes Mellitus patients, the majority of self-acceptance was not good as many as 23 people (92%). After DSME in Diabetes Mellitus patients, the majority of good self-acceptance was 22 people (88%).

Based on self-care before DSME in Diabetes Mellitus patients, the majority of self-care was not good as many as 18 people (72%). After DSME in Diabetes Mellitus patients, the majority of self-care was good as many as 20 people (80%).

difference between self-acceptance before and after Diabetes Self-Management Education (DSME).

Table 2: The Effectiveness of Diabetes Self-Management Education (DSME) on Self-Care for Diabetes Mellitus Patients

Self-care	N	Mean	Std. Deviation	Z	p-value
Before	25	1.28	0.458	-3.606	0.000
After	25	1.80	0.408		

Based on the results of the study, p-value = 0.000 (p-value <0.05). This means that there is a difference between self-care before and after Diabetes Self-Management Education (DSME).

management.¹⁴

Commitment and acceptance therapy are efficacious in improving the psychological well-being of diabetic patients. It can be applied as a useful intervention method to improve psychological adaptation in patients with diabetes.¹⁵ This therapy can be an effective psychological intervention in diabetic patients who are depressed.¹⁴

Discussion

The results of this study found that the self-acceptance of Diabetes Mellitus patients before the Diabetes Self-Management Education (DSME) intervention experienced poor self-acceptance. This is because patients feel anxious about their illness, feel depressed, depressed, and unable to carry out their usual activities. Patients who had poor psychological status, showed that the majority of patients had negative emotions, and a minority had severe pressure on diabetes.¹³

The results of the study obtained self-acceptance after the Diabetes Self-Management Education (DSME) intervention experienced by the respondents became good. This means that there is a difference between self-acceptance before and after DSME. There was an increase experienced by diabetic patients after receiving education, this was marked by patients who did diabetic wound care became more confident, decreased anxiety levels, and felt they received support from others. Good self-acceptance provides benefits for diabetics to manage themselves during the treatment period.¹⁶ The patient's acceptance of the disease can prevent complications in the patient.¹⁷

Diabetic patients who did not have higher self-acceptance had a significant relationship with less active coping, higher diabetic distress, and depression. Assessment of diabetes acceptance can facilitate the detection of high-risk patients.¹¹ One of the mechanisms by which psychological factors influence chronic disease is related to behavioral

The DSME program is highly effective in improving glycemic control and lipid profile. DSME can reduce the risk of developing diabetes

complications. Patients' diabetes knowledge, medication adherence, self-efficacy, and quality of life can also be significantly improved.¹⁸ DSME experienced increased involvement in glucose monitoring. Patient education programs can effectively improve patient self-management and psychological support.¹³

The results of this study found that self-care patients with Diabetes Mellitus before the DSME intervention experienced poor self-care. This is because the patient cannot perform self-care. They need family assistance to carry out treatment to health facilities, take medicine, treat wounds, and check blood sugar levels regularly.

Diabetes Self-Management Education and Support (DSME/S) aims to support informed decision-making, self-care behaviors, problem-solving, and active collaboration with the healthcare team.⁶ Interventions based on the social cognitive model have a positive effect on the self-care of diabetic patients.¹⁹ Self-care behavior increased significantly in the intervention group over time after being given management interventions other than usual care.²⁰ Prioritization of management, with a focus on specific interventions having a large impact on the individual forms the basis of ongoing care.²¹

The results of this study obtained self-care of Diabetes Mellitus patients after the DSME intervention experienced good self-care. This means that there is a difference between self-care before and after DSME. The education program provides the right information following the disease problems experienced by diabetic patients. Patients are more likely to go to the clinic for check-ups and check Blood Sugar Levels (KGD) and seek regular treatment.

The existence of high patient self-care can reduce the severity of complications of Type 2 DM.²² Self-care practices are essential for controlling plasma glucose concentrations in patients. Health workers should improve patient self-care practices to better control plasma glucose and prevent complications related to diabetes mellitus.²³ Good knowledge about diabetic wound care can improve the patient's self-care status regarding diabetic wound care.²⁴ Self-care has a very useful value for people with DM.²⁵

Recommendations

There is a need for special training to improve the skills and knowledge of nurses in conducting integrated

health education by reviewing the existing curriculum on the approach to Diabetes Mellitus patients.

Conclusion

The DSME program requires involvement in glucose monitoring by health professionals. Providing education to patients effectively provides psychological support and patient self-care. The self-acceptance of Diabetes Mellitus patients was found to be good, and the patient's self-care was mostly good. The resulting study was the effectiveness of DSME on patient self-acceptance and self-care.

Acknowledgements

The author would like to thank all nurses in Wound Care Center who have supported and assisted in the research process. We are also grateful to the academic community of the Faculty of Nursing and Midwifery, Universitas Prima Indonesia who always support in this research.

Conflict of Interest: We declare that there is no conflict of interest.

Source of Funding: Self

Ethical Clearance:

The ethical clearance was taken for the present study from the Ethics Commission of Universitas Prima Indonesia.

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Single-dose Acute Toxicity of 4-F-MDMB-BUTINACA Designer Cannabis Drug: LD50 and Histological Changes in Mice

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How to cite this article: Elkhatim H. Abdelgadir ElAmin, Renad H. F. Albloi, Abdulsallam Bakdash et al. Single-dose Acute Toxicity of 4-F-MDMB-BUTINACA Designer Cannabis Drug: LD50 and Histological Changes in Mice. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):145-152.

Abstract

The purpose of this research was to evaluate the single-dose toxicity of 4-F-MDMB-BUTINACA in Swiss albino mice using histopathological analysis of liver and kidney specimens.

The experimental protocol included oral treatment of mice with different doses (5, 50, 300, 2000 mg/kg body weight of 4-F-MDMB-BUTINACA) for 24 hours. At the end of the treatment, blood samples had been drawn, and renal and hepatic tissues have been excised from the experimental mice groups for histological examinations.

The results revealed that dose-dependent treatment with 4-F-MDMB-BUTINACA causes mild tremor clinical signs with low doses and photophobia (sensitivity to light) and even cessation of breathing as a potential cause of death with high doses in treated mice.

The LD₅₀ value of 4-F-MDMB-BUTINACA was 32.60 mg/kg, which is considered as a chemical compound of low toxicity. Histological studies confirmed that liver and kidney toxicities have been manifested in the findings of congestion, necrosis, inflammation, and bleeding within the liver and to lesser extent in the kidneys.

Keywords: Forensic Toxicology; 4-F-MDMB-BUTINACA; Synthetic Cannabinoid; LD₅₀; Histopathology; Mice.

Introduction

The vast majority of new psychoactive substances are synthetic cannabinoids (SCs).¹ SCs were published for the first time in Europe in the early 2000s. Between 2010 and 2011, the popularity of SCs in the USA has increased, and rates of poison control systems in the 2 years have accelerated by 240%.² A number of studies are increasing that associate SCs with acute behavioral and physiological effects.

Numerous in vitro studies have also demonstrated high cytotoxicity in various cell types for some of SCs.³⁻⁷

Acute systemic toxicity is an assessment of adverse effects that occur on a known route (oral, dermal or inhalation) within 24h following exposure to a single or multiple dose of a test substance.⁸ The test material is consumed and dispersed into different parts of the body until a systemic adverse effect is

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achieved after administration.⁹ The regulatory body requires an acute toxicity test study to mark and classify human substances.¹⁰⁻¹²

J. W. Trevan developed the LD₅₀ (median lethal dose) test in 1927 to measure the dose of a test drug that causes 50% mortality in a given species of animals. It is used to calculate the possible dangers of chemicals to humans.¹³

The acute toxic potential of substances must be assessed in order to ascertain the adverse effects that can occur as a result of unintended or intentional short-term exposure.¹⁴ Based on the outcome of the acute toxicity test, the toxicity of the test drug can be determined. LD50 substances below 5 mg/kg are considered highly toxic whereas LD50 substances above 15,000 mg/kg are considered to be relatively inoffensive (Table 1).¹⁵

4F-MDMB-BUTINACA also known 4F-MDMB-BINACA (Methyl 2-[[1-(4-fluorobutyl) indazole-3-carbonyl]amino]-3,3-dimethyl-butanoate) was first reported to the EMCDDA in November 2018. In 2019, the Institute of Forensic Medicine in Erlangen (Germany) analyzed three unlabeled, seized herbal blends and detected 4F-MDMB-BINACA as the active ingredient.

In spite of growing concern about the increased rates of 4F-MDMB-BUTINACA usage and its effects, there is a lack of information on how to cope with these problems. Consequently, the purpose of this study was to investigate the single-dose toxicity, and histopathological changes caused by 4F-MDMB-BUTINACA in Swiss albino mice.

Materials and Methods

Drug Preparation and Animals

4F-MDMB-BUTINACA was purchased from Cerilliant (Round Rock, TX, USA). The drug was first dissolved in dimethyl sulfoxide (DMSO) (final concentration was 2%) and then taken to its final volume with corn oil. As a vehicle control, DMSO and corn oil were also used.

A total of 30 Swiss albino male mice, aged 6 weeks and weighing approximately 20±25 g, were obtained from the Experimental Animal Care Center, King Saud University. The animals were maintained in climate-controlled rooms (23±2°C, relative humidity of 55±5%) with diurnal lighting (12:12-hour light: dark photoperiod) with free access to water and

commercial pelleted diet (Saudi Grains Organization, Riyadh, KSA). Before starting the experiment, the mice were acclimatized to the laboratory atmosphere for 1 week.

LD50 and Acute Toxicity Symptoms

The single-dose study was conducted in accordance with OECD 423 guidelines for testing of chemicals.¹⁶ A total of 30 mice were divided into five groups, one of which was used as a control. Each group consisted of 3 males and 3 females.

In order to assess acute toxicity, five groups of mice were orally gavaged with 4-F-MDMB-BUTINACA at dosages of 5, 50, 300, and 2000 mg/kg body weight (bw) in a final volume of 0.25 mL. A control group received only vehicle control.

The mice were individually observed for their general behavior at 1, 2, 3, 5, and 24 h after treatment. The number of deaths within this period was recorded, and LD₅₀ (the dose that kills 50% of animals) was determined according to the probit method (method of least squares) using the (Software Stat Plus) (Ver. 2015 Build 5.9.8.5 ©2015).

Necropsy was performed on all animals, and renal as well as hepatic tissues were preserved in 10% buffered formalin for histopathological analysis.

Histopathological Procedures

In an automated tissue processor (Tissue-tek VIP-5, from SAKURA), formalin-preserved hepatic and renal tissue samples from 4-F-MDMB-BUTINACA-dosed rats and the control group were processed. The tissues were then processed into paraffin wax blocks using standard procedures. Paraffin sections (4–5 µm) were stained with hematoxylin and eosin, the conventional staining technique. Stained sections were examined for histopathological changes.

Statistical analysis

The significance of variations between means was compared at every time point the use of Duncan's multiple range test (DMRT) after ANOVA for one-way classified data.¹⁷

Results

Acute toxicity symptoms and LD50

The administration of 4-F-MDMB-BUTINACA in low and high preload doses brought about

severe clinical symptoms, including tachycardia, convulsions, and difficulty in breathing. Further, an increase in locomotor activity of mice was also observed. Severe constriction and stiffness were observed for all muscles of the body. Similarly, narrowing of the eyes and prominent blood vessels in the ears were observed. There were deaths after

dosage ranging from 1 to 24 h; however, the animals which survived exhibited normal behavior, similar to the animals in the control group. The toxicity was observed to be a dose-dependent phenomenon. The LD₅₀ value was calculated to be 32.60 mg/kg bw (Table 1, Figure 1).

Table 1: Number of deaths by sex and dose for oral 4-F-MDMB-BUTINACA.

Gender	Dose mg/kg/ BD	Total	No. dead	Log of Dose	Mortality	Corrected Mort.	Probits
Male	2.5	3	0	0.398	0.00	8.33	3.625
	5	3	0	0.699	0.00	8.33	3.625
	50	3	1	1.699	33.33	33.33	4.33
	300	3	3	2.477	100.00	91.67	6.445
	2000	3	3	3.301	100.00	91.67	6.445
Female	2.5	3	0	0.398	0.00	8.33	3.625
	5	3	0	0.699	0.00	8.33	3.625
	50	3	2	1.699	66.67	66.67	5.44
	300	3	3	2.477	100.00	91.67	6.445
	2000	3	3	3.301	100.00	91.67	6.445
Combination	2.5	6	0	0.398	0	4.17	3.25
	5	6	0	0.699	0.00	4.17	3.625
	50	6	3	1.699	50.00	50.00	5
	300	6	6	2.477	100.00	95.83	6.445
	2000	6	6	3.301	100.00	95.83	6.445
Control M+F	0	6	0	0	0.00	0	0

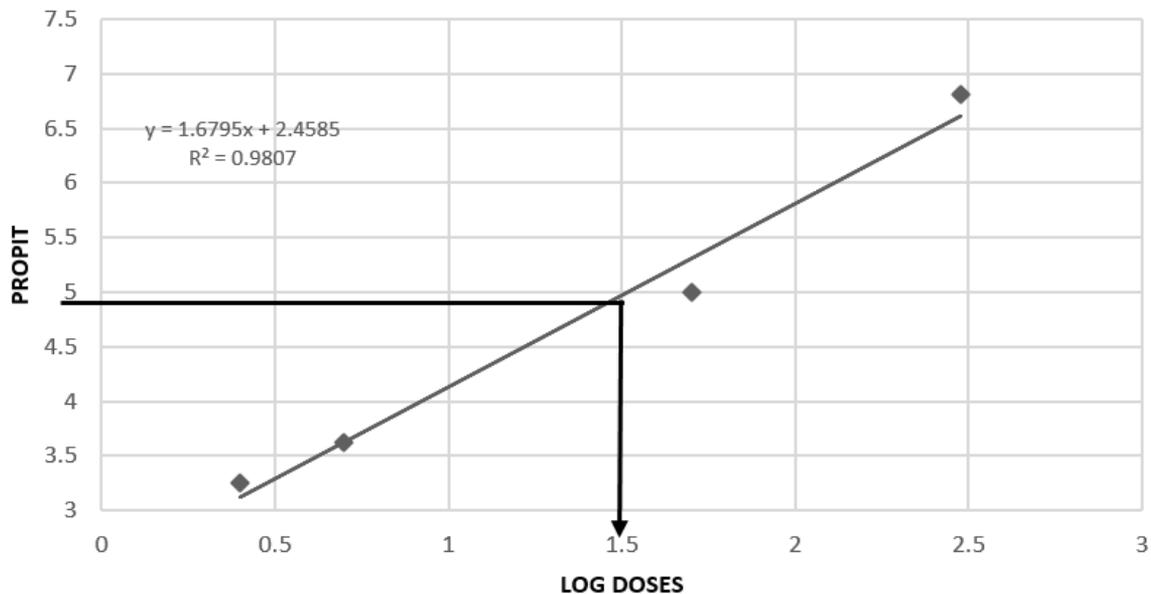


Figure 1: Curve graph for applying propite analysis to male and female mortality outcomes.

Figure 2: Photomicrograph of Liver(H&E-400X).

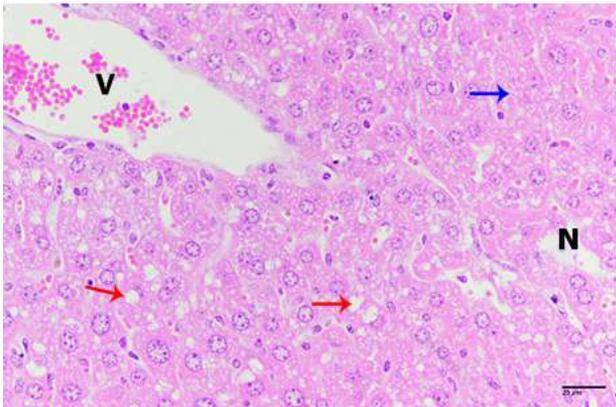


Figure 2a: Male mice liver treated with (5mg/kg) of 4-F-MDMB-BUTINACA.

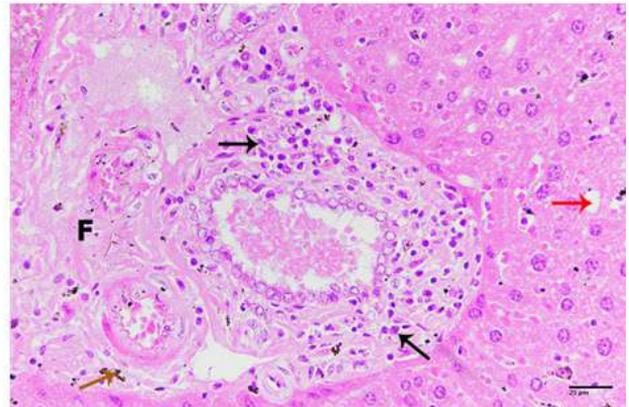


Figure 2b: Male mice liver treated with (50mg/kg) of 4-F-MDMB-BUTINACA.

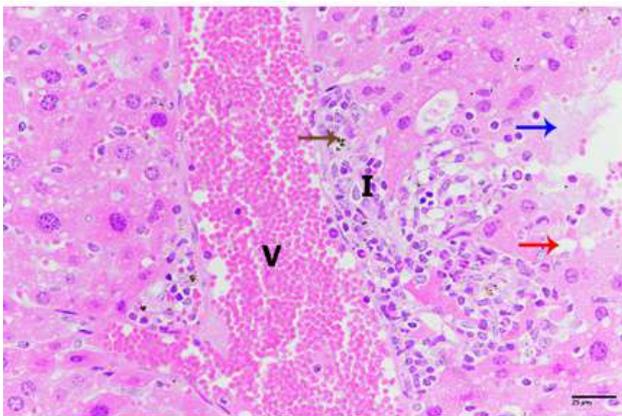


Figure 2c: Female mice liver treated with (300mg/kg) of 4-F-MDMB-BUTINACA.

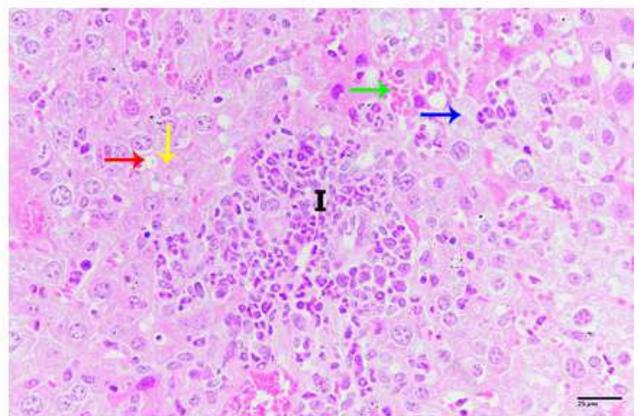


Figure 2d: Female mice liver treated with (2000 mg/kg) of 4-F-MDMB-BUTINACA.

Figure 3: Photomicrograph of Renal(H&E-400X).

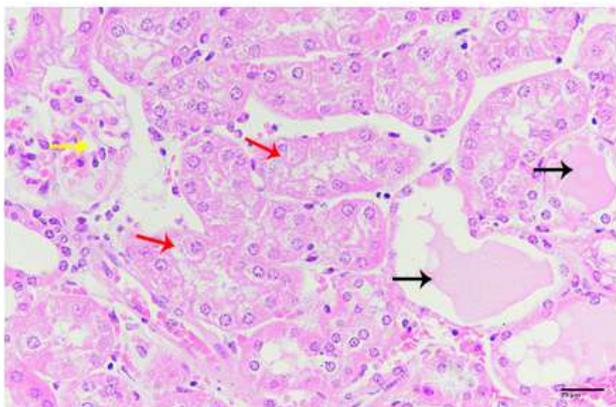


Figure 3a: Male mice kidney treated with (5mg/kg) of 4-F-MDMB-BUTINACA.

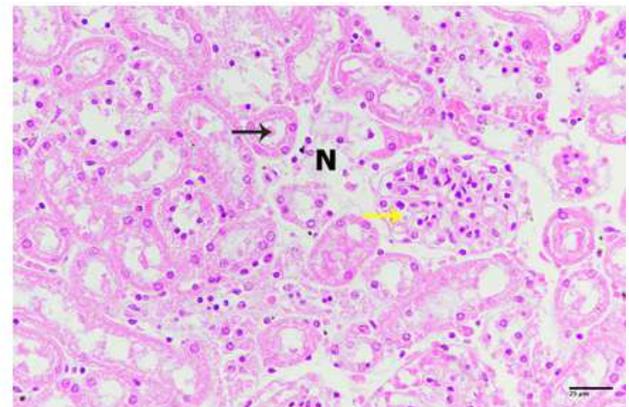


Figure 3b: Female mice kidney treated with (50mg/kg) of 4-F-MDMB-BUTINACA.

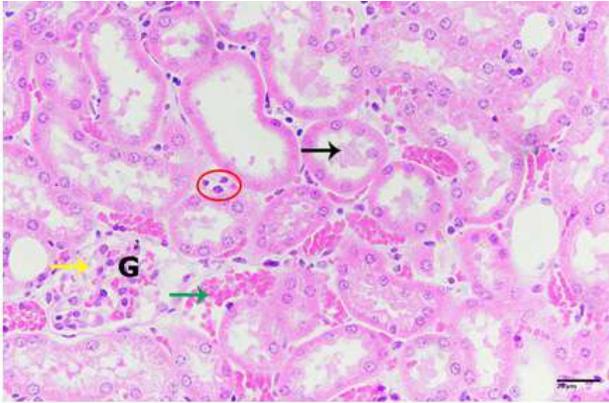


Figure 3c: Female mice kidney treated with (300mg/kg) of 4-F-MDMB-BUTINACA.

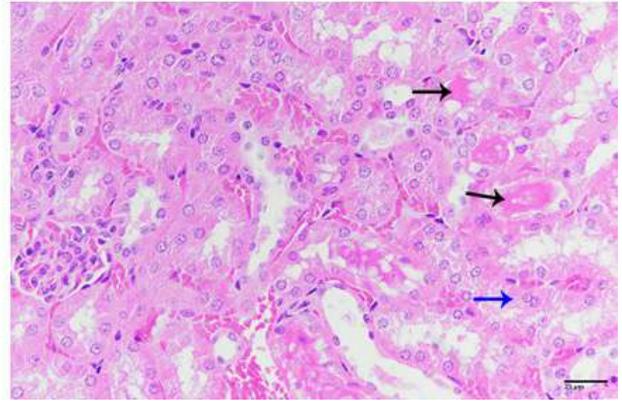


Figure 3d: Male mice kidney treated with (2000mg/kg) of 4-F-MDMB-BUTINACA.

Figure 4: Photomicrograph of Heart(H&E-400X).

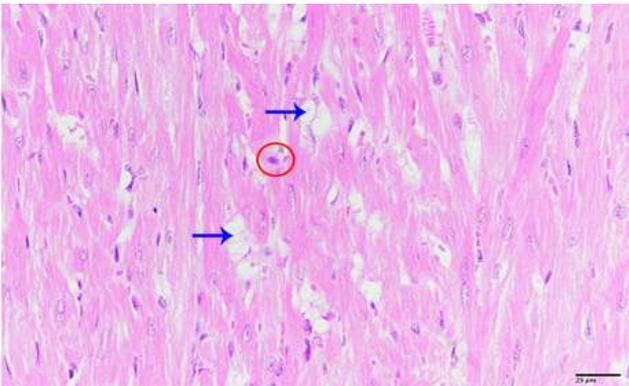


Figure 4a: Female mice treated with (5mg/kg) of 4-F-MDMB-BUTINACA.

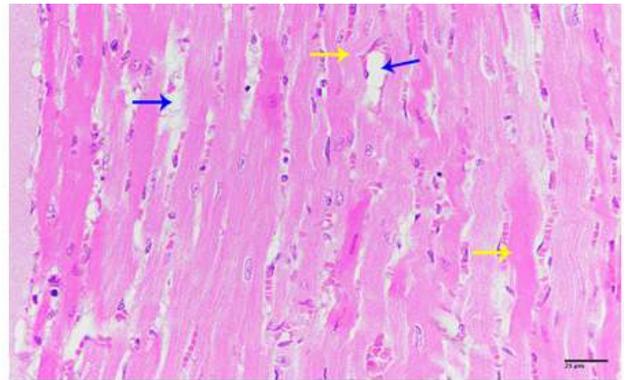


Figure 4b: Male mice cardiac muscles treated with (50mg/kg) of 4-F-MDMB-BUTINACA.

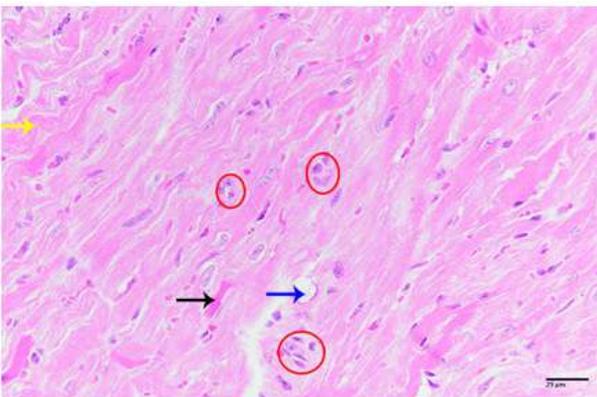


Figure 4c: Female mice treated with (300mg/kg) of 4-F-MDMB-BUTINACA.

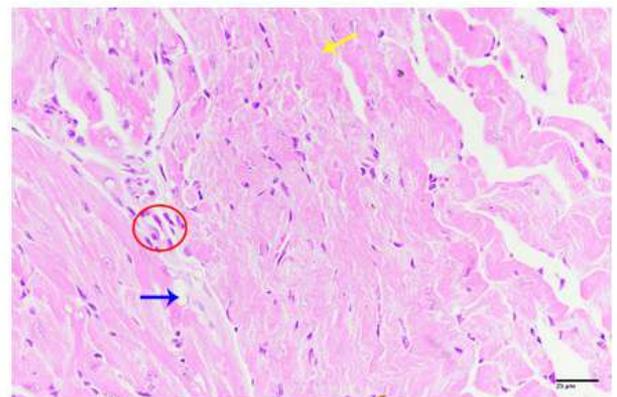


Figure 4d: Female mice treated with (2000mg/kg) of 4-F-MDMB-BUTINACA.

Histological studies

Liver histology after 24 h of treatment. Both male and female mice were treated with low and high doses of 4-F-MDMB-BUTINACA. Control liver

section from mice treated with a vehicle showed normal hepatic structure marked by central vein surrounded with anastomose network of hepatocytes with central abundant nuclei. Sometimes, hepatocytes looked binucleated due to its regeneration, besides

hepatocytes were separated from each other by blood sinusoid that contained Kupffer cells. Liver of both male and female mice were treated with (5mg/kg) of 4-F-MDMB-BUTINACA. revealed more pathological signs as congested veins with erythrocytes, more cytoplasmic degeneration and steatosis, besides too presence of hemosiderin granules, dilated vein with thickened wall, hepatocytes suffered from cytoplasmic degeneration, steatosis and necrosis (Fig. 2a). Liver of both male and female mice were treated with (50 mg/kg) of 4-F-MDMB-BUTINACA posted a great incidence of inflammation and macrophages, wide dilatation of vein filled with edema, hepatocytes showed steatosis and heavy incidence of hemosiderin granules, in addition, wide fibro-granulomatous reaction surrounded the bile duct consisted of inflammatory cells mixed with layers of fibrosis, hepatocytes showed steatosis and depositions of hemosiderin (Fig. 2b). Liver of both male and female mice were treated with (300 mg/kg) of 4-F-MDMB-BUTINACA displayed widely dilated and congested vein with erythrocytes surrounded by inflammation, hepatocytes showed steatosis and hyaline degeneration besides to precipitation of hemosiderin granules, displayed cytoplasmic degeneration, micro and macro-steatosis (Fig. 2c). Liver of both male and female mice were treated with (2000mg/kg) of 4-F-MDMB-BUTINACA exhibited aggregation of leukocytic infiltration besides to hemorrhage, hepatocytes displayed cytoplasmic degeneration and steatosis, others showed hyaline degeneration and some hepatocytes exhibited karyolysis which indicator for apoptosis, congested vein and steatosis besides to hemosiderin granules (Fig. 2d).

Kidney histology after 24 h of treatment. Both male and female mice were treated with low and high doses of 4-F-MDMB-BUTINACA. Control kidney section from mice treated with a vehicle Control renal tissue posted normal structure with abundant glomeruli in the Bowman's capsule, besides too presence of tubules sections as proximal convoluted tubules, distal convoluted tubules rather than collecting tubules. kidney of both male and female mice were treated with (5 mg/kg) of 4-F-MDMB-BUTINACA revealed atrophied and distorted glomeruli and tubular casts, some changed as atrophied glomeruli with foam cells, tubular cells showed marked degeneration and filled with edema (Fig. 3a). kidney of both male and female mice were treated with (50 mg/kg) of 4-F-MDMB-BUTINACA

exhibited wide necrotic areas filled with scattered inflammatory cells, glomeruli showed degeneration and foam cells, tubules showed severe degeneration and tubular casts, atrophied glomeruli with foam cells, in addition to hemosiderin presence (Fig. 3b). kidney of both male and female mice were treated with (300 mg/kg) of 4-F-MDMB-BUTINACA displayed distorted glomeruli filled with foam cells, tubules showed degeneration and casts, additionally, hemorrhage and leukocytic inflammatory exudate were seen (Fig. 3c) kidney of both male and female mice were treated with (2000mg/kg) of 4-F-MDMB-BUTINACA showed severe tubular degeneration and Casts filled degenerated tubules (Fig. 3d).

Heart histology after 24 h of treatment. Both male and female mice were treated with low and high doses of 4-F-MDMB-BUTINACA. Control cardiac section from mice treated with a vehicle showed normal cardiac muscles fibers characterized by disc central nuclei stained blue rather than striations and intercalated discs. Cardiac muscles of both male and female mice were treated with (5 mg/kg) of 4-F-MDMB-BUTINACA displayed many degenerated foci some aggregations of inflammatory cells, faint myocardial fibers associated with inflammatory cells depositions (Fig. 4a). Cardiac muscles of both male and female mice were treated with (50 mg/kg) of 4-F-MDMB-BUTINACA exhibited widely necrotic areas filled with erythrocytes and inflammatory cells and degenerated foci besides to wavy myocardial fibers due to contraction (Fig. 4b). Cardiac muscles of both male and female mice were treated with (300 mg/kg) of 4-F-MDMB-BUTINACA posted marked pathological changes as focal degeneration in the myocardial fibers, other fibers showed wavy appearance, scattered aggregations of inflammatory cells and small infarctions (Fig. 4c). Cardiac muscles of both male and female mice were treated with (2000 mg/kg) of 4-F-MDMB-BUTINACA cardiac muscles of female mice showed more degeneration and necrosis in addition to increase of inflammation and wavy myocardia fibers due to more contraction (Fig. 4d).

4-F-MDMB-BUTINACA -treated mice showed degeneration in liver and kidney tissues to varying degrees. Histological changes in liver included small aggregation of inflammatory cells besides to some cytoplasmic degeneration with karyolysis, vein congested with hemorrhage and edema and steatosis degeneration. Renal tissue showed degeneration of

the cell tubules and appearance of foam cells that looked empty in the glomerulus, tubular degeneration increased besides too presence of tubular casts and glomeruli suffered from atrophy accompanied by increasing the Bowman's space. Cardiac muscle fibers showed vacuolar degeneration of myocardial fibers and aggregations of inflammatory cells.

Discussion

Designer drugs have recently become a sensation and in order to circumvent drugs laws, these compounds are synthesized with subtle changes in their chemical structures compared to conventional psychotropic substances.¹⁸ Since 4-F-MDMB-BUTINACA has a varied legal status in different countries,¹⁹ determining its toxicity and multiple effects in laboratory animals is critical for forensic investigation.

There were many toxic symptoms exhibited by the treated mice that began shortly after administration, indicating that the drug was rapidly absorbed and distributed. 4-F-MDMB-BUTINACA has low toxicity on the Hodge and Sterner toxicity scale of 24-h acute toxicity.²⁰ The LD₅₀ of 4-F-MDMB-BUTINACA was 32.60 mg/kg bw. This is a low value in comparison to natural cannabis drugs, particularly the active ingredient in the tetrahydrocannabinol (THC) cannabis, which has LD₅₀ values of 42 and 482 mg/kg bw when administrated intravenously and orally, respectively. However, it was found that 4-F-MDMB-BUTINACA toxic symptoms were similar to THC cannabis in occurrence, the most important of which is decreased locomotor activities with low doses and stimulated movements and jumping with high doses (Beaulieu 2005). In contrast, 4-F-MDMB-BUTINACA symptoms appear faster and within a shorter time. In comparison to other designer cannabis drugs such as CP-47497, CP-55940, UR-144, JWH-133, JWH-149, JWH 073, and NM-2201 (the orally LD₅₀ values in mice were 5000, 5600, 5600, 5000, 5600, 5600, and 2460 mg/kg/BW, respectively), THJ-2201 is considered more toxic.²¹

Presently, 4-F-MDMB-BUTINACA pharmacological statistics are unavailable, but they should possess affinity to cannabinoid receptors similar to AM-2201.²²⁻²³ There are several individuals who reported their experience on drug-user forums after the oral administration of 4-F-MDMB-BUTINACA. Most of

the users used oil, milk, alcohol, and butter to consume the drug orally. They claim that outcomes began to appear after 50 min, peaking after ~3 h and subsiding after 4-5 h. The user also claimed that effects were extremely similar to dextromethorphan and multiple bong hits. Signs mentioned by most of them covered slamming the head several times into the wall or on the floor, depressed breathing, increased heart rate, and violent shaking.²⁴ Unfavorable reactions along with kidney pains, muscle spasms, and paranoia have been also stated on drug-user forums.²²

This is the first study to explore the cytotoxic effects of 4-F-MDMB-BUTINACA on liver and kidney tissues. This study indicates that 4-F-MDMB-BUTINACA has high toxicity, its toxic effects on liver tissue were close to the effect of cocaine, which is classified as highly toxic and causes liver damage and inflammation.²⁵ The inflammation, necrosis, and congestion seen in kidneys of 4-F-MDMB-BUTINACA-treated mice were similar to the changes found by Dargan et al. (2014), who studied the effects of methoxetamine (analogue of ketamine) in renal toxicity in mice.²⁶

Conclusion

4-F-MDMB-BUTINACA is of high acute toxicity but had rapid effects after administration. Toxic effects began with mild tremors at low doses and progressed to photophobia and even cessation of breathing with high doses. In hepatic tissue, small aggregation of inflammatory cells besides to some cytoplasmic degeneration with karyolysis, vein congested with hemorrhage and edema and steatosis degeneration. Renal tissue showed degeneration of the cell tubules and appearance of foam cells that looked empty in the glomerulus, tubular degeneration increased besides too presence of tubular casts and glomeruli suffered from atrophy accompanied by increasing the Bowman's space. Cardiac muscle fibers showed vacuolar degeneration of myocardial fibers and aggregations of inflammatory cells.

Ethics Clearance

Ethical approval for this study was obtained from Naif Arab University for Security Science Ethics Committee (**Nauss-Rec-21-06**).

Source of Funding: Nill

Conflict of Interest: Nill

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Correctional Institutions as a Place of Guidance to Counter Radicalism for Terrorist Prisoners in Indonesia

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How to cite this article: Erlangga Alif Mufti, Edy Lisdiyono, Retno Mawarini Sukmariningsih et al. Correctional Institutions as a Place of Guidance to Counter Radicalism for Terrorist Prisoners in Indonesia. *Indian Journal of Forensic Medicine and Toxicology* 2022;16(3):153-158.

Abstract

Correctional Institution as a place to house terrorist inmates have not managed to solve the problem of deradicalization to complete for terrorist inmates, so it is necessary to cooperate with other relevant state institutions. But the deradicalization process has obstacles such as inmates being unwilling to cooperate and the absence of synergy with other applicable state institutions. The parameters of the success of correctional institutions in conducting deradicalization are necessary to strengthen the process of returning terrorism convicts to the community, so that this research is important to do. This study uses the research method that is juridical normative by collecting primary data and secondary data. The results of this study describe the correctional institution to be the vanguard in the process of deradicalization of terrorism convicts to be ready to return to the community so that the implementation of effective deradicalization required cooperation from correctional institutions, relevant state institutions, and inmates. Correctional institutions are an important vector in the fight against radicalism. The role of correctional institutions in the deradicalization of terrorism prisoners is to synergize with other institutions to foster terrorist prisoners to become good human beings when they leave correctional institutions.

Keywords: Correctional Institution; Deradicalization; Terrorism

Introduction

To reform the criminal system and implementation, the term prison system in Indonesia has been changed to the correctional system, and the prison term is changed to a correctional institution. Correctional institutions in Indonesia are still in the public spotlight because they often experience various problems that do not end, ranging from overcapacity, the occurrence of illegal levies,

including being a place of terrorist recruitment. Inmates who experience problems will become people who are not accepted by society when they are free to make fertile ground for recruiters of would-be terrorists.¹ Terrorist inmates captured and put into correctional institutions are trying to build new terrorist cells inside the correctional institution. Like persuading a child to follow the wishes of terrorist inmates, of course, non-terrorist inmates are given an encouraging gift if they want to follow it.

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The process of coaching terrorist inmates can not be seen as the same problem as other inmates, such as perpetrators of corruption or drug crimes. Radicalism becomes the seeds that thrive in the womb of terrorism crimes that will further give birth to terrorist convicts.² Other inmates may regret the actions that led to them serving their sentences. That's not the case with terrorist inmates. Some terrorist inmates do not feel remorse for acts that society or the state view as terrorist acts.³

The motives of terrorist inmates are very different from the motives of other inmates. The motive of the actions of terrorist inmates is certain beliefs, ideologies, or understandings that are actualized fanatically as a life choice.⁴ For the sake of belief, ideology, or understanding, "sahid death" is the life choice of the inmates, and they do not hesitate to take actions to actualize those beliefs, ideologies, or understandings.⁵ Even among the perpetrators of terrorist crimes, some seem satisfied and proud after committing actions that the public or the state considers terrorist acts.⁶ They also did not feel pressured by the harsh international condemnation of many bombings that caused severe damage. They resulted in many victims suffering, injuries, hundreds died and caused a widespread impact.⁷

A particular problem in correctional institutions in Indonesia is that they should pay special attention to terrorist inmates. Terrorism convicts have a very different psychological and mental attitude than other case inmates.⁸ Terrorism convicts have an exclusive attitude by only gathering in groups, being uncooperative with the guard warden, and can teach their beliefs to other prison inmates or even to all guards and prison officers.⁹ The main plan to deal with terrorist inmates is to be given supervision and deradicalization coaching. The approach to terrorism convicts cannot be equated with the system to ordinary inmates. A special process or preferential treatment of terrorist convicts is necessary due to the risks and special needs of terrorism convict groups.¹⁰ One particular approach in the deradicalization of terrorism convicts is the placement of terrorist inmates in correctional institutions. As it is known that the characteristics of terrorist inmates are different from other inmates, namely the extreme radicalism and become a life belief that becomes a guide for the behavior of every terrorist convict.¹¹

The deradicalization process in correctional institutions faces several obstacles, including

excess prison capacity, limited personnel resources in quantity and expertise in the deradicalization process, infrastructure, and behavior of terrorist inmates released.¹² These obstacles make it difficult for correctional institutions to place terrorist inmates in prisons with special characteristics and a high-risk level.⁶ The placement of terrorist inmates in correctional institutions should be done considering the level of risk of each terrorist inmate, the implementation of the program of coaching, and the ability of penal institutions to foster terrorist inmates.¹³ The process of coaching on the placement of terrorist inmates is strongly related to the success of the deradicalization process to straighten out the ideology of terrorist inmates and eliminate the understandings of terrorism.¹⁴

Based on the description above of the problems that will be reviewed in this paper, First how the current correctional institution in dealing with terrorism, Second Why has not integrated handling of the perpetrators of terrorism crimes, in the sense that the handling of deradicalization of terrorism perpetrators seems to stop when they have been caught or convicted. The purpose of this paper is to find out how the implementation, consideration, and analysis in support of deradicalization for terrorist inmates in correctional institutions and to know the solutions to the obstacles faced.

Research Methods

The research method is normative juridical, which is done by researching library materials or mere secondary materials.¹⁵ This research is Normative Juridical Research on correctional institutions as a place of development for deradicalization of terrorism convicts in Indonesia. Data analysis methods are carried out by collecting data through the analysis of library sources or secondary data that includes primary legal materials, secondary legal materials, and terrier legal materials, both in the form of documents and applicable laws and regulations relating to the normative juridical analysis of correctional institutions, terrorism convicts and radicalization. To analyze the legal materials that have been collected, in this study using qualitative data analysis method that is normative juridical presented descriptively, namely by describing a regulation related to deradicalization of terrorism narratives in correctional institutions in the legal system in Indonesia and further assessment of whether the application is by the normative provisions.

Result and Discussion

An important key of a terrorist organization is recruitment. Recruit member can use their skills to see, assess, and encourage potential recruits to follow the same path of struggle. The process carried out by terrorist recruitment has four recruitment schemes in extensive networks, information funnels, infecting, and crystal embryos.¹⁶

The recruitment scheme can be successful if the community or target group has a tangent way of thinking; for example, if all targets are interested in getting the same books to read or invited to regular meetings. In this case, the target is seen as having homogeneity, and the homogeneous group can be lured through a specific scripture or verses that are precisely the same in a pouch.¹⁷ Funnel patterns occur when a recruiter takes an incremental approach when they believe the target or population focus segment is the primary target that has a large following. This process requires an individual to have the right motivation and undergo a transformation in identity. Therefore individuals begin at one end of the process and are transformed into dedicated group members at the other end. Infection patterns occur when trusted agents are incorporated into the target population to raise potential recruits through direct personal appeal. Infections tend to succeed where most of the members are ordinary people; this allows intruders to convert disgruntled elected members.¹³ Finally, the embryo crystal pattern occurs when the target is challenging to access and very far away. It can be compared to lowering the temperature of the glass that is flooded with boiling water, waiting until the water in it becomes cold until then the ice crystals form as a form of total freezing or, in other words, an unconditional surrender to the doctrine. Al-Qaeda groups often adopt this approach and are claimed to be most successful in reaching populations in restricted and difficult areas, such as prisons.¹⁸

Terrorism in Indonesia is an extraordinary crime that attacks human rights, the economy and is a severe threat to national sovereignty because terrorism is an international crime that endangers peace, security, and welfare, as stated in the Universal Declaration on Human Rights.¹⁹ A planned and sustainable eradication can solve this act of radicalism to protect and protect human rights. A clear and severe criminal penalty must accompany terrorism as a crime, and there is no bargaining chip for it. A terrorist who has been sentenced to prison for committing a terrorism

offense should receive special attention. In sentencing, it is indiscriminate and does not matter whether terrorist convicts feel like citizens harmed by the state.

The placement of terrorist inmates and the awarding of criminal penalties are one form of punishment for actions that have been committed. There are several theories about the purpose of criminal law in criminal law, namely absolute theory (retributive), relative theory (deterrence/utilitarian), merger theory (integrative), treatment theory, and social defense theory. The theories of criminalization consider various aspects of the target to be achieved in criminal prosecution. Absolute theory (retributive theory) sees that criminalization is retaliation for the mistakes that have been made and is oriented towards the actions of the proceeds of the crime itself. The perpetrator must receive the criminal penalty for the offense he has committed. The basis of punishment must be given according to the crime itself because the crime has caused suffering to others; in return (vergingelding), the perpetrator must be given the suffering and criminal sanctions as well as in prison.

The legislation of both the Criminal Code and the Correctional Act, which is associated with the theory of the purpose of criminalization, has the purpose of criminalization of terrorists using a combined theory (integrative), where terrorist inmates detained in correctional institutions, on the one hand, are a form of punishment and retaliation for acts of terrorism that have been committed, and on the other hand, aim to foster that convicted perpetrators can change and realize their mistakes. Therefore in placing terrorist inmates must be for common criminal purposes and bring about change. The placement of terrorist criminals in correctional institutions is part of the process of coaching inmates.²⁰ The current disciplinary paradigm focuses on the way inmates are trained, not just as a form of criminalization or retaliation for actions that have been taken. by the correctional system based on Pancasila and the Constitution of the Republic of Indonesia 1945, which is the final part of the criminalization system, terrorism convicts are given treatment to be target citizens in correctional institutions.

Inmates must be protected and guided by providing life support to become valuable citizens and accepted by the community after their release from the correctional institution. By giving the claim, it is clear that imprisonment is not intended as an act of revenge from the state. By Article 2 and Article 3 of Law No. 12 of

1995 concerning Correctional Services that correctional institutions as a place of inmate development have the purpose and function of fostering the target citizens to realize their mistakes and prepare the community can be harmonious and united with the district, so that when free inmates can be re-accepted by the community. The principle of imprisonment in a correctional institution is to retaliate against inmates' actions and bring about change to those inmates. By giving the claim, it is clear that imprisonment is not intended as an act of revenge from the state. The system of development in correctional institutions is carried out based on the principles: equal treatment and service, respect for human dignity and dignity, loss of independence, and guaranteed right to stay in touch with certain families and people regulated explicitly in Article 5 Correctional Law, The principles of Coaching in principle include three correctional thoughts that are as goals, processes, and methods. First. As a meaningful goal with the construction of correctional services, inmates are expected to be aware of their actions, straighten their views, and become citizens who obey the applicable laws. Second. As a process means a variety of activities that must be done during Coaching and mentoring. Third. As a method is a way that must be taken to achieve the goal of coaching and mentoring with the correctional system. Coaching is carried out through 3 stages, namely the first stage of the beginning (administration/orientation), the advanced stage (Coaching, assimilation), and the Final Stage (reintegration).

The guidance of inmates in prisons requires serious attention by paying attention to the condition of each perpetrator of the crime, as well as his relatives. The particular problem in dealing with terrorism convicts is integrating the handling of terrorism criminals,²¹ where the handling of terrorism perpetrators seems to stop when caught and sentenced. The construction of inmates of this category can not be seen as the same problem as other case inmates, such as perpetrators of corruption or drug crimes. Terrorist inmates are born from the womb of radicalism and terrorism. Special treatment needs to be given to terrorist inmates, where the treatment of terrorism convicts cannot be equated with the treatment of other case inmates. Their treatment should eliminate the opportunity for them to engage in terrorist activities inside and outside the Prison.

The motives of terrorist inmates compared to the reasons of other inmates' actions are very different. The motive of the activities of terrorist inmates is

certain beliefs, ideologies, or understandings that are actualized fanatically as a life choice. For the sake of religion, ideology, or understanding, "sahid death" is the life choice of the inmates, and they do not hesitate to take actions to actualize those beliefs, ideologies, or understandings. Even among the perpetrators of terrorist crimes, some seem satisfied and proud after committing actions that the public or the state considers terrorist acts.⁵ They also did not feel pressured by the harsh international condemnation of many bombings that caused severe damage. They resulted in many victims suffering, injuries, hundreds died, as well as causing a widespread impact. Special treatment or different treatment of terrorist inmates is also due to the needs and risks inherent in him. The moral basis of such therapy is that other treatment can not always be interpreted as violating the principle of equality of treatment and service (the principle of non-discrimination).

Personality development and self-reliance in line with the purpose of a correctional system that bridges and rehabilitates, changing the attitudes, mental and behavior of terrorist inmates towards a positive life through religious, socio-cultural, and economic approaches is a form of coaching terrorist inmates. The guidance can provide enlightenment of thought and open insight to terrorist inmates with peaceful and tolerant religious knowledge and national understanding within the framework of the Unitary State of the Republic of Indonesia. For Terrorist Prisoners, there is an addition in the process of coaching it while in prison, namely deradicalization, especially from the doctrines of terrorism, which is a program of the National Counterterrorism Agency (Badan Nasional Penanggulangan Terorisme (BNPT)).¹²

Collaboration between government institutions to combat deradicalization plays a significant role in building law and legal awareness or law-abiding culture in the community. Because the radicalism that triggers acts of terrorism has many sleeper cells that at any time can rise and threaten the sovereignty of the country, the problem of radicalism indicated by terrorist inmates should not be looked at in the eyes and is always the primary concern so as not to spread to other inmates in prisons or influence. In contrast, terrorist inmates return to communities that have not been affected by radicalism.

Deradicalization attempts to neutralize radical understandings through interdisciplinary approaches, such as law, psychology, religion,

and socio-culture for individuals who are radically influenced and like violence¹. The primary purpose of deradicalization is to make violent terrorists or groups willing to abandon or disengage themselves from acts and activities of terrorism. In particular, the goal of deradicalization is: first, to make the terrorists ready to leave acts of terrorism and violence. Second, radical groups support moderate and tolerant thinking. Third, radicalizes and terrorists can support national programs in building national and state life within the frame of the Unitary State of the Republic of Indonesia.

The implementation of deradicalization in Indonesia is described as an integrative, whole, and sustainable program with two kinds of coaching, namely deradicalization outside prisons and deradicalization in prisons. Deradicalization outside prison consists of the stage of identification, development of counter-radicalization, and monitoring and evaluation. Deradicalization in Prisons consists of the title, rehabilitation, reeducation, association, and monitoring and evaluation. Deradicalization programs are implemented gradually so that goals and objectives can be achieved effectively. Deradicalization efforts are essential for terrorist inmates in correctional institutions to straighten out the understanding or ideology of radicalized terrorist inmates. Because prison sentences do not necessarily make them aware, even the prison becomes a place to learn more deeply the ideology they believe in and does not close the possibility of disseminating its ideology to fellow inmates.

Prisons are vulnerable places, and it is easy to radicalize. Radicalization is the process by which inmates are recruited, instigated, and involved in extreme groups in prisons or processes where inmates who are already engaged in radical groups become more radicalized and spread that understanding to other inmates. The punishment and placement of terrorist inmates in correctional institutions is a form of punishment for terrorist crimes committed. The sentence given in disciplinary science is not only to create a deterrent effect and eliminate the right to physical independence. Still, it is part of the process of coaching conducted by the Correctional Institution and deradicalization programs that aim to make terrorist inmates aware of their mistakes, straighten out radicals and terrorism, and not repeat their actions and change in a more acceptable direction.

Three main aspects must be considered in the placement of terrorist inmates: the level of risk,

development programs and human resources capabilities, and correctional institutions' infrastructure. The three aspects are interrelated and affect the success of correctional institutions in conducting inmate training. Therefore the placement of terrorist inmates must be carried out carefully and adapted to the needs of coaching.

The constraints of the placement of terrorist inmates are still constrained by several things, such as: First, limited human resources of correctional officers both in quantity, quality and competence to conduct the coaching of terrorist inmates, mainly to conduct profiling and assessment. The standard provision of terrorist inmate training in correctional institutions must be at least four employees for special officers who build 10 - 20 terrorist inmates. The special employee must have competence, knowledge and educational background, among others, legal scholars, religious scholars and psychology scholars and have supporting experience. But in reality, the employees who have such special skills are relatively few and unevenly present in all correctional institutions. Second, Not ideally, most correctional institutions' conditions to foster and place terrorist inmates by the needs and adequate security standards are due to overcapacity, old-model building layout, security completeness, facilities, and supporting infrastructure. Third, Unconscionable and implantation of the system of coaching and placement of terrorist inmates by correctional officers. Fourth, Not optimal cooperation between the Ministry of Justice and Human Rights with other agencies, especially with BNPT in the framework of coaching terrorist inmates. In some prisons, the method of coaching for terrorist inmates that are not yet known by correctional officers, in addition to the limited data and information of convicted terrorists owned by correctional institutions, causes the pattern of inmate coaching does not run optimally. a Fifth is the behavior of terrorist inmates are largely uncooperative, unwilling to participate in coaching programs, be exclusive, closed, and potentially spread radicalism to other inmates or even to officers.

Conclusion

Correctional Institutions are at the forefront of deradicalizing terrorism convicts to be ready to return to society. So that in the implementation of effective deradicalization required the participation of correctional institutions, relevant state institutions, and inmates. Correctional institutions are an

important vector in the fight against radicalism. The role of prisons in the deradicalization of terrorism prisoners is to synergize with other institutions to foster terrorist prisoners to become good human beings when they leave Correctional institutions. The correctional institution in collaboration with BNPT and other institutions synergize with each other to deradicalize and relate terrorism convicts so that they can analyze policies and approaches, identify dilemmas, and create road maps while finding new ideas in countering radicalization and returning terrorist convicts to society.

Acknowledgment

We would like to thank all those who have helped during the research. This research was independently funded by the researchers.

Ethical Clearance: Data taken from the books, websites and use of legal provisions only.

Source of Support: Self

Conflict of Interest: None

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Observation of Tri-allelic Patterns in Autosomal STRs during Establishment of Genetic Fingerprint Database for the Iraqi Security Forces

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How to cite this article: Esam Ghazi Mohammed Salih, Mohammed Ibrahim Nader, Majeed Arsheed Sabbah et al. Observation of Tri-allelic Patterns in Autosomal STRs during Establishment of Genetic Fingerprint Database for the Iraqi Security Forces. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):159-164.

Abstract

We report five cases of tri-allelic patterns observed during establishment of genetic fingerprint database for 354 individual from Iraqi security forces volunteers. These individuals had been typed by using the PowerPlex® Fusion System for the following 22 autosomal STRs: D3S1358, D1S1656, D2S441, D10S1248, D13S317, Penta E, D16S539, D18S51, D2S1338, CSF1PO, Penta D, TH01, vWA, D21S11, D7S820, D5S818, TPOX, D8S1179, D12S391, D19S433, FGA and D22S1045. All five tri-allelic patterns were observed at the Penta D locus and had the genotype 9/11/13, 9.4/13/14, 9/10/11, 7/9/11 and 9/10/14 respectively. All cases belonged to the Type 2 tri-allelic pattern; involve a fairly balanced set of three alleles. Five cases in 354 typed individuals is a frequency for tri-allelic patterns in autosomal STRs of 1.41%.

Keywords: Tri-allelic patterns; DNA typing; Autosomal; Short tandem repeat (STR); PowerPlex® Fusion System; Iraqi security forces; Iraq.

Introduction

Genotyping of polymorphic short tandem repeat (STR) loci is widely used in forensic DNA analysis and kinship testing. Together with off-ladder alleles and primer binding site mismatches yielding silent (null) alleles, tri-allelic patterns are a third category of genotyping irregularities which can be encountered during STR profiling.^{1,2} Current study reported five cases of tri-allelic patterns observed during

establishment of genetic fingerprint database for the Iraqi security forces.

Material and methods

Iraqi Security Forces (ISF) samples (354) were collected. Whole blood samples, buccal swabs and hair follicles were the sample types of choice. Blood is one of the richest sources of DNA. All samples were collected from donors volunteering in the

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AL-Muthana Military hospital by dedicated medical personnel. Three milliliters of blood were collected through venipuncture into 3 ml sterile EDTA blood collection tubes which were labeled with the subject's name, rank, age, sex and the date. Afterwards, the blood stains were made from the collected blood on a FTA™ Classic Card (Whatman™).

FTA cards were packed into individual clean paper envelopes for easy transfer to their final destination in the forensic genetic laboratories in DNA Typing Department of Medico-legal Directorate / Ministry of Health / Baghdad / Iraq. All samples were made anonymous, given serial numbers after collection and analyzed according to standard operating procedures. All possible measures were taken to prevent contamination. DNA profiles consisting of the following 22 autosomal STRs were determined: D3S1358, D1S1656, D2S441, D10S1248, D13S317, Penta E, D16S539, D18S51, D2S1338,

CSF1PO, Penta D, TH01, vWA, D21S11, D7S820, D5S818, TPOX, D8S1179, D12S391, D19S433, FGA and D22S1045, using the PowerPlex® Fusion System (Applied Biosystems). PCR products were separated by capillary electrophoresis on the ABI 3130xl Genetic Analyzer (Applied Biosystems) and alleles were identified using ABI's Genemapper software.

Results

In the present study, five tri-allelic patterns were observed at the Penta D locus of samples 35, 65, 93, 100 and 145 (Table 1). The Penta D tri-alleles are 9/11/13, 9.4/13/14, 9/10/11, 7/9/11 and 9/10/14 respectively as shown in Figures (1, 2, 3, 4 and 5), making these samples an example of a "Type II" tri-allelic pattern, according to the nomenclature of Clayton et al.³ These samples genotyped twice on the genetic analyzer 3130xl to verify the results and the same tri-allelic pattern were obtained.

Table 1: The total tri-allelic patterns observed in present study and reported on STRBase.

STRBase		In present study	
STR Locus	Variant alleles	STR Locus	Variant alleles
CSF1PO	9/11/12; 10/11/12	—	—
FGA	19/20/21; 19/22/23; 19/24/25; 20/21/22; 20/21/24; 20/23/24; 21/22/23; 21/25/26; 22/24/25; 22.2/23/23.2	—	—
TH01	7/8/9	—	—
TPOX	6/8/10; 6/9/10; 6/10/11; 6/10/12; 7/9/10; 7/10/11; 8/9/10; 8/10/11; 8/10/12; 8/11/12; 9/10/11; 9/10/12; 10/11/12	—	—
VWA	11/16/17; 12/18/19; 14/15/17; 14/15/18; 14/16/18; 14/17/18; 15/16/17; 18/19/20	—	—
D3S1358	15/16/17; 15/17/18; 16/17/19; 17/18/19	—	—
D5S818	10/11/12; 11/12/13	—	—
D7S820	8/9/12; 8/10/11	—	—
D8S1179	10/12/13; 10/12/15; 12/13/14; 12/13/15; 13/15/16	—	—
D13S317	8/11/12; 10/11/12; 10/12/13	—	—
D16S539	12/13/14	—	—
D18S51	12/13/15; 12/14/15; 12/16/17; 14/15/22; 15/16/20; 16/17/20; 19/22.2/23.2	—	—
D21S11	28/29/30; 28/30.2/31.2; 29/31/32; 30/30.2/31	—	—

Contd... Table 1: The total tri-allelic patterns observed in present study and reported on STRBase.

Penta D	None reported yet in STRBase	Penta D	9/11/13; 9.4/13/14; 9/10/11; 7/9/11; 9/10/14
Penta E	None reported yet in STRBase	—	—
D2S1338	None reported yet in STRBase	—	—
D19S433	None reported yet in STRBase	—	—
SE33	None reported yet in STRBase	—	—
D6S1043	None reported yet in STRBase	—	—
D1S1656	None reported yet in STRBase	—	—

Applied Biosystems
GeneMapper ID v3.2

ESAM.111818.KH.

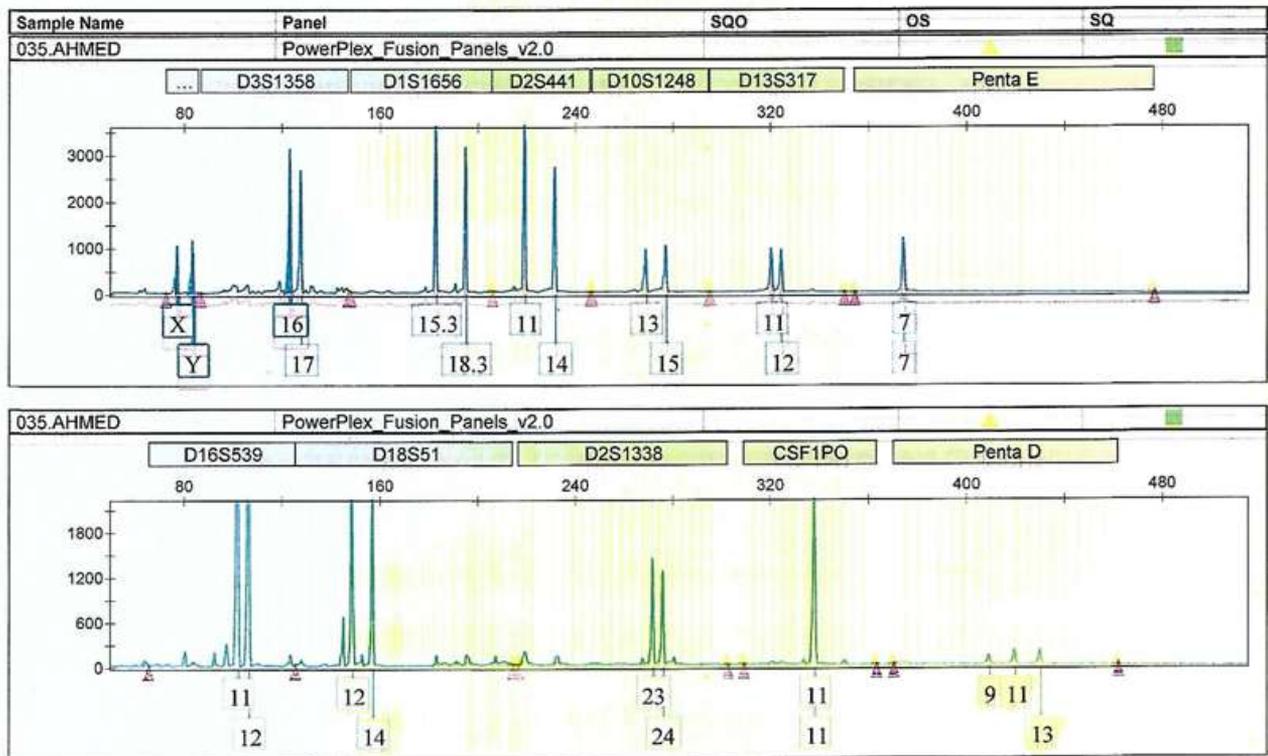


Figure 1: Tri-allelic pattern 9/11/13 observed in Penta D genetic locus from sample number 35.

Applied Biosystems
GeneMapper ID v3.2

ESAM.111818.KH.

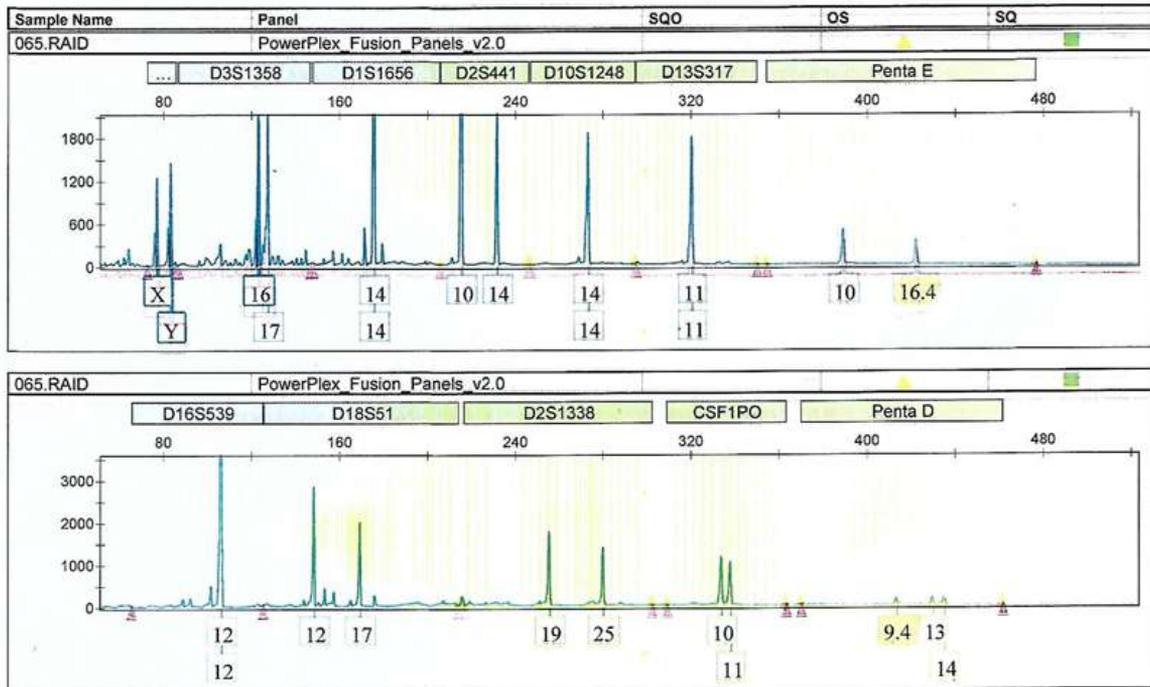


Figure 2: Tri-allelic pattern 9.4/13/14 observed in Penta D genetic locus from sample number 65.

Applied Biosystems
GeneMapper ID v3.2

120318.KH.ESAM.2

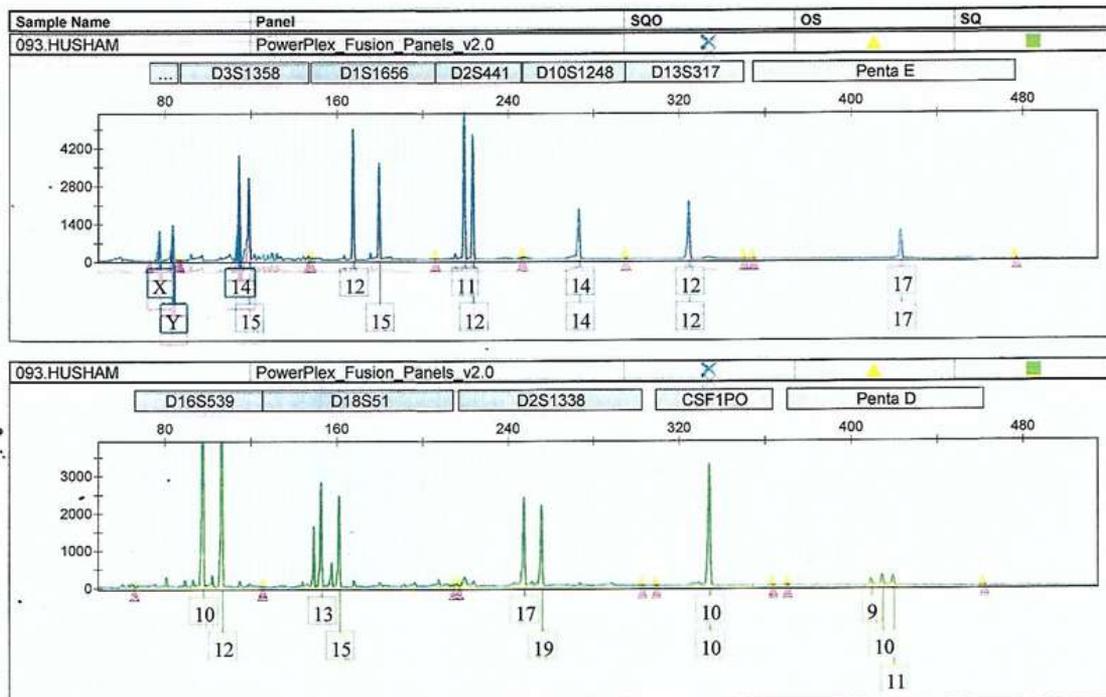


Figure 3: Tri-allelic pattern 9/10/11 observed in Penta D genetic locus from sample number 93.

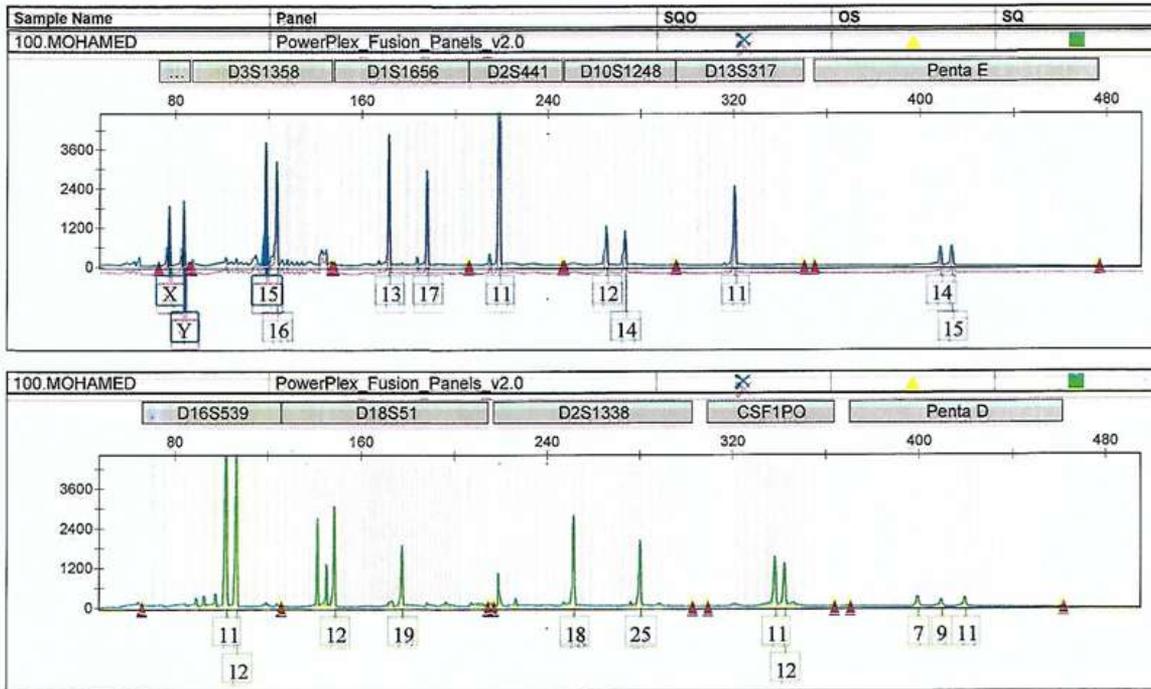


Figure 4: Tri-allelic pattern 7/9/11 observed in Penta D genetic locus from sample number 100.

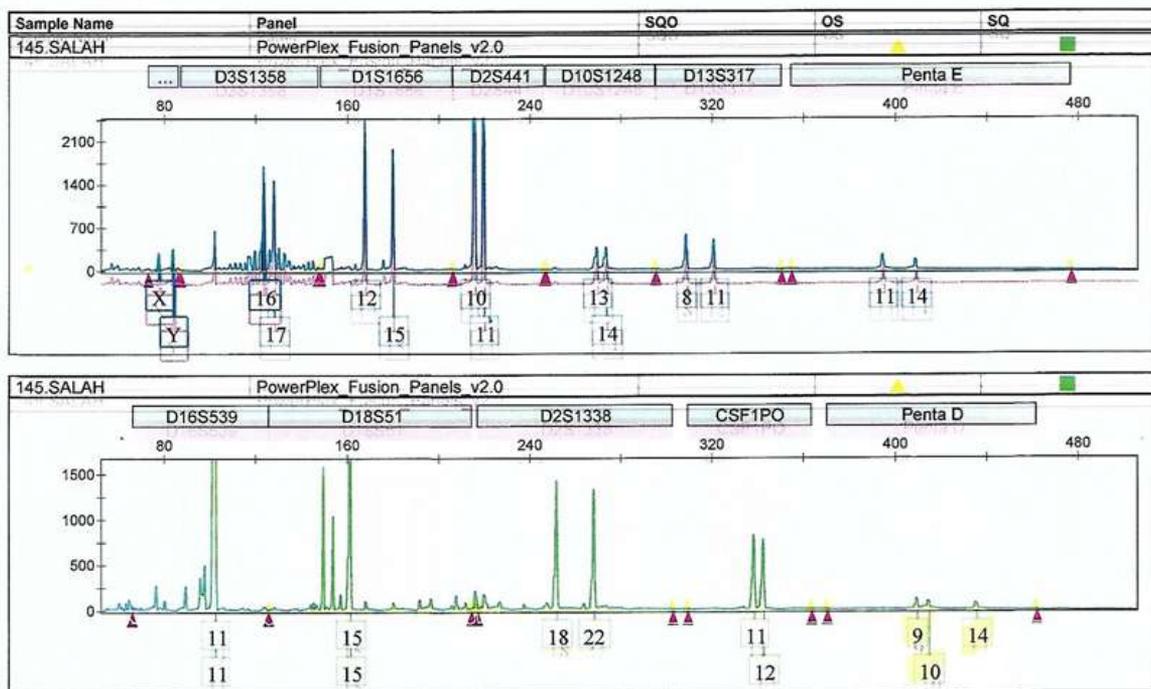


Figure 5: Tri-allelic pattern 9/10/14 observed in Penta D genetic locus from sample number 145.

Discussion and conclusion

The five cases of tri-allelic patterns we observed were all examples of the type 2 pattern. In a series of 15 tri-allelic patterns published by Huel et al.⁴, there were 12 Type I and 3 type II (three peaks of even height) patterns. Thus, the type I pattern occurs significantly more frequently than the type II pattern. A type I pattern is explained by somatic mutation of one allele during an individual's development, resulting in a chimera with some cells containing the original allele and others the mutant allele. A type II pattern is caused by a localized intrachromosomal duplication event or chromosomal aneuploidy. In case of an intrachromosomal duplication event, it is likely that the two resulting alleles would be tightly linked and therefore inherited in progeny. A case of tri-allelic inheritance at locus D3S1358 was reported by Vidal and Cassar.⁵ 179 patterns of tri-allelic variants of autosomal STRs have been reported on the STR Internet Database of NIST.⁶ Five cases in 354 typed individuals in our study, is a frequency for tri-allelic patterns in autosomal STRs of 1.41%. Two other published series reported frequencies of 0.05%⁴ and 0.18%.⁷

Conflict of Interest: None

Funding: self

Ethical Clearance: Not required

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The Occurrence and the Characteristics of Liver Injury, and its Impact among Hospitalized Covid-19 Patients in Basrah City-Iraq

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How to cite this article: EFateh Al-khaqani, Yasser Alaa Sabeeh, Ali Raheem Hashim et al. The Occurrence and the Characteristics of Liver Injury, and its Impact among Hospitalized Covid-19 Patients in Basrah City-Iraq. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):165-172.

Abstract

Background: The respiratory system is most affected by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV2). However, COVID-19 can appear in a variety of ways. It has found a link between higher liver enzymes and COVID-19 infection, implying that the virus can induce liver damage through direct hepatotoxic injury, medication toxicity, or an immune-mediated response.

Materials and Methods: In the southern Iraqi province of Basra, a cross-sectional observational study is being conducted in a single center (Basra teaching hospital) to estimate the prevalence of liver damage among hospitalized patients and identify those at risk.

Results: The study found that most of the patients have a normal liver function test at the time of diagnosis and or admission, but after hospitalization, there is a statistically significant increase in the liver enzymes that is positively related to the disease severity and cytokine storm and also lead to more extended hospital staying and further mortality. Fortunately, the degree of liver damage is of mild severity in the majority of patients. Still, the severe form of liver damage was also noticed in some patients, especially those with a higher degree of lung involvement and severely desaturates with raised inflammatory markers.

Conclusions: Elevated liver enzymes are prevalent, but the majority is mild with COVID-19 disease. Liver function abnormalities, particularly increased levels of AST and ALT, are not only common in COVID-19, but they are also linked to poor outcomes, mainly if severe liver damage has occurred.

Keywords: Liver injury; liver Enzymes; COVID-19.

Introduction

The "severe acute respiratory syndrome coronavirus-2" (SARS-CoV-2) has posed new health risks and difficulties in the world since December 2019, according to the International Classification

Committee of Viruses. The World Health Organization (WHO) labeled the coronavirus disease 2019 (COVID-19) a global pandemic on March 11, 2020.¹ The respiratory system is most affected by the severe acute respiratory syndrome coronavirus

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2 (SARS-CoV2); however, as we learn more about this novel disease, several published research have revealed its organotropism and multisystem organ inflammatory nature.^{2,3}

COVID-19 can appear in a variety of ways, ranging from mild cases with minimal symptoms like a sore throat and loss of smell or taste to disease those results in death.⁴ Other organ injuries, such as acute kidney injury, liver damage, cerebrovascular stroke, and gastroenteritis, have been recorded in addition to respiratory tract involvement.⁵ Although the liver has been implicated in adult patients with COVID-19 infection, the actual prevalence of hepatic abnormalities, the kind of liver enzyme derangement, and its association to outcomes are yet unknown.⁶

Researchers have found a link between COVID-19 infection and higher liver enzymes, suggesting the virus may damage the liver either directly or through medication toxicity, or through an immune response. The SARS epidemic has been linked to 60 percent of patients developing liver damage in the past.⁷ We believe SARS-CoV-2 may induce liver impairment since it belongs to the same coronavirus family as SARS-CoV-1. The angiotensin-converting enzyme (ACE-2) receptor, which is also the binding site for SARS-CoV-2, is expressed in the biliary epithelium.⁸ The expression of the ACE2 receptor in hepatocytes has been found to be increased.^{9,10}

It is unclear whether liver enzyme abnormalities are a prevalent symptom of COVID-19. The prevalence, extent, and rate of progression to liver failure in patients who have a simultaneous liver enzyme derangement with COVID-19 are unknown. In light of the fact that the evidence isn't conclusive. Many of the COVID-19 patients had abnormal liver enzymes to variable degrees. Most of the injuries were minor, and they seemed to be related to the severity of the COVID-19 infection. It is uncommon for a severe liver injury, to result in major liver damage, liver failure, or death.¹¹ Furthermore, In the current COVID-19 clinical trials, mild to moderate liver injury has been recorded, including increased aminotransferases, hypoproteinaemia, and prothrombin time lengthening.¹²

Regarding liver injury, Acute liver failure occurs when significant complications arise soon after the initial signs of liver disease (such as jaundice) and suggests that the liver has been severely damaged (80-90 percent of liver cells have lost function).

Hepatic encephalopathy and decreased protein synthesis are two of the side effects (as measured by the levels of serum albumin and the prothrombin time in the blood). The 1993 categorization defines hyperacute as occurring within one week, acute as occurring between eight to twenty-eight days, and subacute as occurring from four to twelve weeks, both the speed with which the disease develops, and the underlying cause have a significant impact on prognosis.¹³

Many clinical investigations on the effects of hepatic involvement in COVID-19 have been reported recently. However, the majority of them differ due to differences in the definition of liver injury, clinical presentations, and disease severity in separate research.¹⁴ Furthermore, Acute liver injury and liver function abnormalities, particularly high AST and ALT values are not only common in COVID-19 but are also linked to poor outcomes.¹⁵

Objective

This study aims to estimate the prevalence of liver damage among hospitalized COVID -19 patients and try to identify those who are at risk.

Methods

This cross-sectional observational study took place in a single center in the Basra governorate in southern Iraq (Basra teaching hospital, which specializes centre for the treatment of COVID 19 patients) from March 15, 2020, to August 15, 2021. The study covers 280 hospitalized patients who have been diagnosed with COVID 19 on clinical, radiological, and laboratory levels. The training and human development center in the Basra health directorate gave its permission. Information was gathered through medical records in hospitals and intensive care units, direct interviews, and examinations of patients, and then plotted on a standardized questionnaire.

The variables used in the study include:¹⁶

- The **age** (which is further categorized into three groups: <35, 35-65, and above 65 years).
- The **sex** (male and female).
- The **comorbidities**: "diabetes mellitus, hypertension, cerebrovascular disease, ischemic heart diseases, heart failure, atrial fibrillation, chronic kidney disease, bronchial

asthma, or chronic obstructive pulmonary disease, hemoglobinopathies, malignancy, and immunocompromised patients" and are plotted as absent if the patient has no comorbidities or present if the patient has any.

- The **ward** in which the patients were admitted (Intensive care unit and infectious ward).
- The **duration** of hospitalization (less than five days, from five to ten days, more than ten days).
- The presence of **cytokine storm** or release syndrome, which is measured clinically and biochemically, and plotted as present or absent.
- The extent of **lung involvement** as determined by a chest CT (below 50 percent and higher than 50 percent).
- The **oxygen saturation**, which is divided into three categories (below 70 percent, from 70 percent to 93 percent, and above 93 percent).
- The **clinical manifestation**, which includes epigastric pain, jaundice, hepatomegaly, nausea or vomiting, diarrhea and was plotted as present or absent.
- The levels of **liver enzymes** were taken on two occasions in this study: At admission prior, on the first day of hospitalization prior to any medical intervention, and on the last day of admission after receiving the full course of medical treatment.
- The **pattern of liver damage** was classified into three categories according to the degree of elevation of liver enzymes: No elevation: if the readings are within the normal laboratory reference range. Mild to moderate elevation: if the readings are less than three times of the standard laboratory reference range. Severe elevation: if the readings are more than three to five times of the normal laboratory reference range.
- In this study, the **patient's outcome** was evaluated and classified as follows: Respiratory failure or another COVID-19-related consequence caused death. Alternatively, patients may be discharged with full recovery (as measured by improvements in O₂ saturation, respiratory rate, general condition, and inflammatory biomarkers such as C-reactive protein, ESR, Ferritin, and LDH) or with morbidity (for those who are infection-free but still have respiratory problems due to lung fibrosis).

All patients involved in this study have received the COVID-19 protocol, which includes steroids,

the antiviral drug, remdesivir, and antibiotics in a particular situation with supplemental oxygen in addition to other supportive therapy.

The statistical analysis was done by using SPSS (Statistical Package for the social sciences) version 20. The quantitative variables were calculated by the mean, and the standard deviation and one-sample t-test was used for the two-group comparison while the categorized variables were expressed by count and percentage. The results were expressed in the form of tables, the association between the variables were assessed by using Chi-square test (or fissure exact test) and the significance threshold was set at a P value less than 0.05.

Results

The total number of patients involved in this study is (280); their mean age is (57.1) years, with a standard deviation of (1.28). regarding sex distribution, they were 161 (57.5%) male and 119 (42.5%) females. while 66 (27.5%) have no comorbidities, the other 203 (72.5%) patients have comorbidities. Moreover, the degree of lung involvement was less than 50% in 66 (23.6%) only. In comparison, it was more than 50% in 214 (76.4%) of patients. for oxygen saturation, 158 (65.4%) of patients their SP_{O2} ranging from 70 - 90% but 122 (43.6) their average oxygen saturation was below 70%. Finally, concerning the presence of cytokine storm, the result shows that 272 (93.6) of patients have clinical and laboratory evidence of cytokine storm, while the remaining 18 (6.4%) have no such evidence.

The gastrointestinal manifestation of patients enrolled in the study showed that the most frequently reported complaint is anorexia that presented in 169 (60.4%) followed by abdominal pain (epigastric and right hypochondria pain) in 154 (55%). Vomiting was the 3rd most common symptom in 150 (53.5%). Regarding the altered bowel motion, diarrhea was found in 57 (20.4%) patients, while constipation was found in 40 (14.2%) patients. Regarding jaundice, it was presented in 14 (5%) patients, and hepatomegaly by clinical examination was detected in 19 (6.8%) patients. Malena is reported only in two patients (0.7%), while hematemesis and hematochezia were not reported during this survey.

For the total number of patients, eighty-seven (31.1%) have liver damage through the evaluation of liver enzymes and total serum bilirubin at the time of admission. Moreover, one hundred ninety-

two patients (68.6%) have liver damage through the evaluation of liver enzymes and total serum bilirubin at time discharge, the majority have only

mild elevation in the liver function test, and the degree of liver damage at admission and discharge is summarized in the Table 1 below.

Table 1: The degree of liver damage at time of admission and discharge

The Pattern of liver damage at admission	The frequency	
	No.	%
No evidence of liver injury	193	68.9
Mild liver damage	84	30.0
Moderate liver damage	1	0.4
Severe liver damage	2	0.7
The Pattern of liver damage at discharge	The frequency	
	No.	%
No evidence of liver injury	88	31.4
Mild liver damage	162	57.9
Moderate liver damage	17	6.1
Severe liver damage	13	4.6

The mean difference between the level of liver enzyme and total serum bilirubin at time of admission and time of discharge are summarized in

the Table 2 below and the significance of difference was calculated by using one sample t test.

Table 2: The difference between liver function test at admission and discharge

The parameter		Statistical numbers				95% Confidence Interval of the Difference	
		Mean	SD	SE	P value	Lower	Upper
ALT	Admission	47.85	21.8	1.30	0.001	44.85	49.99
	Discharge	83.62	58.99	3.52		76.68	90.56
AST	Admission	52.14	34.08	2.03	0.001	48.13	56.15
	Discharge	101.16	75.55	4.51		92.27	110.05
TSB	Admission	0.84	0.36	0.02	0.001	0.80	0.88
	Discharge	1.88	6.01	0.35		1.17	2.58
ALP	Admission	39.71	19.79	1.18	0.001	37.38	42.04
	Discharge	54.43	19.95	1.19		52.09	56.78

The relationship between liver injury at the time of discharge and the study variables (sex, age,

medical illnesses, severity of respiratory disease, cytokine storm) are summarized in Table 3 below.

Table 3: The association between the liver injury and patients' characteristics

The variables		Liver injury				Total
		Absent (%)	Mild (%)	Moderate (%)	Sever (%)	
Sex	Male	51 (31.7)	97 (60.2)	8 (5.0)	5 (3.1)	161
	Female	37 (31.1)	65 (54.6)	9 (7.6)	8 (6.7)	119
Statistical numbers		P value: 0.387				
Age	<35	8 (57.1)	6 (42.9)	Zero	Zero	14
	35-65	64 (36.8)	97 (55.7)	7 (4.0)	6 (3.4)	174
	>65	16 (17.4)	59 (64.1)	10 (10.9)	7 (6.7)	

Contd... Table 3: The association between the liver injury and patients' characteristics						
Statistical numbers		P value: 0.002 (Chi Square) / P value: 0.003 (Fishers exact)				
Medical illnesses	Absent	41 (53.2)	34 (44.2)	Zero	2 (2.6)	77
	Present	47 (23.2)	128 (61.1)	17 (8.4)	11 (5.4)	203
Statistical numbers		P value: 0.001 (Chi Square) / P value: 0.001 (Fishers exact)				
Saturation	70-93	77 (48.7)	79 (50.0)	2 (1.3)	Zero	158
	<70	11 (9.0)	83 (68.0)	15 (12.3)	13 (10.7)	122
Statistical numbers		P value: 0.001 (Chi Square) / P value: 0.001 (Fishers exact)				
CT	<50 %	41 (62.1)	25 (37.9)	Zero	Zero	66
	1. >50%	47 (22.0)	137 (64.0)	17 (7.9)	13 (6.1)	214
Statistical numbers		P value: 0.001 (Chi Square) / P value: 0.001 (Fishers exact)				
Cytokine storm	Absent	13 (72.2)	5 (27.8)	Zero	Zero	18
	2. Present	75 (28.6)	157 (59.9)	17 (6.5)	13 (5.0)	262
Statistical numbers		P value: 0.001 (Chi Square) / P value: 0.005 (Fishers exact)				
Site of admission	ICU	18 (12.9)	92 (66.2)	16 (11.5)	13 (9.4)	139
	Ward	70 (49.6)	70 (49.6)	1 (0.7)	Zero	141
Statistical numbers		P value: 0.001 (Chi Square) / P value: 0.001 (Fishers exact)				
Total		88	162	17	12	280

The impact of liver damage on the survival and the duration of hospitalization are shown in Table 4 below.

Table 4: The association of the liver injury with the outcome and the duration of hospitalization

The variables		Outcome		Duration of hospitalization			Total
		Discharge	Death	<5	5-10	>10	
Liver injury	Absent	87 (98.9)	1 (1.1)	33 (37.5)	51 (58.0)	4 (4.5)	88
	Mild	141 (87.0)	21 (13.0)	32 (19.8)	110 (67.9)	20 (12.3)	162
	Moderate	11 (64.7)	6 (35.3)	Zero	12 (70.6)	5 (29.4)	17
	Sever	4 (30.8)	9 (69.2)	Zero	6 (46.2)	7 (53.8)	13
Total		243	37	179	36	36	20
Statistical numbers		P value (Chi & Fisher): 0.001		P value(Chi & Fisher): 0.001			

Discussion

To start with, COVID-19-related hepatic damage is defined as alanine aminotransferase (ALT) or aspartate aminotransferase (AST) levels above three times the upper limit of normal, and alkaline phosphatase (ALP) or total bilirubin levels exceeding two times the upper limit of normal.¹⁷ This was referred to in our study as severe elevation of liver enzyme or liver damage.

As it was shown in our study, the gastrointestinal symptoms are common suffering or common complaint in hospitalized patients with COVID-19, especially the Anorexia followed by abdominal

pain, nausea, and vomiting, and diarrhea, then constipation, to a lesser extent to other GIT complaints as jaundice or Malena. These results were consistent with the results of many studies, which showed that COVID-19 patients have GIT signs in a range of 11.4–61.1 percent of cases. Also stated, is that Anorexia, diarrhea, nausea, vomiting, and abdominal pain/discomfort are among the COVID-19-related GI symptoms that are minor and self-limiting.¹⁸

Regarding the demographic features of the patients enrolled in the study, our results found high severe liver injury among females. In contrast, the mild elevation of the liver enzyme was higher among males, but both these findings are not

statistically significant. Nevertheless, regarding the age group categories, a significant association was shown between the severity of the liver injury and advancing age, while no reported case of severe liver injury was under 35 years. Our finding is not similar to other studies regarding gender distribution as it is shown that male patients were more likely to have a liver function injury than females.¹⁹

Both hollow and solid gut organs express the ACE2 receptor. The neutral amino acid transporter B0AT1 (SLC6A19) present in the intestinal epithelium stabilizes ACE2 messenger RNA (mRNA), which is abundantly expressed in the GI tract (Xiao et al. 2020). As a result, one idea for COVID-19 patient's liver impairment is that the SARS-CoV-2 virus destroys Cholangiocytes via ACE 2 receptors. Furthermore, it was discovered that in cases of liver injury, the expression of ACE 2 in hepatocytes increased.²⁰ Therefore, it is expected to find elevation of liver enzyme and some sort of liver damage even at the initial days of diagnosis or hospitalization in which the virus itself is blame as a cause of liver injury due to ACE2 receptor effect and according to our results, we found that the majority of patients about 70% has no evidence of liver damage but, approximately 30% show elevation of liver enzyme and despite the majority is mild to moderate, but severe liver damage is found in one patient also.

It is worthy to note that liver damage is increased during the days of hospitalization. As shown in the results above, there is a noticeable increase in the percentage of mild to moderate liver damage, which reaches up to 60%. Furthermore, the rate of severe liver injury also increases to reach up to 5% compared with less than 1% at the time of admission. These findings are of close similarity to a large cohort study that shows at the time of entry, 41.0 percent of the patients showed abnormal liver function tests and liver damage, but those Patients with abnormal liver function tests and liver injury climbed to 76.3 percent after two weeks in the hospital.²¹ Many ways may explain this. The first of them is increasing the progression of diseases and respiratory disease severity in addition to the cytokine storm, and our results show a significant difference in the manifestation of liver injury and its severity among those with severe respiratory disease; ICU admitted patients who severely desaturated and among those with a high level of inflammatory markers who labelled as patients with cytokine storm or release syndrome. It was known that cytokine storm frequently results in

a rapid deterioration of the patient's condition when the patient enters multiple organ failures, and the systemic inflammation generated by it might result in additional liver injury.^{22,23} The other mechanism that might explain the progression of liver damage during hospitalization is the side effects of the commonly used drug during admission, such as the antiviral and paracetamol, or maybe the antibiotics that might be used for the secondary bacterial infections in addition to tocilizumab that used in the treatment for the cytokine storm. As a result, one of the reasons for COVID-19-related liver impairment could be pharmacological activities. Therefore, monitoring changes in liver function and medication timing in COVID-19 patients throughout hospitalization may help determine the causal association between medicines and liver harm.²⁴

In addition to what was mentioned above, this study also showed a highly significant difference between the levels of the enzyme at the time of admission comparison with their levels at times of discharge as well as there is an obvious increase in the level of AST higher than the level of ALT and this is consistent with the findings of the majority of research that have found raised AST and ALT are the most common causes of abnormal liver function tests, with elevated AST being more common than ALT. Increased GGT and total bilirubin are less prevalent than elevated AST and ALT.²⁵

Finally, according to our findings, the higher level of liver enzymes and the more severe liver injury is associated with increased mortality and poorer outcome in addition to more extended hospital stays. This might be related to the higher severity of infection that resulted in a liver injury which may exaggerate the occurrence of poorer outcomes. Also, this may give a hint to monitor the liver function test frequently during hospitalization. In addition, the clinician may depend on the liver enzyme as mortality predictors. This finding is argued with Henry and colleagues. They did a meta-analysis to see if liver function and coagulation function were considerably more significant in severe and critical patients than in non-severe patients. It was hypothesized that liver injury could be linked to severe COVID-19 infection. Also, the clinical features of individuals with and without liver function damage were compared. The findings revealed that those with liver injury spent much more time in the hospital.²⁶ Furthermore, Increased AST values are related to 3 times more risk of bad outcomes in COVID-19 patients, according to

a meta-analysis of 12 studies that presented data on raising AST and outcomes, resulting in a total sample size of 5135 patients for evaluation.¹⁵

Conclusions

1. Elevated liver enzymes are prevalent, but the majority is mild with COVID-19 disease.
2. Liver function abnormalities, particularly increased levels of AST and ALT, are not only common in COVID-19, but they are also linked to poor outcomes, mainly if severe liver damage has occurred.
3. The etiology of the liver injury is unknown, although it appears to be complex, with a cytokine storm and immunological dysregulation playing a role. Hypoxia, sepsis, numerous medications, direct viral action, and ICU-related infections are all possibilities.

Recommendations

1. Future research should focus on determining the etiology of liver impairment during COVID-19 infection, particularly if it is caused by a therapeutic medicine.
2. In clinical practice, it's essential to know whether the aberrant liver function appears at the time of diagnosis or later on during treatment.
3. Regular follow-up for the patients is required for the liver function test to avoid serious complications.

Limitations

This study is an only cross-sectional study and involves a small sample size. In addition, it did not include the evaluation of the levels of coagulation profile or protein levels that may give more idea about the state of liver injury. Moreover, there are differences in how liver dysfunction and acute liver injury are classified. There are different cutoff values for increased liver enzymes and varied definitions of Covid-19 disease severity and outcomes among studies.

Additional Information

The authors declare that they have no competing interests (financial AND nonfinancial interests)

Ethical clearance

It is taken from the college of medicine ethical

committee in Basrah university and the development and training committee in Basrah health directorate.

Source of funding: It was self-funding from the budget of the researchers.

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The Effect of Playing Collage Therapy in Increasing Motor Skills Development in Preschool Children

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How to cite this article: Ferasinta Ferasinta, Nurhayati Nurhayati, Lussyefrida Yanti. The Effect of Playing Collage Therapy in Increasing Motor Skills Development in Preschool Children. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):173-175.

Abstract

Background: The stages process development of each child is the same, which is the result of the maturation process.

Aims: The aim of this study was to compare the effectiveness of playing collage therapy in improving motor skills development in preschool children.

Material and Methods: This research was a quasi-experimental study using a pre-test and post-test research design. Playing collage therapy intervention was given to the 15 respondents, which has been chosen by using a purposive sampling technique.

Results: The results of this study indicate that most of the children experienced motor skills development beyond expectation (43.8%) before being given playing collage therapy. Then their motor skills development increasing as very well developed (100%) after being given playing collage therapy. Furthermore, the results of the t-test calculation obtained a p-value of 0.000 (α 0.05), which is means that there is a significant increase in motor skills development through preschool children aged 3-5 years in PAUD Tunas Harapan of Bengkulu after being given playing collage therapy.

Conclusion: From the results of this research, we conclude that playing collage therapy can be used as an alternative therapy to improve motor skills development in pre-school children..

Keywords: Preschool children; playing collage therapy.

Introduction

Preschool children aged between 3-6 years. At this age, children generally join in the children's program (ages 3-5 years) and playgroup program (ages 3 years), while at the age of 4-6 years they usually attend kindergarten program. Children in his age are expected to have several skills that require motor

skills, such as using scissors well even though they are not straight in cutting, tying shoelaces, coloring neatly, and others skills according to the motor skills development that must be achieved. The activities of childhood in this age should be directed by their parents to improve their skills in these matters. This is important to give the opportunities and continuous practice in improving the children's skills.^{1,2}

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The United Nations Children’s Fund (UNICEF, 2011) report that the incidence of growth and development disorders in children aged 3-6 years, especially for motor skills development disorders is still high (27.5%) about 3 million children. Around 16% of children aged 3-6 years in Indonesia also experiencing developmental disorders in the form of intelligence due to brain development disorders, hearing problems and motor skills disorder.^{3,4}

The process of development stages of each child is the same, which is the result of the maturation process. However, each child has a different pace in achieving the stage. The development stages are divided into several stages, namely prenatal period (from the conception to birth), infancy (from 0-1 years), early childhood (ages 1-3 years), preschool (ages 3-6 years) and school period (ages 6-18/20 years).^{5,6}

Growth and development stage have the same meaning of changes, but in particular they are different. Growth shows quantitative changes as the result of physical maturation which is characterized by the complex system of muscle tissue, nervous system and other measurable functions of organ systems. As the result of maturity stage, the physical organs are ready to carry out their activities according to the stage of individual development.⁷

According to the initial survey conducted by the researchers on October 20, 2020 at PAUD Tunas Harapan of Bengkulu, there were 163 children join in this preschool, 148 children in 2019 and 252 children in 2018. Based on the data available in the background of study, the authors were interested in conducting a research entitled “The effectiveness of playing collage therapy in improving motor skills development in preschool children at PAUD Tunas Harapan of Bengkulu”.

Methods

The method of this study was a quasi-experimental study with a pre test and post test design. This research also carried out a descriptive observational study.

Results

Univariate Analysis

This study report that the respondents age was 3 years old (66.6%) and 4 years old respondents (33.4%) (Table 1).

Table 1: Frecuency Distribution of Respondens Based on Ages in PAUD Tunas Harapan of Bengkulu

Ages	F	(%)
3 years old	10	66.7
4 years old	5	33.3
Total	15	100

Bivariate Analysis

This study found that the mean of motor skills development was 16.13 with a standard deviation 1.675 before being given playing collage therapy. While the mean of motor skills development increase gradually about 8.87 with a standard deviation 2.625 after being given playing collage therapy. From the t-test results shows that the p-value of before and after being given playing collage therapy was 0.000 (smaller than the p-value 0.005), which interpreted that there was a significant effect of playing collage therapy intervention in increasing the motor skills development in preschool children in PAUD Tunas Harapan of Bengkulu (Table 2).

Table 2: The Effectiveness of Playing Collage Therapy Before and After Intervention

Variabel	Mean	SD	P Value	N
Sebelum Intervensi	16.13	1.675	0.000	15
Setelah Intervensi	8.87	2.625		

Discussion

Distribution of Respondents Based on Ages

The results of this study describe that majority of the respondent’s aged from 3 to 4 years. The respondents with the most age of 3 years was 10 respondents (66.6%). This is in line with Suhartini’s research (2019) which found that the inhibiting developments occur in early childhood, around the age of 2 to 4 years. Children who get better attention will develop a sense of confidence in their abilities.

This is as same as Fazrin’s research (2017) which report that there were obstacles in the children’s skills motor development, including being slow in concentration, getting bored quickly, and lack of eye and hand coordination. To get better motor skills development in children, the parents ave to train their activity in motor skills development.

The Effectiveness of Playing Collage Therapy Before and After Intervention

Based on the results of dependent t-test analysis, it shows that the mean of motor skills development was 16.13 with a standard deviation 1.675 before being given playing collage therapy. While the mean of motor skills development increase gradually about 8.87 with a standard deviation 2.625 after being given playing collage therapy. The p-value of before and after being given playing collage therapy was 0.000, which presented that there was a significant effect of playing collage therapy intervention in increasing the motor skills development in preschool children

This study in line to Muarifah research (2019), which states that motor skills development increase after implementing collage therapy especially in the terms of hands and eyes coordination, so hand movements in this study developed properly. This study suggest that the parents should pay more attention in control, coordination of using hands and fingers in preschool children. This study also as same as Rufaida results (2019) which found that children who get a lot of stimulation develop their motor skills faster than children who get less or even no stimulation.

Conclusion

To conclude, the frequency distribution of preschool children was 3-4 years old (66.6% and 33.4%). The average mean value of motor skills development before playing collage therapy intervention was 16.13 and 8.87 after playing collage therapy intervention. In addition, there was a significant effect of playing collage therapy on the improvement of motor skills development in preschool children at PAUD Tunas Harapan of Bengkulu.

Conflict of Interest: The author(s) declare that there is no conflict of interest.

Ethical Clearence: This study has approval from the Komite Etik Riset Kesehatan, Politeknik Kesehatan Kemenkes Bengkulu.

Funding: The research funding of this study was supported RISTEKBRIN 2021.

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A Comparison between the Effectiveness of Platelet-Rich Plasma Injection and Corticosteroid Injection in Plantar Fasciitis Patients: A Systematic Review

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How to cite this article: Fikri Rasikh Pritanto, Rwahita Satyawati, Mouli Edward et al. A Comparison between the Effectiveness of Platelet-Rich Plasma Injection and Corticosteroid Injection in Plantar Fasciitis Patients: A Systematic Review. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):176-182.

Abstract

A systematic review using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses chart (PRISMA) was used in this study. About 7 articles have been included. A month post-injection, corticosteroid injection reduced pain more than PRP injection (Mean: 1.22; 95% CI:-1.10 to 3.54; p=0.30). However, PRP injection reduced pain significantly more than corticosteroid injection in three months after injection (Mean: -0.77, 95% CI:-1.46 to -0.96; p=0.03), and in six months later (Mean:-1.22, 95% CI:-2.02 to -0.42; p=0.003). The effectiveness difference between Platelet-Rich Plasma injection and corticosteroid injection in patients with plantar fasciitis varies depending on peak period and facial thickness.

Keywords: Plantar fasciitis; Corticosteroid injection; Platelet-rich plasma Injection; Systematic review; Effectiveness.

Introduction

Plantar fasciitis is a disease characterized by inflammation of the plantar fascia. Patients with *plantar fasciitis* usually feel non-spread pain that feels stabbing.¹ The pain worsens when patients begin to stand up after resting, and the symptom will primarily come in the morning. The pain gradually decreases in line with the use of the foot in activities, although it does not completely heal. Pain can be worsened if the patient stands or walks for too long, does strenuous activities, or lifts heavyweights for a long time.²

The leading cause of plantar fasciitis is still undetected in specific ways.³ However, some researchers believe that one of the causes is the formation of small tears in the plantar fascia, which repeatedly cause inflammation. Factors related to these tears include obesity, excessive running, a sedentary lifestyle, the shape of the foot is too flat (*pes planus*), the foot angle is too high (*pes cavus*), or work that requires walking or standing for a long duration.⁴

Plantar fasciitis has a self-limiting character;

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hence, the rigid standard of the therapy has not been defined yet.⁵ Several therapeutic modalities, such as administering non-steroidal anti-inflammatory drugs, stretching, orthotics, and shock waves, can accelerate healing and improve the patient's quality of life, aiming those patients to carry out normal daily activities.⁶ When these noninvasive modalities are unsuccessful, injections of drugs such as corticosteroids can be performed.

Corticosteroids are one of the most effective drugs to treat inflammation. The strong anti-inflammatory effect of corticosteroids can help heal the inflammation quickly, although it can cause some side effects and complications.⁷ Despite being considered an effective treatment in reducing pain, the side effects and complications have led many researchers to look into alternative drugs, which are more effective and efficient for managing plantar fasciitis.⁸

Platelet-Rich Plasma (PRP) has become another safer alternative compared to corticosteroid injection for curing plantar fasciitis.⁹ PRP therapy has recently become famous for the healing of various tissues in various medical fields. PRP is a biological product taken from the patient himself in plasma, which has a platelet concentration above the average.¹⁰ It can release growth factors and cytokines that are useful in the healing process. Because of that, the effectiveness for specific tissues, which have low healing potentials such as tendons, ligaments, fascia, and plantar fascia, has been proven (Wu, Diaz, and Borg-Stein, 2016). The concept of PRP, which is taken from the patient itself, can minimize the possibility of rejection reactions, side effects, and complications. Hence, this theory has made PRP a safer alternative compared to corticosteroids.⁸ However, many practitioners are still debating whether PRP injections are more effective in treating plantar fasciitis than corticosteroid injections.¹¹

The purpose of this study was to systematically analyze the comparison of the therapeutic effectiveness of Platelet-Rich Plasma injection when compared to corticosteroid injections in treating patients with plantar fasciitis.

Methods

A systematic review using a meta-analysis approach was used in this study. The results of the primary studies were synthesized using a statistical (quantitative) approach. The journal search

protocol was adjusted to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses chart (PRISMA). Collecting articles was conducted using the PubMed and ScienceDirect databases by applying several keywords: 'Platelet-Rich Plasma' or 'Corticosteroids', and combined with 'Plantar fasciitis'. The selected period was the last ten years, namely from 2010 to 2020.

Quality and bias assessment was carried out using the COCHRANE Risk of Bias Tools which was used to assess bias in research using the RCT (Randomized Controlled Trial) method.¹² The risk of bias was assessed in 5 areas: the randomization process, deviations from predetermined interventions, missing data output, measuring output, and reporting results.¹³

The population was primary research data on Plantar fasciitis patients who have undergone Platelet-Rich Plasma (PRP) injection or corticosteroid injections and published in the PubMed and ScienceDirect databases. The samples were research journals that met the inclusion criteria: reporting differences between Platelet-Rich Plasma (PRP) injections and corticosteroid injections in Plantar fasciitis patients; using the RCT (Randomized Controlled Trial) method; using the Visual Analog Scale (VAS) pain measurement instrument; in a maximum period of 10 years, evaluating post-injection therapy after 1, 3 or 6 months; the most recent and most complete studies. Meanwhile, the exclusion criteria used include: Studies without good method validation; Studies are not written in Indonesian or English; Studies with a period longer than the last ten years, in poor condition, and incomplete.

The instrument used was a search database for research journals, namely PubMed and ScienceDirect, which are used to search for research journals with predetermined topics. COCHRANE Risk of Bias Tools Version 2 (RoB 2) was also used to conduct a biased assessment on the journal to be used. To perform quantitative analysis, COCHRANE Review Manager (RevMan) version 5.4 was used.

Results

According to the accumulated articles, as many as 1534 journal articles were founded on the database. To obtain appropriate articles, multiple screening was done by using several predetermined criteria, such as duplication, the specified time span of 10 years, the Randomized Controlled Trial

(RCT) method, language used, the use of Visual Analog Scale in measuring pain, the duration of out post-therapy evaluation. There were 17 articles indicated duplication, 37 articles provided short-text and without abstract; 617 articles exceeded from the length of 10 years period; 785 articles without using Randomized Controlled Trial (RCT) method; 2 articles eliminated as they do not write in English or Indonesia; 65 articles were out of topic discussed; and 2 articles were not using Visual Analog Scale. Finally, about seven journal articles have been included in a systematic review for extraction and analysis, thereby drawing conclusions from the journal data.

The risk assessment results of bias in the seven journals used can be seen in Figures 1, which state that the risk of bias is relatively small. Several areas have a high risk due to lack of information in journals about the related area.

Articles Description

The research design in each study was carried out in parallel in each group. All studies used patients with plantar fasciitis who failed conservative treatment for at least three months and had never had surgery.

The total sample of all studies used was 497, which varied from 25 samples to 60 samples in each group. All studies used clinical features and physical examination to diagnose Plantar fasciitis in each sample used. Four studies (Jain, Khurana, Shery and Uğurlar)^{7,9,11,14} performed further imaging using

either ultrasonography or MRI to confirm a further diagnosis of Plantar fasciitis. Each group in the study received one injection each in both the PRP and corticosteroid injection groups, except in the study by Uğurlar, which gave three injection sessions seven days apart for each injection. Apart from 2 studies (Khurana and Uğurlar)^{9,14}, all studies included patients in a post-injection stretching exercise program.

Pain Felt a Month Post-Injection

Meta-analysis and Forest Plot showed that a month post-injection, corticosteroid injection reduced pain more than PRP injection, but it was not significantly (Weighted Mean Difference: 1.22; 95% CI: -1.10 to 3.54; p= 0.30). The heterogeneity of the studies was assessed as the highest (Chi²=33.01; I²=94%), which may be due to the small number of studies used in a month post-injection analysis. Besides, the study by Uğurlar used a different route of administration of the injection (Table 1).

Pain Appeared 3 Months Post-Injection

Of the seven studies selected for analysis, six studies had pain measurement data 3 months post-injection, as can be seen in Table 2. Meta-analysis and Forest Plot showed that PRP injection reduced pain significantly more than corticosteroid injection (WMD: -0.77, 95% CI: -1.46 to -0.96; p=0.03). Heterogeneity in the study was considered quite high (Chi²=15.63; I²=68%).

Table 1. Meta-analysis evaluation of pain measurement one month after injection using a visual analogue scale (VAS).

Study of subgroup	PRP injection		Corticosteroid Injection		Weight	Mean Difference IV, Random 95% CI	
	Mean	SD	Mean	SD			
Jain et al, 2018	6.5	1.7	5.7	2.7	34.9%	0.80 [-0.19, 1.79]	
Uğurlar et al, 2018	7.8	6.5	3.2	2.4	28.1%	4.60 [2.43, 6.77]	
Khurana et al, 2020	3.98	1.03	4.93	1.07	36.9%	-0.95 [-1.33, -0.57]	
Total (95% CI)					100%	1.22 [-1.10, 3.54]	
Heterogeneity: Tau ² = 3.75; Chi ² = 33.01, df=2(P<0.00001) I ² = 94% Test for overall effect Z=1.03(P=0.30)							

Figure 2: Meta-analysis evaluation of pain measurement 3 months after injection using a visual analog scale (VAS)

Study of subgroup	PRP injection		Corticosteroid Injection		Weight	Mean Difference IV, Random 95% CI	Mean Difference IV, Random, 95% CI
	Mean	SD	Mean	SD			
Jain et al., 2015	3.5	3.3	2.83	3.44	10.2%	0.67 [-1.04, 2.38]	
Sherpy et al., 2015	0	2.5	1	2.25	13.7%	-1.00 [-2.32, 0.32]	
Mahindra et al., 2016	2.52	1.71	3.64	1.62	18.4%	-1.12 [-2.04, -0.20]	
Jain et al, 2018	5	2.5	4.3	2.8	15.4%	0.70 [-0.46, 1.86]	
Ugurlar et al, 2018	2.7	0.3	4.4	3.5	16.3%	-1.70 [2.79, -0.61]	
Khurana et al, 2020	1.45	0.75	2.72	0.98	26%	-1.27 [-1.58, -0.96]	
Total (95% CI)					100%	-0.77 [-1.46, -0.08]	

Heterogeneity: Tau²= 0.45; Chi²= 15.63, df=5 (P=0.008) I²= 68%
 Test for overall effect Z=2.20 (P=0.03)

Table 3: Meta-analysis evaluation of pain measurement 6 months after injection using a visual analogue scale (VAS).

Study of subgroup	PRP injection		Corticosteroid Injection		Weight	Mean Difference IV, Random 95% CI	Mean Difference IV, Random, 95% CI
	Mean	SD	Mean	SD			
Jain et al., 2015	3.7	3.58	3.28	3.55	12.4%	0.42 [-1.38, 2.22]	
Ugurlar et al, 2018	2.6	0.3	5.2	3.6	20.3%	-2.60 [-3.72, -1.48]	
Jain et al, 2018	3	2.6	3.3	2.8	19.4%	-0.30 [-1.48, 0.88]	
Shetty et al., 2019	3.3	2.9	4.8	2.9	15.7%	-1.50 [-2.97, -0.03]	
Khurana et al, 2020	0.52	0.6	1.92	1.03	32.2%	-1.40 [-1.70, -1.10]	
Total (95% CI)					100.0%	-1.22 [-2.02, -0.42]	

Heterogeneity: Tau²= 0.49; Chi²= 11.623, df=4 (P=0.02) I²= 66%
 Test for overall effect Z=3.00 (P=0.003)

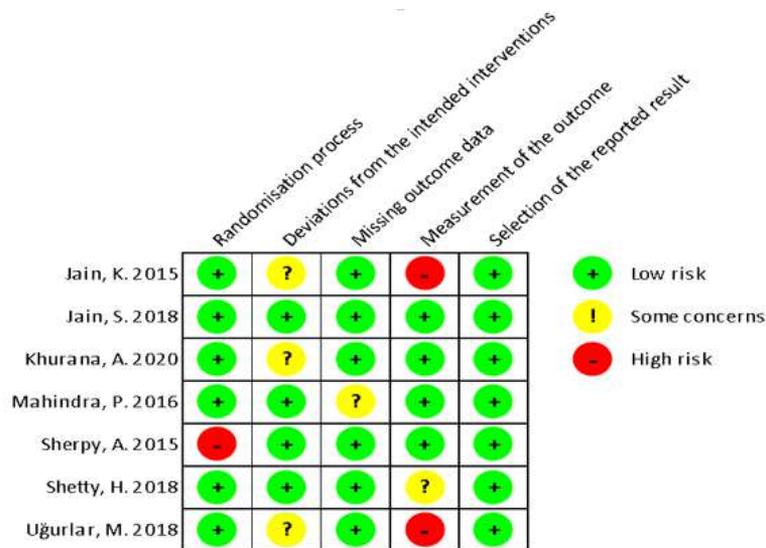


Figure 1: Summary of risk of bias

Pain Appeared 6 Months Post-Injection

We found five studies with pain measurement data at 6 months post-injection, as shown in Figure 1

and Table 3. Meta-analysis and Forest Plot showed that PRP injection reduced pain significantly more than corticosteroid injection (WMD: -1.22, 95%

CI: -2.02 to -0.42; $p=0.003$). Heterogeneity in the study was considered relatively high ($\text{Chi}^2=11.62$; $I^2=66\%$). Of the seven studies that the author uses in a systematic review study, 2 journals examine the thickness of the Fascia plantaris before and after injection, namely the study by Sherpy(2015)¹¹ and Jain (2018).⁷

A study conducted by Jain revealed that corticosteroid injection can reduce fascia thickness better in the first one month with a mean difference of 0.8 for PRP and 1.4 for Corticosteroids. In the 3rd month, corticosteroid injection (mean difference 1.9) was better than PRP injection (mean difference 1.5). Until the 6th month of corticosteroids (mean difference 2.2) was also still better than PRP injection (mean difference 2.1), although when compared to 3 months, PRP injection was better in reducing thickness. It is stated that corticosteroid injection is better in reducing thickness than PRP injection significantly in the first month and 3rd month after injection. Meanwhile, at the 6th month, there was no significant difference in thickness reduction.

Meanwhile, according to a study conducted by Sherpy, PRP injection can reduce fascia thickness better than corticosteroid injection, both at month 1.5 with a mean difference of 0.5 for PRP and 0.22 for corticosteroids, also at month 3 with a mean difference of 0.99 for PRP and 0.53 for corticosteroids. However, it was stated that the difference between the two injections in reducing thickness was not significant.

All studies reported that there were no complications for neither PRP injection therapy nor corticosteroid injection therapy.

Discussion

Plantar fasciitis is a disease of the feet in pain that greatly interferes with daily activities.² Although it is self-limiting or can heal on its own, sometimes plantar fasciitis does not go away even with a combination of conservative therapy, thus requiring continued treatment.⁴ Corticosteroid injection is one of the advanced therapies that can be used. Corticosteroid injections have been shown to reduce inflammation and pain in patients with plantar fasciitis quickly and effectively with anti-inflammatory effects that can accelerate the inflammatory process in patients.⁷ Even so, complications from corticosteroid injections in the form of fat atrophy and plantar fascia to the occurrence of fascia rupture make researchers look

for safer, more effective and efficient alternatives for plantar fasciitis treatment options that do not improve with conservative therapy. Platelet-Rich Plasma or abbreviated PRP is an alternative therapy that can reduce inflammation and pain quickly, and has a low possibility of complications.⁸

This study used the VAS to perform comparisons of pain reduction regardless of the arguments that questioned the subjectivity of the VAS, which resulted in results according to how data takers in each study described pain on the VAS scale. In addition, pain measurement using VAS is also very simple so that it can be done anywhere easily.¹¹

The meta-analysis data support the assumption that PRP injection has a better outcome in reducing pain over a longer period of time. In a very short period of 1-month PRP injection and corticosteroids were equally as effective in reducing pain (WMD: 1.22, 95% CI: -1.10 to 3.54; $p=0.30$). In the medium term of 3 months, PRP injection reduced pain more than corticosteroid injection (WMD: -0.77, 95% CI: -1.46 to -0.96; $p=0.03$). Meanwhile, in a long period of 6 months, PRP injection also reduced pain more than corticosteroid injection (WMD: -1.22, 95% CI: -2.02 to -0.42; $p=0.003$).

Based on the findings, PRP injection and corticosteroids were as much pain relief in the evaluation of the 1st-month post-injection, while at the 3rd and 6th months, PRP injection was more in reducing pain. In the 1st month, RP injection and corticosteroids were equally effective in reducing pain because their function was to inhibit inflammatory genes such as Interleukin and Nuclear Factor kappa B, thereby reducing inflammation, and in the process, inhibiting cyclooxygenase-2, which resulted in reduced pain. In addition to the inhibitory function of inflammatory genes, growth factors in PRP can also increase collagen production, which will help in strengthening fascia and speeding up the healing process. Growth factors can also accelerate cell activities such as cell proliferation, communication, and differentiation which can accelerate the formation of new cells in the healing process.¹⁵ Both processes will work by accelerating the healing process of microtears or small tears that are the main cause of inflammation so that in the longer term, PRP reduces inflammation more optimally. The healing process takes longer, so at the 3rd and 6th-month evaluation after Platelet-Rich Plasma injection, it can reduce pain more than corticosteroid injection.¹⁶

In addition to comparing pain relief, measurement of thickness in the plantar fascia can also be used to analyze which therapy is better in treating the plantar fascia. Thickening of the plantar fascia is one of the signs that often appears in patients with plantar fasciitis. According to a study conducted by Jain and colleagues, plantar fasciitis sufferers are 100 times more likely to experience abnormal thickening of the plantar fascia (>4.0 mm) compared to normal individuals (Jain et al., 2018). The thickness of the plantar fascia is caused by inflammation of the fascia itself and the response of the body with growth factors that increase fibroblast proliferation and collagen production which causes thickening of the tissue e.¹⁵

From the two journals that analyzed fascia thickness, the authors concluded that corticosteroid injection reduced thickness more than Platelet Rich Plasma injection in plantar fasciitis patients. This is due to the mechanism of corticosteroids reducing inflammation and swelling and inhibiting fibroblast proliferation and collagen production from reducing fascia thickness more maximally.¹⁷ In contrast to PRP, which although it can reduce inflammation, thereby reducing fascia thickness, PRP also has a growth factor mechanism that increases fibroblast proliferation and collagen production, which actually thickens the fascia, causing the opposite effect and causing PRP to not be able to reduce fascia thickness maximally.¹⁵

Following one of the researchers' considerations, the use of PRP injections compared to corticosteroid injections is a high possibility of decreasing complications.⁸ All studies used reported that there were no complications after PRP injection therapy or corticosteroid injection therapy. Thus it can be concluded that PRP injection is as safe as corticosteroid injection in plantar fasciitis patients.

This study has several limitations. First, there is fairly high heterogeneity, especially in the pain variable; therefore, a random effect model is used. This heterogeneity can be explained by differences in the type of agent used, the method of preparation of the agent (amount, duration and centrifugation system), additional agents, method of administration of the agent, and the volume of agent used. Second, the use of the slightly subjective Visual Analog Scale pain scale as previously described. Third, the number of studies used to use only 7 RCT studies, from 2 databases. In addition, not all studies used

to measure pain simultaneously, so in the analysis, all studies in the month that have been determined cannot be used. Fourth, although only RCTs were used to reduce the possibility of bias arising from not randomization in this study, there was one journal that did not sufficiently meet the randomization criteria by Cochrane. In addition, there are 2 journals that do not meet the final outcome measurement criteria according to the criteria determined by Chocrane, for the assessment of bias in RCT studies.

Conclusion

The difference of effectiveness between Platelet-Rich Plasma injection and corticosteroid injection in patients with plantar fasciitis is varied depending on peak period and facial thickness. Corticosteroid injection reduces pain more than Platelet-Rich Plasma injection in the 1st month. In contrast, in a more extended period, namely the 3rd and 6th month, Platelet-Rich Plasma injection is much more effective to reduce pain. Meanwhile, corticosteroid injection can decrease fascial thickness more than Platelet-Rich Plasma injection. Neither Platelet-Rich Plasma injection nor corticosteroid injection effect the complication of plantar fasciitis. Hence, the safety level of Platelet-Rich Plasma injection is as safe as a corticosteroid injection.

Funding: This research has not received any funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflict of Interest: The authors declare no conflict of interest

Ethical Clearance: Not applicable.

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Sex identification based on lipprint patterns: A Review

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How to cite this article: Fiqna Amalya, Devi A. A. Nasution, Myrtati D. Artaria et al. Sex identification based on lipprint patterns: A Review. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):183-188.

Abstract

Background: Many researchers have conducted studies of lip prints to aid in human identification. The distinctiveness of the lip print pattern will be a distinguishing feature from one person to the next.

Purpose: This study analyzes the dominant types and patterns of lip print in males and females.

Results: Most males—7 research out of 20—were type III lip print pattern, and 6 out of 20 belong to the type II lip print pattern. Females mainly were typed II pattern—11 out of 20, and type I pattern—7 out of 20.

Conclusion: When the patterns are type I or type III, the sex of the individual can be easily identified; however, when the pattern is type II, the individual could be either a male or a female, with a higher probability of being a female. Other forms of identification should be available to support sex identification in this circumstance.

Keywords: Crime; crime scene; lip-print; lip-print pattern; lip-print type; sex identification.

Background

Humans are large groups of living beings who exist on this planet. Genetics and environmental adaptation are to blame for the emergence of these variances.¹ Human physical variances are influenced by the environment and genetics, according to Glinka in 2008.² The presence of morphological variances can be utilized to determine a person's identity, for example, through the use of fingerprints, ears, retina, or lip prints. The identification process is an attempt to determine an individual's identity.

Anthropologist R. Fischer, in 1902 has described the lip print of human lips; then, this was

recommended the first time for personal identification in criminal cases by the French criminologist named Edmond Locard.³ The wrinkles and grooves in the red part of the transitional zone of the lips are referred to as "*sulci labiorum rubrorum*," each individual has wrinkles and grooves that will not be the same because there are specific characteristics in each individual.⁴ Like fingerprints, lip prints can be found or left behind because they are visualized with the help of lipstick on a glass surface, tissue, handkerchief, or on objects at the crime scene in cases of crime and murder.⁵ According to Gagliardi⁵ this visualization can be used as a guide at the crime scene and provide convenience to forensic personnel who play an active role in identifying a murder case.

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Lip prints have unique and stable characteristics, which is a strong reason why lip prints are used as objects to solve crimes and murder cases. In Poland (1985-1997), lip prints were used to identify 85 cases, and 34 cases were successfully resolved. Thus, it further strengthens why lip print is used to solve criminal cases.² The presence of lip prints is a supporting medium for investigation, and if the sex category is known, it will make it easier to shortlist the list of suspects with the motive for their crimes.⁶ Another compelling reason is that lip print does not change from the individual's age to the sixth week of life.⁷

Qomariah et al.⁸ conducted a study using lip prints to find out the dominant patterns and types of the male and female sex using the Suzuki and Tsuchihashi classification for the analysis process. It is known that the lip print of the dominant type III male is as much as 40%, then the lip print on the female sex category types I' as much as 35%. In 2019 Mahampang⁵ also conducted the same study, and the result was that the dominant type in men was type II, while in women, type I. The actual use of lip prints is not only a method of sex category identification, and as is the case with research conducted by Arisetiawan⁹, which uses lip prints to reveal the inheritance of patterns from father and mother to child, his research shows the inheritance of lip prints from parents to children. In addition, there is an exciting study to find out the relationship between lip print patterns and ancestry, whether the descent is pure Malay ethnicity or not, in the Malay ethnic group in Riau. The results of Afandi and Mandatasari's study¹⁰ showed no significant relationship between lip prints and ancestry.

Based on previous research or research, there are varying results regarding the type of dominant pattern in males and females. Therefore, we would like to study the type of lip prints, whether they could differentiate males from females by utilizing literature from previous studies that have been done.

Methods

We conducted a literature review on the many forms of lip prints, which we discovered in several databases. To begin, keywords such as lip prints, cheiloscopy, sex category identification, and type of lip print patterns were identified. Scientific publications, thesis outcomes, theses, and books were all sources of data from prior study. Then, according to the study topic, we carried out a selection process.

All prior studies on sex category identification based on lip print patterns that employed Suzuki and Tsuchihashi's lip print classifications were included in this analysis. We only look at research that was published between 2009 and 2020. The lipstick method of taking lip prints was the most common.

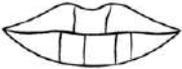
Findings

Classifications of Lip Prints

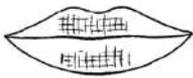
The existence of lip print classifications such as those of Suzuki and Tsuchihashi will facilitate the analysis of sex identification. In addition, there are also other lip print classifications; however, they are not used as a reference in the analysis of this study.

According to Suzuki and Tsuchihashi's¹¹ classification divided into six types according to the shape and path of the grooves (Table 1).

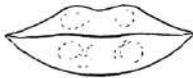
Table 1: Classification of Lipprint Patterns According to Suzuki and Tsuchihashi¹¹

Classification	Grooves Type
	Type I (Vertical complete) is a straight groove and crosses the entire width of the reddish part of the lips.
	Type I' (Vertical incomplete) is a straight groove but does not cover the entire lip.
	Type II (Branched) is a lip print whose groove is straight vertically and has a branch at the top of the groove.
	Type III (Intersected), namely the grooves of the lips that seem to cut from one another.

Contd.. Table 1: Classification of Lipprint Patterns According to Suzuki and Tsuchihashi¹¹



Type IV (Reticular), that is, the grooves of the lips look like boxes like a net.



Type V (Undifferentiated), the grooves do not belong to I-IV and cannot be differentiated morphologically.

Lip prints retrieval method

Lip prints at the crime scene must be identified quickly in order to determine if they belong to the criminal suspect or the victims. Lip prints can be

taken and documented immediately; however, the precise approach must be used to achieve the best results.

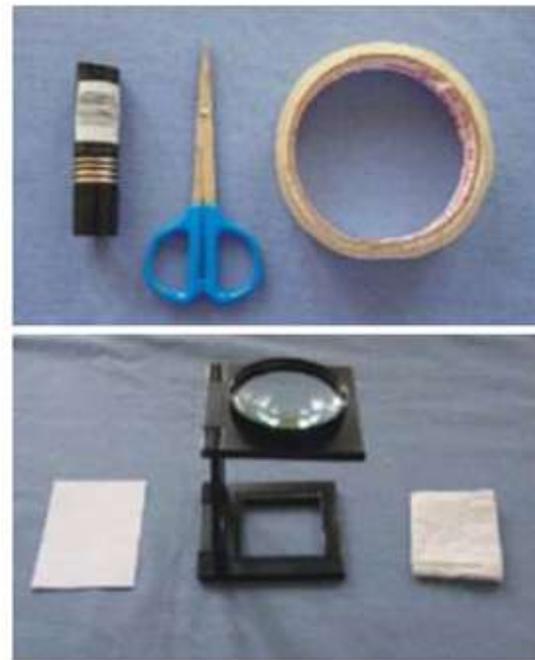


Figure 1: Tools, Materials, and Techniques Using the Lipstick Method

Source: Atmaji et al.²

Lip prints were obtained from the fissures, wrinkles, and grooves of the labiorum sulci on the labial mucosa (Figure 1). Research by Mishra et al.¹² described that lip print results were obtained from a substantially moving part of the lips and from that it would produce a different lip print even in the same individual, this was according to the direction, pressure, and method used in making the lip print. The development of odontology has contributed significantly to the emergence of cheiloscropy studies as a unique, individual, and stable identification tool. Both research¹³ by Budowle et al. and Rai & Anand suggest that forensic investigations are concerned with individual identification, which combines the application of lip prints, anthropology and odontology, and DNA profiling techniques. Suzuki and Tsuchihashi¹¹ confirmed that each lip print is

unique, and that a traumatized lip has no effect on the lip print.

According to Reddy¹⁴, cheiloscropy studies need to be refined into a unified system for use in forensic dentistry. Lip prints as proof of personal identification and criminal investigations are the subject of limited research papers and information. It will need additional research to uncover more facts from lip prints before they can be used as valid proof.

Classification by Suzuki and Tsuchihashi¹¹ is divided into six types of lip prints based on shape and groove so that it is used as the standard classification of lip prints. Furthermore, this strategy provides a clear representation, making it easier to comprehend and master. Based on the aforementioned classifications, this study prefers to adopt Suzuki and Tsuchihashi's

classifications because they are used more frequently than others. Renaud's classification is considered a full classification of lip prints because there are 10 sorts of patterns, however it has only been used in a few previous investigations. Many prior research have preferred Suzuki and Tsuchihashi's more easy classifications over Renaud's because of the greater number of lip print type patterns.

A total of 20 scientific articles that have been analyzed have similarities and differences among them. Some of the differences included the number of research samples, the location of the study, the lip print technique--Single motion or Prabhu's method, and the method of data analysis. In addition, several scientific articles explained analysis techniques to make it easier to visualize into several divisions into 4, 6, 8, 10 quadrants on the subject's lips.

Ranjan et al.⁶, Vatchala et al.¹⁵, and Rastogi & Parida¹⁶ performed a lip print analysis by splitting the lips into four quadrants. Unlike previous studies, Costa & Caldas¹⁷ and Moshfeghi et al.¹⁸ divided lip print analysis into six quadrants. Mahampang⁵ conducted a lip print study by dividing the lips into eight quadrants, whereas Sunday et al.¹⁹ between the inner labial mucosa and outer skin. Examination of this lip prints is known as cheiloscopy. Some authors have worked on lip print in the past and made some striking points on its application in forensic studies and human identification. The study was carried out to identify and compare the lip prints patterns among the Igbos. The study was done to investigate and document the characteristic cheiloscopy pattern of Igbos and to assess the distribution of lip print patterns among males and females. A total of 300 subjects were used for the study. 150 were males, 150 were females, all of which were normal subjects. Subjects were selected and identified based on an oral interview. These subjects were selected through purposive convenient sampling method. The males and females had their highest percentage distribution in type I (35.73% divided the lip quadrant into 10. Mahampang⁵ also explained that accurate analysis results would be obtained using the eight quadrant observation method by showing all the variations in each individual's type of lip print pattern. As a result, researchers can employ the eight quadrant approach as a way of extensive observation, making it easier to assess and detect sex. In the analysis of cheiloscopy procedures, it can be said that there is no apparent standardization of quadrant division.

The tools used to generate the total lip print impression: lipstick and masking tape, are identical to the information gained from these scholarly studies. This tape is used to transfer the printed results so that they may be visualized and analyzed more easily. It is clear from the findings of the investigation that each person's lip print is distinct. It is also supported by Yadav's²⁰ study, which claims that lip prints are similar to fingerprints and bite marks in that they're unique to each person and can be easily studied using the cheiloscopy procedure.

In cases of sexual crimes, lip prints are generally found at the crime scene and on the victim's body--starting from the face, neck, and other body parts.²⁰ Kasprzak²¹ stated that identifying persons based on the imprints formed by the lips is difficult since the effect of the wound on the lips might obscure the lip trace because of a stain covering the lips, but this can be exposed by thorough chemical investigation. Lip print traces discovered at a crime scene can be used to deduce details about what happened, such as the number of persons involved, the type of cosmetics used, work habits and nature, sex categorization, and the existence of specific pathologies on the lips.

According to Karki's²² research, the outcomes of lip print prints are permanent and unique to each individual. As a result, if a suitable method cannot be developed, the lip print approach can be utilized as a substitute for other forensic investigative methods, and lip prints have the potential to be a supporting tool in identification.

This statement from Karki²² is backed up by Abedi et al.²³, who claim that lip prints aren't the same as fingerprints because there are still flaws, such as the lack of validation and quality in defining specific lip print procedures. Lip prints, on the other hand, can sometimes help provide answers and act as extra evidence in relation to the events being investigated. More research is needed to continue developing and discovering appropriate procedures, as well as to increase the validity of lip print evidence as a tool in criminal justice delivery. The importance of attempts to undertake lip print recording on all individuals, according to Domiaty et al.²⁴, is to develop a database that may be utilized to provide answers to civil and criminal cases.

Another study conducted by Eldomiaty et al.²⁵ lower middle and lower left tried to investigate the stability of the lip print pattern. Although the

study took almost three years to complete, lip print data was collected from September 2009 to October 2012. For reasons that are frequently observed at crime scenes, this study focuses primarily on the lower lip print. The old lip print data is maintained in envelope paper for three years before being compared to the fresh lip prints. The results revealed that 89.6 percent of the old and new lip prints had identical grooves, while the remaining 10.4 percent had a comparable pattern in one or more regions. In line with Eldomiaty et al.²⁵ lower middle and lower left, Ludwig and Page²⁶ conducted a study that was designed to determine whether or not the details of lip prints with lipstick were available, and the results of lip prints were then compared to determine whether these two things could be linked between each other. It was done to see if the flow patterns of each subject were similar and to confirm the use of cheiloscropy in forensic investigations.

Conclusions

The diversity of the analysis technique in visualizing lip prints, which splits the quadrants on the lip print into different categories, including 4, 6, 8, and 10 quadrants, is revealed by a review of 20 prior scientific works. Finally, the outcomes of lip print kind and pattern are dominant for both male and female sexes. As a result of the differences and similarities in information gathered from the analysis of 20 prior scientific works, it is possible to infer that this knowledge is useful for this study.

The dominating lip print type data from each previous research sample, both male and female, were then used to create groupings. Seven out of twenty scientific publications belong to the type III lip print pattern group, which is dominated by men. There are around 11 scientific papers on the female sex category that belong to the type II lip print pattern category. Six scientific publications in male lip prints belong to the type II lip print pattern group, while seven scientific articles in female lip prints belong to the type I lip print pattern group. Suzuki and Tsuchihashi classified lip prints into six categories: type I, I', II, III, IV, V. Based on the study of the grouping of lip print results in Table II, there is one scientific publication in the type V group for both males and females. We found the type IV lip print pattern mostly in women.

The following conclusions can be taken from the findings of study and analysis conducted on 20 prior scientific studies linked to sex determination

based on the type of lip print pattern belonging to Suzuki and Tsuchihashi: The type II lip print pattern is represented in six research articles. 1) Type III wrinkles and lip prints (lip prints) were the most common type and pattern of wrinkles and lip prints (lip prints) in men (Intersected). As a result, type III predominates in as many as seven of the prior twenty scholarly articles. The type I lip print group includes seven scientific articles. 2) The female had the dominant type and pattern of wrinkles and lip prints, which was type II – Branched. As many as 11 of the last 20 scientific articles demonstrate the dominance of type II research.

The following conclusions can be taken from the findings of study and analysis conducted on 20 prior scientific studies linked to sex determination based on the type of lip print pattern belonging to Suzuki and Tsuchihashi: 1) Type III lip print pattern group is dominated by males, and type I lip print pattern group is dominated by females. 2) As many as 11 of the last 20 scientific articles demonstrate of type II is dominated by female, but that type II is dominated by male is represented in 6 research articles. Therefore, when an individual's pattern is type II, the individual could be either a male or a female, with a higher probability of being a female.

Conflicts of Interest: None

Source of Funding: None

Ethical Clearance: FISIP Universitas Airlangga has granted the permission to accomplish this research

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Estimate the Prevalence of Fatty Liver in Overweight and Obese Children in Ilam

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How to cite this article: Gholamreza Kalvandi, Reza Najafi, Kourosch Sayehmiri et al. Estimate the Prevalence of Fatty Liver in Overweight and Obese Children in Ilam. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):189-193.

Abstract

Introduction: Obesity worldwide is considered as the single largest cause of chronic illness. The children of the present generation are the only people throughout history who die because of problems with overweight before their parents. Obese children are prone to fatty liver disease. Fatty liver is one of the chronic liver disorders that is associated with lipid accumulation in hepatocytes. The aim of this study was to estimate the prevalence of fatty liver in overweight and obese children in Ilam and to investigate some of its possible causes, in order to consider the need for planning for educational interventions for behavioral change.

Method: This cross-sectional study was carried out in Imam Khomeini Hospital, Ilam, in 2017, and among 106 children aged 6-18 years who were referred to the center for evaluation, a randomized sampling was performed and divided into two groups of overweight and obese based on their BMI.

Conclusion: Regarding the relatively high prevalence of non-alcoholic fatty liver disease in school-age children, and especially in adolescents, the need to educate parents and their children for proper nutrition and daily physical activity with the correct method is still felt.

Keywords: Prevalence; Non-Alcoholic Fatty Liver Disease; Overweight; Obese; Children.

Introduction

Obesity worldwide is considered to be the single largest cause of chronic diseases.¹ Type 2 diabetes, high prevalence of cardiovascular disease, increased number of cancers and the occurrence of a group of mental illnesses associated with obesity.² Obesity and the resulting problems impose significant costs

on societies. Obesity is predicted in the 21st century as the most important threat to human health.³ Children of the present generation are the only people throughout history who die for reasons of overweight problems before their parents.⁴

Obese children are prone to fatty liver disease. Fatty liver is one of the chronic liver disorders that

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is associated with lipid accumulation in hepatocytes, and is usually caused by accumulation of fat in the liver of more than 5% by weight of the liver. This disorder is due to steatosis (a simple fatty liver, which is a benign disorder) to non-alcoholic steatosis hepatitis, in which fat accumulation accompanied by inflammation and damage to the fibrosis of the liver tissue, ultimately progressive fibrosis and cirrhosis.⁵ Fatty liver disease is often off, sometimes with increased liver enzymes.⁶ The prevalence of fatty liver in obese children has been reported from 42.6 to 77.1 in various studies,⁷⁻⁹ and in the near future fatty liver disease is expected to be the most common cause of chronic liver disease in children. Awareness of Fatty Liver Disease can strengthen the child's and his family's motivation for diet and exercise, as well as new therapies for Fatty Liver Disease, helping to relieve the disease.¹⁰

The aim of this study was to estimate the prevalence of fatty liver in overweight and obese children in Ilam and to investigate some of its possible causes, in order to consider the need for planning for educational interventions for behavioral change.

Methods

In a cross-sectional study that was conducted in Imam Khomeini Hospital of Ilam city in 2017, 106 children were randomly selected among children between the ages of 6 and 18, who were referred to the center for general evaluation. They were divided into three groups: overweight (85 to 95th percentile), obese (above 95 percent), and patients with severe obesity (more than 120 percent of 95th percentile or BMI greater than 35) based on their BMI. The three

groups included 41 (38.7%) overweight, 46 (43.4%) obese and 19 (17.9%) with severe obesity.

Students entered the study after their consent, and their parents. In case of dissatisfaction, another person entered the study. All children and adolescents were examined by a pediatrician. For all participants in the study, a preliminary questionnaire including age, sex, moderate to severe daily activity and clinical examinations including height, weight, blood pressure and examinations for diabetes, liver disease, and endocrine diseases were completed. Students who had mental retardation, chronic drug use, chronic medical condition, genetic symptoms or syndromes, signs of liver dysfunction and signs of endocrine disease were excluded.

Individuals Body mass index (BMI) of the patients were measured, and was calculated based on the BMI percentile charts for age and gender. All of these patients were undergo liver ultrasound by 3 radiologists who were aware of the goals and type of study, but did not have any information on how to divide them. Data were analyzed by SPSS-24 software and P values <0.05 were considered significant.

Results

In this study, 106 children and adolescents 6-18 years old with a mean age of 10.03 ± 2.65 years were studied. The subjects were divided into 3 groups based on their BMI including 41 people (38.7%) with overweight, 46 people (43.4%) with obesity and 19 people (17.9%) with severe obesity. Of the total number of participants, 22 (20.8%) were boys and 84 (79.2%) were female. Table 1 shows the prevalence of NAFLD in different groups of this study.

Table 1: Distribution of NAFLD in overweight and obese children

Variable	Fatty Liver (+) N (%)	Fatty Liver (-) N (%)	Total	P Value
Overweight	5 (12.2)	36 (87.8)	41 (38.7)	≤0.001
Obese	9 (19.6)	37 (80.4)	46 (43.4)	
Severe Obesity	11 (57.9)	8 (42.1)	19 (17.9)	
Total	25 (23.6)	81 (76.4)	106 (100)	

According to Table 1, the prevalence of non-alcoholic fatty liver disease in the whole population was 23.6%. Also, 12.2% of overweight patients, 19.6% of patients with obesity and 57.9% of patients

with severe obesity had NAFLD. In a more general comparison, it can be said that the prevalence of fatty liver was 12.2% in overweight people and 30.8% in obese people.

In the sonographic survey, 9 out of 15 boys and 16 out of 91 girls had NAFLD disease. According to the analysis, there was a significant relationship between the risk of fatty liver and sex and the chance for NAFLD was higher in boys. ($P < 0.05$)

In the study of age groups, these subjects were divided into two age groups of less than 12 years of age and over 12 years old to determine the relationship between age and the probability of non-alcoholic liver disease, in which there was no significant relationship between the risk of disease Non-alcoholic fatty liver disease and age ($P = 0.469$).

In this study, the relationship between body mass index and non-alcoholic fatty liver disease was measured, which indicated a strong correlation between the increase in BMI and the increased risk of NAFLD disease ($P < 0.05$).

In this study, the mean level of AST and ALT liver enzymes was measured in NAFLD patients based on fatty liver grade and also based on body mass index. In measuring the level of enzymes based on the

disease grade, according to the results, the mean of AST and ALT in the Grade 1 disease was higher than the normal maximum, but with increasing severity of the disease (from Grade 1 to 2), no significant increase was observed in the mean level of enzymes (Figure 1).

Also, in assessing the level of liver enzymes based on the severity of obesity, according to the results, the mean levels of AST and ALT increased with an increase in obesity, and this figure also reached 2 times the normal maximum in people with severe obesity (Figure 2).

It should be noted that these measured values only represent the average level of enzymes in some subjects, because despite the presence of NAFLD in some patients, they had no increase in the level of enzymes.

Figure 1: shows the mean liver enzyme levels based on the disease grade. According to this chart, the average level of enzymes in Grade 1 is higher than the Upper Limit of normal.

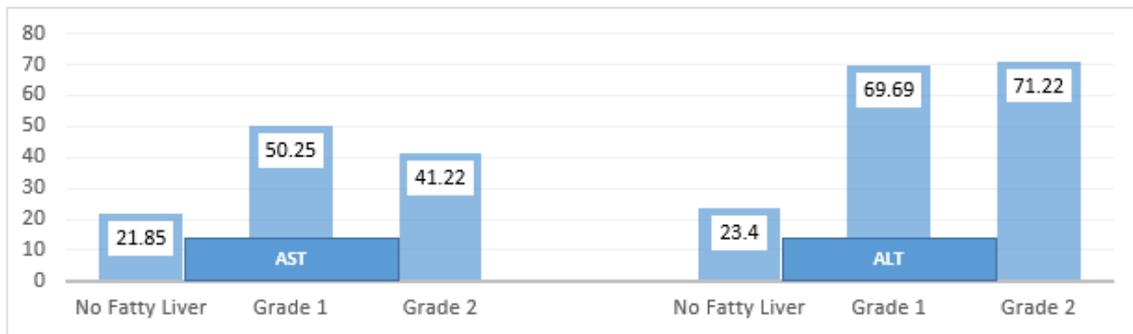


Figure 1: Comparison of mean levels of liver enzymes in NAFLD patients based on disease grade

Figure 2: shows the average level of liver enzymes based on the severity of obesity. According to this chart, the average level of enzymes increases with

an increase in the severity of obesity and in severe obesity it reaches about 2 times the normal maximum.

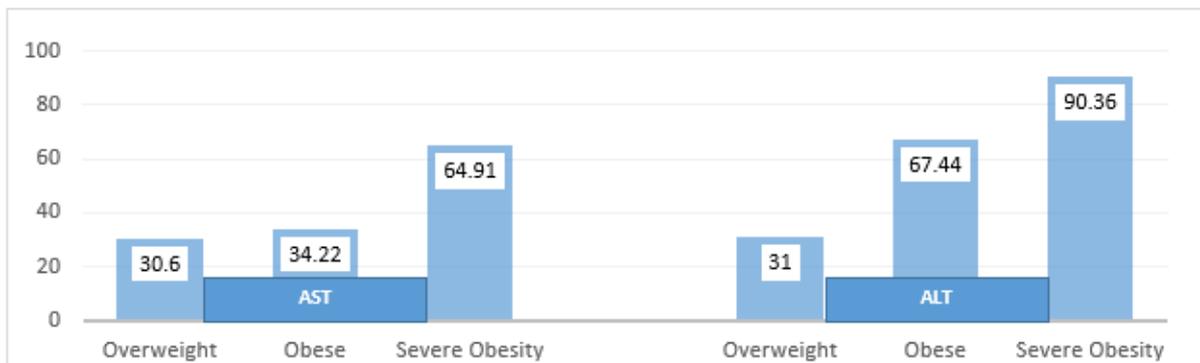


Figure 2: Comparison of mean levels of liver enzymes in NAFLD patients based on severity of obesity

Discussion

Awareness of Fatty Liver Disease can strengthen the child's and his family's motivation for diet and exercise, as well as new therapies for Fatty Liver Disease, helping to relieve the disease.¹⁰ In this regard, our study was the first study in children in Ilam province.

According to the information obtained from this study, most of the patients with overweight and obesity were below the age of 12 years (73.6%) and the prevalence of fatty liver in the studied population was 23.6%, which in the group with overweight was 12.2%, in the group with obesity was 19.6% and in the group with severe obesity was 57.9%. Also, if we divide people into overweight and obesity groups, the prevalence was 12.2% in overweight people and 30.8% in those with obesity. In a study conducted by Dr. Adibi et al., The prevalence of fatty liver in overweight children was 10.5 and in obese children was 54.4%.¹⁰ In the study of Shiasi Arani et al., The prevalence of non-alcoholic fatty liver in obese children was 55.3%.⁶ The prevalence of non-alcoholic fatty liver disease in the United States is estimated to be between 16% and 23%, and in some studies it has reached 31%.¹¹ According to studies, the prevalence of non-alcoholic fatty liver disease in overweight and obese people is lower in this study than in other studies in Iran, which can be attributed to climatic conditions and nutritional status of the area. It should be noted, however, that the study population was smaller than other studies, and the vacancy of a wider study is also felt by the presence of people without overweight and obesity.

In the study of Pan and colleagues, the prevalence of fatty liver in some cases was higher in women and in some cases was higher in males.¹² In this study, there was a significant relationship between the risk of NAFLD and sex. The likelihood of having a NAFLD is greater in the boy's sex. Of course, this can be linked to the lower number of boys referring to the clinic, and more studies are required to review them.

In our study, there was no significant relationship between fatty liver disease and age of patients. In a study by Schwimmer and colleagues, there was a significant relationship between age and the risk of developing fatty liver disease.¹³ However, we should note that our study was conducted on children, as

well as the larger statistical population can change the results.

In our study, in patients with NAFLD, the mean level of liver enzymes was higher than that of healthy individuals, but with an increase in disease severity, there was no significant increase in the mean level of liver enzymes. Also, in this study, with an increase in the severity of obesity, the mean level of liver enzymes was higher in people with NAFLD and in the group with severe obesity it was up to 2 times the normal upper limit. In the study of Sanial et al., The level of ALT and AST enzymes in people with fatty liver disease is 1 to 4 times of normal, which is consistent with our study⁽¹⁴⁾, but with regard to the level of enzymes there is a need for more studies based on the disease grade. However, in the study of Strangers et al., T was a significant relationship between BMI and serum levels of AST and ALT.¹⁵

Conclusion

Regarding the relatively high prevalence of non-alcoholic fatty liver disease in school-age children, and especially in adolescents, the need to educate parents and their children for proper nutrition and daily physical activity with the correct method is still felt, but the results of this study indicate that the prevalence of non-alcoholic fatty liver in the city of Ilam is lower than in other cities studied in Iran, and this can be due to various causes, including the climatic conditions of the city, the nutritional and social culture of the people of this region, Attention to physical activity and even genetic differences. Probably more studies to investigate the causes of overweight and obesity in Ilam can be helpful in identifying the factors affecting NAFLD disease in this area.

Conflict of interest statement: No potential conflict of interest relevant to this article was reported.

Acknowledgement

This article is the result of a GP thesis, GP96153, and it was approved by the University Ethics Committee under IR.AJUMS.REC.1396.1083.

Funding

This research was sponsored by the Vice-Chancellor of the research and technology deputy of Ilam University.

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Menstrual Disorders Following Tubal Ligation

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How to cite this article: Hyaam A Lafta Al-Assadi, Sajida Al-Rubai, Huda Qahtan Fouad. Menstrual Disorders Following Tubal Ligation. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):194-198.

Abstract

This is a prospective case – control study that was carried out at Basrah Maternity and Child Hospital from 1st of January 2019 till 1st of June 2020.

Objective: The objective of this study is to find an association between menstrual disorders and bilateral tubal ligation.

Design: It is a case-control study of 30-45 years old women setting at Basra governorate.

Material and Method: Three hundred twenty four patients were included in the study of age [30- 45] years, they were divided into two groups. group one [156] with history of tubal ligation 1-3 years ago compared to a control group of [168] without tubal ligation a non-medical contraception was used.

Result: In group one menorrhagia was more common when compared with non-tubal ligation group [68.5% Vs 29.7] respectively and the difference was statistically significant.

Conclusion: Our findings show that menstrual disorders were more common in women tubal ligation. This study shows that tubal ligation may influences irregular menstruation and causes menorrhagia. In other words, tubal ligation is associated with an increased risk of menstrual disorders.

Keywords: Menstrual disorders; tubal ligation.

Background

Menstrual disorder is one of the problematic effects of tubal ligation, although the results of related studies have been inconsistent and inconclusive.^{1,2} Some women who have completing family planning choose tubal ligation (TL) as a method of contraception.³ The occurrence of abnormal bleeding after TL was first described by Williams et al.⁴ The amount of blood loss can range from slight spotting to

80 mL and the average being 30mL. Loss of more than 80mL of the blood is considered abnormal. It has been hypothesized that ligation may increase incidence of menstrual disturbances among women receiving TL. Several studies about the side-effects of TL on menstrual function have been conducted,^{5, 6} yet the existence of a post TL syndrome has been debated.

Abnormalities reports associated with TL surgery include the entire spectrum of menstrual disorders,

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such as: more frequent menstrual periods, irregular menstrual cycles, menorrhagia, metrorrhagia, spotting, dysmenorrhea and oligomenorrhea.⁷ However, some studies^{1,8} showed no increase in menstrual disorders in women undergoing TL as compared with a control. The usual duration of the menstrual flow is 3-5 days, but flows as short as 1 day and as long as 8 days can occur in a normal female.

Around the world, sterilization is the chosen option to more than 220 million couples desiring contraception.⁹ Sterilization was the most common method of contraception used in the United States, utilized by 47.3% of married couples.^{9, 10} Tubal ligation accounted for 30.2% and vasectomy for 17.1%^{9, 11} For those who have completed childbearing, sterilization using tubal ligation is a safe and effective contraceptive option. The aim of this study is to compare the occurrence of menstrual disorders at women with or without tubal ligation (TL), identify the type of menstrual disorder in women with tubal ligation, resolving the debate about menstrual disorder after tubal ligation.

Methodology

This is a prospective case control study conducted at Basra maternity and child hospital. The study was carried out from 4th of January 2019 till 1st of June 2020. 156 women underwent tubal Sterilization [1-3] year ago and to assess the effect of tubal sterilization on menstrual cycle. All been subjected for diagnostic curettage due to abnormal uterine bleeding compare to 168 healthy women using condom as a contraceptive, pills at least for 3 months where assigned as tubal ligation and non-tubal ligation group respectively.

All the patients were multiparas, of Age group between 30-45 years, there is no chronic medical

illness such as diabetic mellitus, hypertension, thyroid disorder, cardio vascular disease, no gynecological disease and there is at least three normal cycle before tubal ligation. We compared the distribution of social demographic characteristics, obstetrical as well as menstrual bleeding between the two studied groups and a validated pictorial blood loss assessment chart [PBAC] was also used for the evaluation of menstrual blood loss [MBL], which used to assess blood lost depending on certain criteria such as presence of clots, number of stain pads which should be of the same type. All our participants are volunteers and were studied, most MBL abnormalities were taken.

- Normal menstrual| blood loss: which a menstrual interval of 21-35 days and flow with duration of 7 days or less.
- Oligomenorrhoea: bleeding interval longer than 35 days.
- Polymenorrhoea: menstrual interval shorter than 21 days
- Menorrhagia: When PBLAC scored 2 100.
- Metrorrhagia: vaginal bleeding between the expected menstrual period
- Hypermenorrhoea: flow extend beyond 7 days
- Menstrual irregularity: Which is a menstrual interval shorter than 21 days and longer than 35 days, and amount of blood lost is varied.

All the statistical analysis where done by SPSS version 20 student- t - test, and chi square was performed and revealed statistical differences between studied groups. A P. value less than 0.05 was regarded to be statistically significant.

Result

Table 1: Comparison of socio-demographic characteristics between TL and Non TL groups

	TL [156]	%	NON-TL (168)	%	P Value	
Age	38.6±1.8		39.2±1.1		0.477	
AGE OF MENARCHE	12.6±1.8		12.9±1.1		0.574	
PARITY	6.3±2.3		7.1±1.8		0.267	
BMI [kg/m ²]	30.8±3.4		31.1±3.8		1	
Educational level	Illiterate	91	58.33333333	71	42.2619048	0.23
	Educated	65	41.66666667	97	57.7380952	0.46

		TL [156]	%	NON-TL (168)	%	P Value
PREVIOUS CONTRACEPTIVE METHODS	PILLS	110	70.51282051	88	52.3809524	0.23
	CONDOM	35	22.43589744	42	25	0.317
	OTHERS	11	7.051282051	36	22.6190476	0.17
MODE OF DELIVERY	NYD	92	58.97435897	132	78.5714266	0.072
	CS	64	41.02564103	36	21.4285714	0.005
SMOKING	YES	30	19.23076923	48	28.5714286	0.022
	NO	126	80.76923077	120	71.4285714	0.847

Table 1 shows the comparison of the socio-demographic characteristics between tubal ligation (TL) and non-tubal ligation groups (Non TL). Table 2

shows menstrual disorder between the two groups, and table 3 shows the histological comparison between tubal sterilization group and control group.

Table 2: Menstrual Disorder between the two groups

Menstrual pattern	TL [156]	%	NON-TL (168)	%	P Value
Oligomenorrhea	42	26.9230769	149	88.69047619	0.41
Polymenorrhea	21	13.4615385	23	13.69047619	0.763
Menorrhagia	107	68.5897436	50	29.76190476	0.001
Metrorrhagia	43	27.5641026	39	23.21428571	0.659
Dysmenorrhea	31	19.8717949	34	20.23809524	0.71
Hypermenorrh	55	35.2564103	20	11.9047619	0.05
Menstrual Irregularity	38	24.3589744	35	20.83333333	0.725
PBLAC	138	88.4615385	83.9	49.94047619	0.001

It was found that; menorrhagia was more in TL group when compared with non TL, PBAC also

was found elevated in the first group and they were statically significant.

Table 3: Histological comparison between tubal sterilization group and control group

		TL [156]	%	NON-TL (168)	%	P Value
Hysterectomy		26	16.6666667	14	8.33333333	0.058
Endometrial tissue result	Proliferative	50	32.05128205	48	28.5714286	0.756
	Secretary	61	39.1025641	48	28.5714286	0.763
	Others	45	28.84615385	52	19.047619	0.133
Mid luteal serum progesterone	< 1ng/ml	14	8.974358974	24	14.2857143	0.297
	1-10ng/ml	51	32.69230769	67	39.8809524	0.413
	>10ng/ml	91	58.33333333	77	45.83333333	0.239

There is no statistical difference between two studied group in regarding to endometrial tissue result and serum progesterone only there is

statistically difference in the rate of hysterectomy among tubal ligation groups.

Discussion

From the review of the literature we found an impact of TL on menstrual cycle characteristics in spite comprehensive, and they have been inconsistent.¹² Our findings suggest that patient with TL are more likely to develop menstrual abnormalities when compare with Non TL, and the difference was statistically significant. When we compare the study finding with other studies it shows a significant increase in the incidence of menstrual disorder. In women underwent tubal ligation when compared with control group, we found there is increase in the duration of the menstrual period [hypermenorrhea], and the amount of bleeding [menorrhagia], and these finding was in agreement with other studies done be.¹³ This can be attributed and explained that tubal ligation might cause damage to the ovary due to increase in the pressure in the utero-ovarian arterial loop.¹⁴

In contrast several other studies concluded that, the duration of bleeding, volume of menstrual flow, menstrual cycle length and cycle irregularity are similar in women with or without TL and these were confirmed by^{12,15}; although, as we mentioned it has been hypothesized that menstrual disorder are caused by the damage effect of tubal ligation on ovarian function through disruption of the ovarian blood supply.¹⁶ Menorrhagia identified as the most common bleeding disorder noticed after tubal ligation which was confirmed in our study by using PBLAC, we found a significant increase PBLAC score in women undergoing tubal ligation when compared with a non-tubal ligation, while several studies showed that there was no significant difference regarding menorrhagia between the tubal ligation and non-tubal ligation groups.¹⁷ In other study done by Wilcox et al. They reported heavy menstrual flow after five years following tubal ligation.¹⁸

Our study assessed the correlation between method of delivery and menorrhagia. Our finding indicated that menorrhagia more common in women with history of caesarian section (CS). Harlow et al¹⁵ concluded that menstrual irregularity, length of the menstruation, length of cycle and other menstrual disorders are similar in women with or without TL but women of CS and TL are more prone to having menstrual disorders and this can be attributed to that women with CS and TL may have endometrial defects at CS scar site and the weakness of uterine contraction as a cause of these menstrual disorders.¹⁵

Hysterectomy was noticed as a major indication of menstrual dysfunction or gynecological problems after TL which was also confirmed in our study when compared by other studies done.^{19, 20} Much of this could be explained by the fact that sterilization procedure has been performed for medical reasons and pre-existing gynecological problems contributed to the hysterectomy rate, regardless of whether sterilization produced the problem that led to hysterectomy, or once childbearing is no longer desired, so the presence of the uterus is much less important to many women and menstrual disorders are not tolerated as welling.

Conclusion

So our findings show that menstrual disorders were more common in women tubal ligation. There are still many important questions to be investigated about probable effect of tubal ligation on menstrual disorders. This study converges an important message that tubal ligation may influences irregular menstruation and causes menorrhagia. Hence women should be informed and instructed by health providers such as midwives and gynecologist regarding the advantage and disadvantage of tubal ligation.

Ethical clearance: Taken from Basrah Teaching Hospital, Basrah, Iraq

Source of funding: Self

Conflict of Interest: None

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Quantitative and Qualitative Determination of Gliotoxin and Acetaldehyde Toxins in Yeasts Isolated from some Respiratory Patients and Study of Yeast Resistance to some Antibiotics

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How to cite this article: Iman. H. Al Fayyadh, Mohammed Hashim al-Yasiri. Quantitative and Qualitative Determination of Gliotoxin and Acetaldehyde Toxins in Yeasts Isolated from some Respiratory Patients and Study of Yeast Resistance to some Antibiotics. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):199-207.

Abstract

The concentrations (64, 128, 32, 16, 8, 4, 2, 1, 0.5, 0.25 and 0.125) mg/ml were used to calculate the lowest inhibitory concentration of antibiotics for *Candida* yeast by microdilution method. The antibiotics fluconazole and itraconazole were used to complete this experiment, which is based on the results of testing the API *Candida* system by demonstrating the resistance of yeasts using several mechanisms, which serve to phenotype each organism selected in the framework. In this test, it was found that there is resistance to the antibiotics itraconazole and fluconazole in the types of yeasts under experiment. The toxin-producing yeasts extracted from yeasts showed that the toxin-producing yeast species were only 27 out of 30 samples of yeast, despite the growth efficiency of all samples. While 30 samples of yeast isolated from patients' sputum, it was found that all samples under the current study contain different amounts of acetaldehyde toxins with different concentrations.

Keyword: Gliotoxin; acetaldehyde; candida; itraconazole; fluconazole.

Introduction

During the past two decades, the incidence of diseases has become high and The species of pathogenic fungi increased significantly. The species of *Candida* yeast became the cause of The infection that is frequently encountered, especially at the present time, and which can cause the spread of a group of diseases that cause injuries in humans and animals, especially in the mucous membranes of the respiratory tract.¹ *Candida albicans* yeast is considered one of the main causes of death, especially in immunocompromised persons, while other species belonging to the genus *Candida* such as *C. glabrata*

and *C. krusei* are also considered pathogens, but to a lesser extent than *Candida albicans*.² The ones that cause infections of yeasts, especially *Candida*, are the possession of multiple virulence factors that enable them to attack the host and to attach more, such as changes in physiology, hydrolysis, and adhesion.³

Aims of study:

1-Detection of Gliotoxin and acetaldehyde from yeasts isolated from the respiratory tract.

2-Determination of the minimum concentration of fluconazole and itraconazole inhibitors for *Candida* yeasts isolated from the respiratory tract.

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Material and Method

Antibiotic Agents

The antagonists used in this study are 150 mg/ l for fluconazole and 100 mg/1 for Itraconazole .

Preparation of solutions

RPMI-1640 Medium It is a growth medium used in cell culture, with acidity up to, this medium was used for the purpose of testing the sensitivity of *Candida* yeasts.⁴

Sensitivity test to determine minimum inhibitory concentration (MIC)

The standard method described here aims to describe the method of performing a sensitivity test used for *Candida* yeasts and measuring their PURPOSE To determine the ability of yeasts to resist antifungals or susceptibility to antifungals used in the experiment in order to obtain data matching known laboratory standards (NCCLS) in fungal control. This test is also consistent with the results of the US National Committees for Clinical Medicine, and is among the most important internationally approved standards M27-A2.⁵ BROTH MEDIUM RPMI 1640 medium supplemented with 2 mM Glutamine, 4.5 g glucose with pH meter⁶ was used.

Fungicides

Antifungal drugs, including itraconazole and fluconazole, were prepared from the drug's manufacturing bottle, which was provided with the generic name of the drug, biological activity expressed in international units, micrograms per milligram of antifungal powder, expiration date as well as storage conditions. Prepared antifungals can be stored in the refrigerator as recommended by the manufacturers.⁷

Prepare stock solutions I prepared solutions for antifungal drugs by equation

$$\text{Weight (mg)} = \frac{\text{volume (mL) desired concentration (mg/mL)}}{\text{antifungal potency (mg/mg)}}$$

Detection of mycotoxins Acetaldehyde and Gliotoxin

Mycotoxins were detected using HPLC technology for Gliotoxin and absorption spectroscopy for acetaldehyde toxins as follows

High-performance liquid chromatography(HPLC) for Gliotoxin

Gliotoxin separation was performed in a column made of C18 (250X4.6) particles of 5 µm size (Knauer, Germany) according to⁸, 20 µl of each biological sample was analyzed using reverse phase HPLC technology with UV detection (RP-HPLC). -UV) by HPLC system from Knauer (Germany) as shown in the table below.

Table 1: shows the methods for detecting Gliotoxin toxins using HPLC technology

No.	Component	Model or version
1	Binary high pressure gradient pump	P6.1L
2	Diode array detector	DAD 2.1L
3	Sample loop (20 µl) and injector	D1357
4	Analyses and system control software	Claritychrom , V 7.4.2.107

Micro-Detection of Acetaldehyde

A. solutions used in produce

1. Reagent solution 0.2% DNPH in 2m h HCL
2. 2 of chelating solution (also called plank) 2 molar hydrochloric acid.
3. 20% w/v Trichloro-acetic acid
4. 50% absolute ethanol
5. Ethyl acetate.
6. A solution of 6.0 M quinidine hydrochloric acid dissolved in a 0.5 M solution of potassium phosphate (5.2 PH).
7. Quartz cell.

B. Colorimetric method for the determination of Acetaldehyde

Colorimetric determination of acetaldehyde with a formula of dinitrophenylhydrazone in aqueous solution was carried out by quantitative drawing in carbon tetrachloride by following the working method that included Divide the 20 mL volume between 5 and 35 µg of the acetaldehyde-containing sample, 5 mL of 2 4-dinitrophenylhydrazine reagent was added and left for 30 min at room temperature. The solution was shaken vigorously with 20 ml of carbon tetrachloride for one minute, after which the formed aqueous

layer was withdrawn again and placed with 5 ml of carbon tetrachloride, the sample was shaken again for 15 seconds, the formed tetrachloride extracts were transferred and titration was done to 50 ml, then 2 ml of ethanolic sodium hydroxide were added. Measure the optical density of the red solution formed within 10 minutes after adding the alkali.⁹

3.3.8. ELZA The ELISA device consists of two parts, one is the analysis base and the "reassembly base" with the absorbance of the material according to the specified wavelength.¹⁰

Result

Determination of the minimum inhibitory concentration of *Candidasp*(MIC)

The concentrations (64, 128, 32, 16, 8, 4, 2, 1, 0.5, 0.25 and 0.125) mg/ml were used to calculate the

lowest inhibitory concentration of antibiotics for *Candida* yeast by microdilution method as in Table (4.6) the antibiotics fluconazole and Itraconazole were used for completing this experiment, which is based on the results of the API *Candida* system test by showing the resistance of yeasts by using several mechanisms, which serve to determine the phenotype of each organism selected in the framework of this test, including In yeasts such as *Candida*, and when the test results interfere with the method of determining the minimum inhibitory concentration, they correspond to a database of no more than a phenotype and its distributions. These patterns correspond to the defense mechanisms of the organism. This process allows correcting the biological and therapeutic course by giving correct treatment reports about the possibility The use of antibiotics and changes in the permissible.

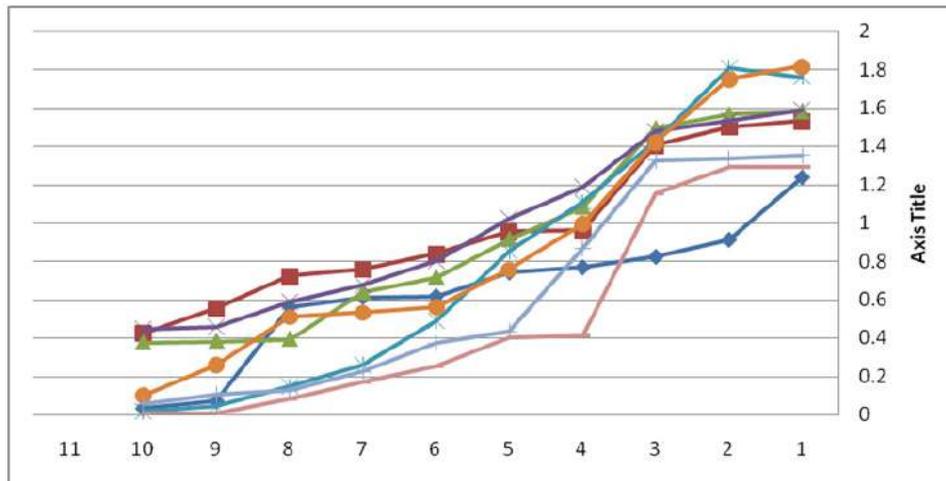


Figure 1: Shows the inhibition and resistance curves of Itraconazole by *Candida* yeasts

It is clear from the above Figures that there is resistance to the antibiotics Itraconazole and fluconazole in the types of yeasts under experiment, and according to the concentrations used, it starts from the highest concentration of 128 mg in some types of yeasts such as fungi, which are characterized by their ability to resist azole antifungal compounds in most cases, while the lowest inhibitory concentration for most yeast species of Itraconazole antifungal was 16 mg/ml. The MIC values (g/ml) of Itraconazole in RPMI at concentration 16 mg/ml for *Candida* yeasts were as follows: 0.851, 1.454, 1.216, 1.46, 1.391, 1.365, 1.418, 1.338, 0.823, 1.404, 1.492, 1.478, 1.429, 1.421, 1.324, 1.152, respectively, for *Candida albicans*, while

the MIC values (g/ml) for *Candida tropicalis* were as follows: 1.339, 1.409, and for *Candida dubliniensis* as follows: 1.486, 1.403, 1.471, 1.591, 1.586. while the MIC values (g/ml) for *Candida glabrata* were 1.554. As for the MIC values (g/ml) of Fluconazole in the RPMI at the concentration of 32 mg/ml they were as follows: 0.484, 0.435, 0.46, 0.431, 0.489, 0.519, 0.302, 0.243, 0.575, 0.55, 0.036, 0.266, 0.398, 0.362, 0.394, 0.257, for *Candida albicans* while the MIC values (g/ml) for *Candida tropicalis* were as follows: 0.425, 0.424 and for *Candida dubliniensis* as follows: 0.447, 0.412, 0.569, 0.447, 0.507, while it was The MIC values (g/ml) for *Candida glabrata* are: 0.507. as shown in Table 2.

Table 2: Shows the minimum inhibitory concentration MIC of *Candida* spp

N0	Isolate	MIC (g/ml) of Itraconazole	MIC(g/ml) of fluconazole
1	C. albicans	0.851	0.484
2	C. albicans	1.454	0.435
3	C. albicans	1.216	0.46
4	C. albicans	1.46	0.431
5	C. albicans	1.391	0.489
6	C. albicans	1.365	0.519
7	C. albicans	1.418	0.302
8	C. albicans	1.338	0.243
9	C. albicans	0.823	0.575
10	C. albicans	1.404	0.55
11	C. albicans	1.492	0.036
12	C. albicans	1.478	0.266
13	C. albicans	1.429	0.398
14	C. albicans	1.421	0.362
15	C. albicans	1.324	0.394
16	C. albicans	1.152	0.257
17	C. dubliniensis	1.339	0.461
18	C. dubliniensis	1.409	0.431
19	C. dubliniensis	1.486	0.512
20	C. dubliniensis	1.403	0.426
21	C. dubliniensis	1.471	0.577
22	C. trupicallis	1.591	0.469
23	C. trupicallis	1.586	0.548
24	C. glabrata	1.554	0.557

The above table shows that most of the *Candida* showed resistance to Itraconazole antibiotics at a concentration of 8 mg/ml, while *Candida* showed resistance to fluconazole at a concentration of 16 mg/ml, and this indicates that *Candida* yeasts show a high resistance to antibiotics, especially the azole group.

Detection of mycotoxin

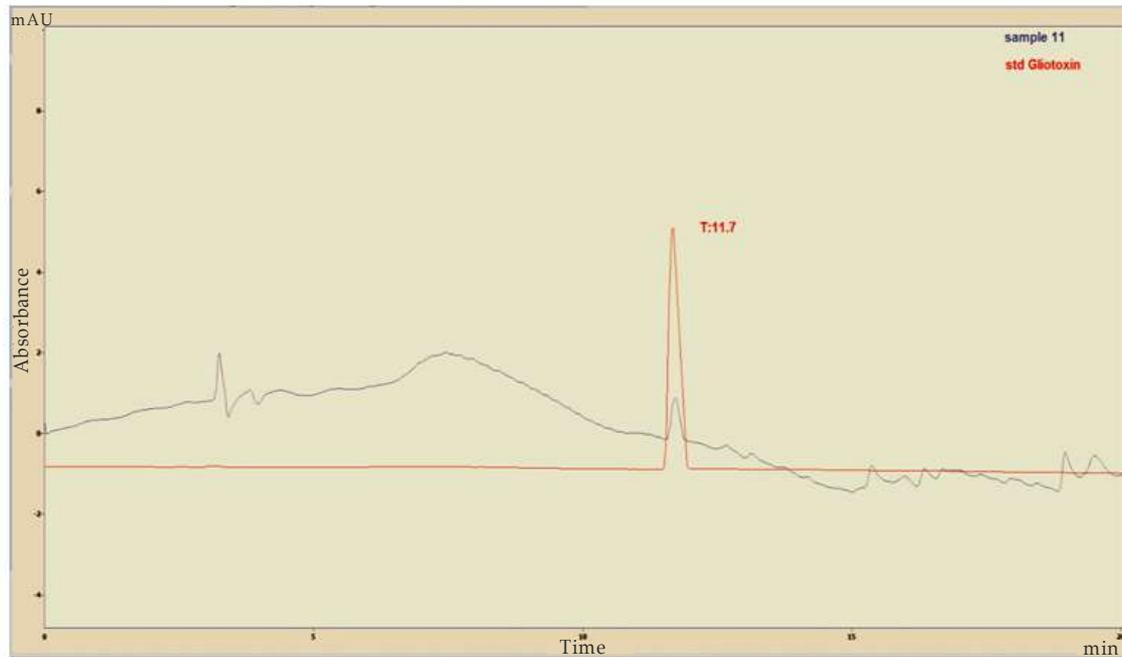
Gliotoxins using HPLC

The specific liquid chromatographic (LC) parameters were as follows: mobile phase (A) water, TFA (99.95: 0.05 v/v); (b) Acetonitrile, either mobile phase flow rate 1 mL/min; As for the degree

program, it was as follows : 10% b for 2 minutes, 10 to 90% B in 18 minutes, 90% B for 1 minute then 90 to 10% b in 1 minute Re-equilibrate to 10% B for 3 minutes. All analyzes were performed at 30°C. The detection wavelength (λ) was set at 254 nm. Gliotoxin was identified by its retention time and UV/VIS matching with standard substances. Then a quantitative measurement of 30 yeast samples isolated from sputum samples of patients with respiratory diseases in Thi-Qar Governorate was carried out by measuring the integrated peak area. The content was calculated using a calibration curve by drawing the peak area against the concentration of the peak area with the respective standard sample as it is shown in the following standard curve:

The HPLC gradient protocol is shown as in Figure 3, where the representative standard curve chromatogram of Gliotoxin of yeast samples cultured on RPMI medium shows the standard curve of Gliotoxin extracted from yeasts compared to the representative curve of Gliotoxin extracted from yeast species in the presence of the toxin in 27

samples Only from 30 samples of yeasts, despite the growth efficiency of all samples. Figure (4) shows the detection of Gliotoxin in small quantities and others ranged at higher rates. The cause may be due to the different genetic nature or environmental conditions specific to the production of Gliotoxin depending on the fungal type and yeast.



Figures 2: Show HPLC chromatograms for gliotoxin of some yeasts, where the retention time of glial toxins is determined in each chromatogram. HPLC chromatogram of *Candida albicans* isolate culture.

The results of the qualitative and quantitative detection of gliotoxin toxins using HPLC technology showed that 27 out of 30 samples contained gliotoxin

toxins with rates ranging from 0.23-2.06 $\mu\text{g}/\text{ml}$ as shown in Table 3:

Table 3: The amount of gliotoxin in yeasts

<i>Candida</i> spp	peak area	$\mu\text{g}/\text{ml}$ (working solution)	$\mu\text{g}/\text{ml}$ media
<i>C. albicans</i>	110.45	11.30	1.13
<i>C. albicans</i>	48.35	4.95	0.49
<i>C. albicans</i>	86.52	8.85	0.89
<i>C. albicans</i>	39.13	4.00	0.40
<i>C. albicans</i>	0.00	0.00	0.00
<i>C. albicans</i>	27.22	2.78	0.28
<i>C. albicans</i>	65.00	6.65	0.66
<i>C. albicans</i>	28.91	2.96	0.30
<i>C. albicans</i>	41.84	4.28	0.43
<i>C. albicans</i>	0.00	0.00	0.00

Contd... Table 3: The amount of gliotoxin in yeasts			
C. albicans	55.84	5.71	0.57
C. albicans	22.88	2.34	0.23
C. albicans	39.43	4.03	0.40
C. albicans	31.93	3.27	0.33
C. albicans	35.19	3.60	0.36
C. albicans	80.46	8.23	0.82
C. albicans	27.39	2.80	0.28
C. albicans	101.05	10.34	1.03
C. albicans	127.25	13.02	1.30
C. albicans	108.68	11.12	1.11
C. albicans	94.56	9.67	0.97
C. albicans	99.35	10.16	1.02
C. albicans	60.20	6.16	0.62
C. glabrate	58.23	5.96	0.60
C. dubliniensis	59.61	6.10	0.61
C. dubliniensis	201.70	20.63	2.06
C. dubliniensis	24.92	2.55	0.25
C. dubliniensis	142.73	14.60	1.46
C. dubliniensis	0.00	0.00	0.00
C. dubliniensis	67.68	6.92	0.69

Acetaldehyde

Using the spectrophotometer method for measuring the percentage of acetaldehyde in the 30 samples of yeast isolated from the sputum of patients it was found that all samples under the current study contain different amounts of acetaldehyde toxins with different concentrations.

When applying the recommended method for standard acetaldehyde solutions purified by ammonia derivatives and under the conditions required for detecting acetaldehyde, which start from the method of culturing samples on saline phosphite medium, it was found that the concentration of acetaldehyde ranged from 1.68 - 8.66mg /100 ml and As shown in Table 4.

Table 4: Show the concentration of Acetaldehyde toxin in yeast

No.	Yeasts species	Concentration of Acetaldehyde mg/100 ml
1	C. albicans	4.556575
2	C. albicans	5.382263
3	C. albicans	1.926606
4	C. albicans	2.232416
5	C. albicans	4.847095
6	C. albicans	2.293578
7	C. albicans	3.807339

Contd... Table 4: Show the concentration of Acetaldehyde toxin in yeast		
8	C. albicans	3.929664
9	C.dublinsiensis	3.180428
10	C.dublinsiensis	1.681957
11	C.dublinsiensis	5.290520
12	C.dublinsiensis	4.633028
13	C.dublinsiensis	5.856269
14	C. kruzii	4.908257
15	C. kruzii	3.960245
16	C. kruzii	5.733945
17	C. kruzii	4.816514
18	Naganishiadiffluens	4.113150
19	Naganishiadiffluens	3.134557
20	Naganishiadiffluens	3.486239
21	Naganishiadiffluens	3.256881
22	C. tropicalis	4.418960
23	C. tropicalis	4.204893
24	Candida lusitanae	4.571865
25	C.lusitanae	4.954128
26	Magnusiomycescapitatus	4.40367
27	Magnusiomycescapitatus	8.669725
28	Magnusiomycescapitatus	4.051988
29	Magnusiomycescapitatus	4.678899
30	Magnusiomycescapitatus	3.501529

Discussion

MIC identification:

Antifungal susceptibility testing plays an important role in testing antimicrobial drugs such as fungi, as an aid in drug development and as a means for the purpose of tracking antifungal resistance in most epidemiological studies¹¹, clinical and laboratory studies. The subcommittee establishes criteria for the purpose of testing antifungals in a standardized manner, including the minimum growth inhibitor concentration test, which provides clinical information that can provide important information about antifungal resistance.¹² The curves of Candida antigens are shown according to the concentrations used in this experiment and the minimum inhibitory concentration (MIC) for yeasts,

and according to the antigens used which included itraconazole and fluconazole, the MIC values were based on a point recommended by CLSI.¹¹

Detection of mycotoxin:

Gliotoxin using HPLC:

The HPLC method confirmed that Twenty-seven samples containing gliotoxin toxins were obtained from a sample of only 30, according to what was mentioned in the results. isolated from clinical samples cultured on RPMI medium, and also consistent with what was found by ⁽¹²⁾ who was able to isolate gliotoxin from 30 yeast samples, mostly containing Candida yeasts. Gliotoxin is considered a carcinogenic and deadly poison when exposed to it, as it was set within the recommendations of the World Health Organization that the lethal

dose of gliotoxin is 50: 67 mg/kg, While the lethal intraperitoneal dose reached: LD50: 32 mg/kg depending on the recommendations of the World Health Organization, and these concentrations are considered lethal. By causing body dysfunction or causing cancer, especially in the lung, exposure to gliotoxin is also a major cause of cancer and highly immunosuppressive that exacerbates infectious diseases or causes immunosuppression and the development of malignant lymphomas such as breast tumors, i.e increase by 10 to 100 times compared to normal cases of the disease, or may increase lung tumors and lung weakness. In the case of low concentrations of the toxin.¹⁴ These results are consistent with¹⁵ stated, when they found that out of 100 strains of *Candida*, there are approximately 60% of the strains capable of producing gliotoxin toxins, especially the clinically important strains that were detected by HPLC technology.

Acetaldehyde

From the results obtained and as shown in Table (4), these results are consistent with what the researcher¹⁶ mentioned and found. That all *C. albicans* isolates produced acetaldehyde at a concentration (>100 µM), which was isolated as a carcinogen in most smokers compared to non-smokers within the scope of the study and the differences were significant in most of the studied isolates. The results are also consistent with that reported,¹⁷ who found that all *Candida* and other *Candida* isolates produce Acetaldehyde toxins and that all *Candida tropical* isolates produce acetaldehyde at higher concentrations than other species, with rates as high as *C. krusei* (54.6 ± 2.9). NS). *C. tropical* and *C. parapsilosis* also produce by yeast high amounts of Acetaldehyde toxins with high moral variations. Acetaldehyde is one of the carcinogenic toxins which is the main cause of cancers such as lung cancer, oral cancer and respiratory system, which have spread recently and in very high rates in most smokers and when yeasts or fungi are present in their respiratory system. The reason may be due to the fact that smoking helps metabolize Acetaldehyde toxins produced by most of the *Candida* species that spread in the upper cavity of the respiratory system, which are considered microorganisms that mainly inhabit the moist parts of the body.¹⁸

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: Not required

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Overview of Lifestyle and Anthropometry of Students of the Faculty of Medicine Airlangga University During COVID-19 Pandemic

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How to cite this article: Jihadna Prima Santika Ruslan Musanip, Hermina Novida, Gadis Meinar Sari et al. Overview of Lifestyle and Anthropometry of Students of the Faculty of Medicine Airlangga University During COVID-19 Pandemic. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):208-213.

Abstract

Background: The COVID-19 pandemic has spread worldwide, forcing governments to implement quarantines as a preventive measure against the spread of the virus. Quarantine causes changes in lifestyle and anthropometry status among communities.

Objective: To describe the lifestyle and anthropometric status of medical students batch 2018 FM UA during the pandemic. Methods: This was a descriptive study using online questionnaires from September 2020 to February 2021.

Results: There were variations in students' anthropometry. Students' average height is 161.110 cm, the average weight is 60.308 cm, the average body mass index is 23.193 cm, the average upper arm circumference is 27.846 cm, and the average abdominal circumference is 79.621 cm. Furthermore, 145 respondents (81.9%) stated that they experienced changes in their lifestyle during the pandemic. A total of 89 respondents (50.3%) continued to exercise and the other 88 respondents (49.7%) did not. Regarding the sleep pattern, 108 respondents (61%) had quite good sleep quality and 50 respondents (28.2%) had quite poor sleep quality. For the food consumption pattern, 46 respondents (26%) ate more sweet foods. Furthermore, 156 respondents (88.1%) did sedentary activities, with 77 respondents (43.5%) doing sedentary behavior for more than 6 hours a day.

Conclusion: During the COVID-19 pandemic, the anthropometric status of medical students batch 2018 FMUA varied; and tend to maintain their physical activity, have quite good sleep quality, prefer eating sweet foods, and do a sedentary behavior for a longer duration

Keywords: COVID-19 pandemic, quarantine, lifestyle, anthropometry.

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Introduction

Coronavirus Disease 2019 (COVID-19) is an acute respiratory syndrome due to SARS-CoV-2 infection. Its first transmission was suspected from animals at the Huanan seafood market, Wuhan, China in December 2019. Eventually, this outbreak continuously spreads among communities throughout the world through human-human transmission and quickly progresses to involved worldwide populations.¹⁶

On March 11, 2020, the World Health Organization (WHO) declared that COVID-19 became a pandemic after resulting in 1,776,867 cases and 111,828 confirmed deaths.¹ In Indonesia, as of June 23, 2020, a total of 47,896 cases and 2,535 confirmed deaths of COVID-19 have been reported by the National COVID-19 Task Force.¹¹

As a measure toward this pandemic, all countries in the world, including Indonesia, have implemented a policy to restrict physical interaction and mobility through quarantine periods to break the chain of COVID-19 transmission.⁶ Prolongation of this quarantine, however, can affect the anthropometry status and lifestyle of the society, including college students, which is in line with diverse responses, either positive or negative responses.⁵ Following this public health concern, this study aimed to find out the description of the lifestyle and anthropometry status of medical students at the Faculty of Medicine, Universitas Airlangga, during the COVID-19 pandemic.

Materials and Methods

This was a descriptive study with a cross-sectional design carried out by using online questionnaires delivered to medical students batch 2018, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia. This study was conducted from September 2020 - February 2021. The questionnaire contained a set of questions assessing general characteristics of the research subjects, anthropometric data of respondents (height, weight, arm circumference, abdominal circumference), and lifestyle changes during this pandemic. A total of 29 questions were adapted from the prior study by Renzo et al. (2020). Data were obtained from subjects after gaining their consent and then served in the form of narratives, tables, graphs, or any other representative illustrations. This research has been reviewed and approved by the Health Research Ethics Committee,

Faculty of Medicine, Airlangga University, Surabaya (No. 111/EC/KEPK/FKUA/2021).

Results

A total of 177 medical students batch 2018 at the Faculty of Medicine, Universitas Airlangga met the criteria of research respondents. Anthropometric status of interest involved height, weight, body mass index (BMI), upper arm circumference (UAC), and abdominal circumference. The average of respondents' height was 161.1 cm. The height ranged from 131 - 182 cm, with the most observed student height was 155 cm (14.1%). The average body weight was 60.3 kg and ranged from 31.4 kg - 118 kg. Bodyweight of 48 kg and 56 kg was the most frequently reported weight (5.6%). Regarding BMI, the average BMI of students was 23.1 and varied from 13.2 - 52.4 BMI units. Additionally, BMI 19.5 was the most prevalent observed BMI (2.3%). According to the Ministry of Health of Indonesia classification, as many as 64.4% of respondents are categorized as had a normal BMI, while 2.8% of students were thin, and 15.7% of students were overweight. The average student UAC was 27.85 cm, varying from 14 cm - 60 cm. UAC 30 cm was the most commonly observed UAC (11.9% of students). Meanwhile, the average waist circumference was 79.6 cm with data distribution ranged from 32 cm - 160 cm. About 9.6% of students had a waist circumference of 80 cm. Furthermore, referring to the Ministry of Health Criteria, 45.5% of respondents were obese (Diagram 1).

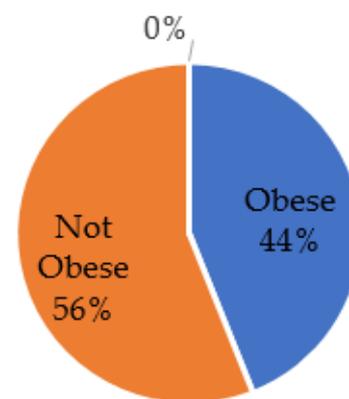


Diagram 1: Student obesity status at the Faculty of Medicine Universitas Airlangga based on Abdominal Circumference

Analysis of physical activity patterns found that 40.68% of respondents admitted did not perform exercise during the pandemic. However, the rest of the respondents declared that they still exercised

although its duration varied among students. The further data is presented in Diagram 2.

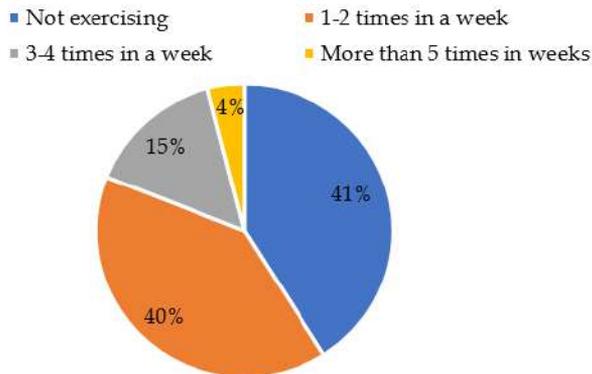


Diagram 2: The proportion of students exercised during the COVID-19 Pandemic

In the domain of sleep patterns, about 61% of students claimed to have quite good sleep quality

and 8.5% of students said to have very good sleep quality. Meanwhile, about 28.2% of respondents admitted to having quite poor sleep quality and 2.3% of respondents declared having poor sleep quality.

Regarding the pattern of frequently consumed foods, most of the respondents admitted that they ate more sweet foods. Conversely, referring to a pattern of rarely consumed food, respondents stated that they ate fewer vegetables. A detailed description of the diet is presented in Diagram 3.

Furthermore, concerning sedentary behavior, 88.1% of respondents stated that they watched TV/played computer/played video games/similar activities more frequently during the pandemic. Meanwhile, 43.5% of respondents even spent more than 6 hours a day doing these sedentary activities. The details are presented in Graph 1.

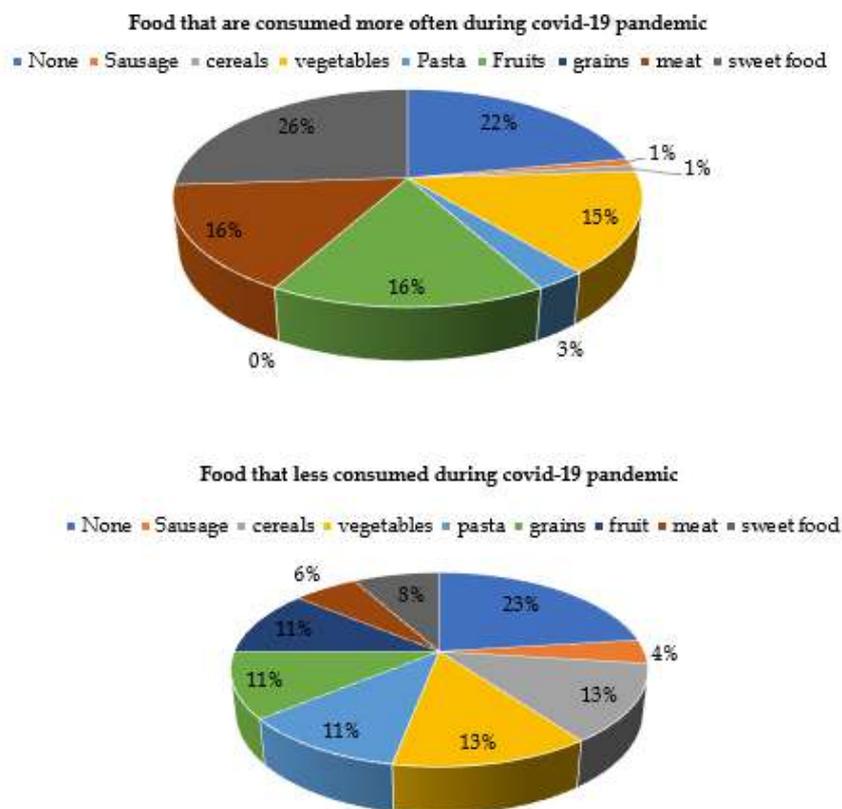


Diagram 3: Commonly and less commonly consumed foods by students during the pandemic

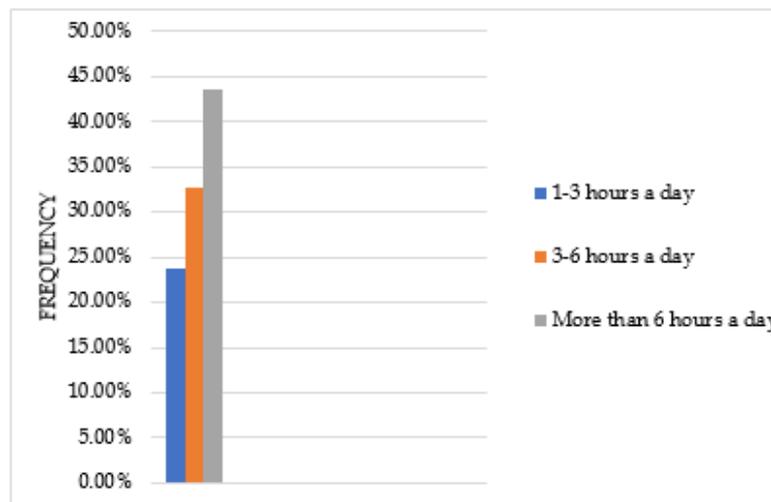
Discussion

Increased stress and depression have been associated with negative impacts on physical activity, nutritional habits, and health status. The high level of depression and anxiety might lead individuals to

preferentially consume sweeter foods and snacks, and probably reduce fruits and vegetable intakes. Unhealthy foods, such as candies, are associated with increased body weight and BMI.¹² Lack of physical activity and sedentary behavior (sedentary activity >2 hours) affects body weight, where an individual who

engages in sedentary behavior for more than 2 hours per day will have an increased risk of unhealthy body composition¹⁴. Another influencing factor of body anthropometry status is sleep patterns. Poor sleep quality due to short sleep duration (<6 hours) is a risk factor for the development of obesity and its complications². The findings of this study found the average body weight of the subjects was 60.31 kg, height was 161.11 cm, and BMI was 23.13 (this BMI was classified as above normal according to WHO but classified as normal according to the Ministry of

Health). The subject's mean waist circumference was 79.62 cm and the subject's mean arm circumference was 27.85 cm. The anthropometric status of subjects tended to be normal, concurring the subjects responded that though the subjects did sedentary behavior with longer duration and consumed sweet foods, most respondents stated that they maintain their routine exercise. In addition, based on the results of the study, respondents had quite good sleep quality.



Graph 1: Duration of student on Watching TV/Playing Computers/Playing Video Games/Similar Activities During the COVID-19 Pandemic

Regular physical activity and exercise are deemed to improve physical fitness, help to reduce the risk of various diseases and hold psychological benefits for a better individual condition.³ Nevertheless, the existence of quarantine has forced individuals to modify their behavior, including their physical activities such as exercise. Nonetheless, Brand et al. (2020) reported that quarantine did not dramatically reduce the frequency of exercise. Instead, it tended to show a positive response among individuals who did not routinely perform an exercise before the quarantine³. Brand's work indicated that 44.2% of individuals maintaining their sports activities, other 31.9% individuals even increased their exercise intensity, and only 23.7% individuals stated a decrease in their exercise frequency. This study is in line with the present study in which medical students appeared to experience positive responses regarding physical activity as 89 students (50.3%) said that they continued to exercise during the COVID-19 pandemic. This might occur as an influence of self-efficacy response, an individual's belief that he/she is

capable of acting to achieve a goal, that has a positive effect on mood. Additionally, psychophysiological processes may involve in this phenomenon as performing exercise could lead to changes in hypothalamic-pituitary-adrenal activity, the stress axis, allowing individuals to better in coping with stress.³

However, Duncan et al (2020) also found a negative response to physical activity during the quarantine period of the COVID-19 pandemic. This negative response was also shown by study subjects as 88 students (49.7%) did not exercise during the pandemic. The decrease in physical activity during quarantine might be influenced by a fear of being exposed to COVID-19, limited sports facilities and recreational areas closure, as well as work-from-home programs.²²

Traumatic events as the COVID-19 pandemic could also result in psychological distress and anxiety symptoms which own a negative effect on an individual's sleep quality. Stress, indeed, is a major

trigger of sleep disorders. The level of stress exposure that disrupts sleep and the subsequent sleeping difficulty is referred to a sleep reactivity.^{9,10,13} However, each individual could show a different response.¹³ As in this study, the respondent's responses to sleep patterns were varied. This study found that 28.2% of respondents claimed to have quite poor sleep quality and 2.3% of respondents even had poor sleep quality. This might be a consequence of increased stress perceived during quarantine, particularly for limited space to perform dynamic activity for a long duration. Therefore, quarantine policy could predispose to sleep pattern disturbances and insomnia through the pathopsychological mechanisms of the stress. In addition, decreased physical activity and higher use of digital media such as mobile phones before bedtime could also lead to sleep disturbances.^{7,20}

However, our study found that the majority of respondents had a quite good sleep quality (61% of respondents). A possible explanation is that individuals with good sleep quality possess a lower sleep reactivity which is linear to lower stress levels. In agreement with stress mechanism, physical activity might also play a role in sleep patterns as this study found that respondents tended to perform physical activities, leading to a good response on sleep quality. Further research, however, is needed regarding good sleep quality during the COVID-19 pandemic.

Furthermore, stress refers to a process involving the perception, judgment, and response to emotional or physiological stimuli. Stress is a challenge to the individual homeostasis process in which the individual has to produce an optimal physiological response to regain the disrupted homeostasis due to stress. One manifestation of disturbed homeostasis comprises the individual eating behavior. Stress leads individuals to consume "calming foods" that contain higher sugar and fat level. Accordingly, these mechanisms suggest that stress can promote disturbed eating patterns.^{16,21} This brief explanation is hypothesized to explain the findings of 26% of respondents who stated that they consumed more sweet foods during quarantine. COVID-19 quarantine might result in daily routine disruption, including prolonged sedentary behavior.²² The total sedentary behavior during quarantine is higher compared to before the pandemic era since people spend more time at home. Medical students also performing school from home which leads them to be in front of

the screen for a longer duration.²² This is following the results of this study in which 43.5% of respondents favored performing sedentary behavior by staring at the screen for more than 6 hours a day. Regarding stress levels, there was no relationship between sedentary behavior such as watching television or playing computer with stress¹⁹. For this reason, further research is needed concerning sedentary behavior and stress relationships, especially during the quarantine period of the COVID-19 pandemic.

Nevertheless, this research possesses several limitations. This study was conducted online during the COVID-19 pandemic and researchers could not directly interact with respondents to collect data. Questionnaires containing questions about anthropometry and lifestyle during COVID-19 have not yet existed in Indonesia and posing a challenge in conducting this research.

Conclusion

During the COVID-19 pandemic, the anthropometric status of medical students batch 2018 Faculty of Medicine, Universitas Airlangga varied; and tend to maintain their physical activity, have quite good sleep quality, prefer eating sweet foods, and do a sedentary behavior for a longer duration.

Conflict Of Interest: No conflict of interest

Ethical Clearance: This research had an ethical clearance that was approved by Faculty of Medicine Airlangga University No.111/C/KEPK/FKUA/2021.

Source of Funding: This study was supported by the authors.

Acknowledgment

The authors would like to medical student batch 2018, Faculty of Medicine, Universitas Airlangga who were willing to participate in this study.

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Framingham Risk Score and Coronary Artery Calcium Score: How Good they Relate

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How to cite this article: Johanes Nugroho, Revi Adheriyani, Ardyan Wardhana et al. Framingham Risk Score and Coronary Artery Calcium Score: How Good they Relate. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):214-217.

Abstract

Background and Aims: Screening patients for coronary arterial disease can be through risk stratification using the Framingham Risk Score (FRS). Direct visualization of subclinical atherosclerotic lesions through coronary artery calcium scores (CACS) can be an additional strategy. Therefore, we want to know the relationship between FRS and CACS in asymptomatic individuals.

Method: A cross-sectional study involving 110 asymptomatic participants who undergoing health screening was conducted in the National Hospital, Surabaya from November 2015 until January 2016. Risk stratification was evaluated using Hard Coronary Heart Disease (10-year risk) outcomes model score and the Agatston-Janowitz's coronary calcium score.

Results: A significant positive correlation was observed between CACS and FRS (Spearman's correlation coefficient $r=0.51$, $P<0.0001$). Age and systolic blood pressure were also positively correlated with CACS. Total cholesterol was the only parameter that showed a negative correlation with CACS. No difference in CACS value was shown in gender and smoking status.

Conclusion: There was a strong correlation between FRS and CACS in asymptomatic individuals.

Keywords: Framingham Risk Score; coronary calcium score; computed tomography; coronary atherosclerosis; coronary arterial disease.

Introduction

Coronary artery disease (CAD) is known to be the biggest contributor of all death caused by cardiovascular disease.¹ Early detection of patient

through risk stratification method should be the focus in daily practice in order to prevent high-cost burden in therapy. Framingham Risk Score (FRS) is commonly used risk stratification algorithms and

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has been proved to provide good risk prediction.^{2,3} However, FRS has not yet been able to accommodate some other important risk factors playing a role in cardiovascular events such as family disease history.

Direct visualization of the sub-clinical atherosclerotic lesions such as coronary artery calcium score (CACS) assessment has become an additional strategy suggested in patient screening. CACS was reported as better independent predictor for CAD than FRS.⁴ However, most of the studies and recommendation regarding CAC focuses on Western society. In this article, we try to know the relationship between FRS and CACS in asymptomatic Indonesian individuals.

Methods

Our study was conducted in *National Hospital, Surabaya* in the period of November 2015 until January 2016 after ethical clearance was obtained. This cross-sectional study was performed to 110 asymptomatic participants for comprehensive health screening. Participants with a clinical history of angina, cardiovascular disease, and coronary revascularization were excluded. All participants voluntarily underwent medical examination and CACS screening by computed tomography (CT).

FRS was based on Hard Coronary Heart Disease (10-year risk) outcomes model including age, sex, smoking history, systolic blood pressure, history of taking antihypertensive medication, total cholesterol, and HDL cholesterol.⁵ Blood pressure measurements were made on the left arm of the seated participants with a mercury-column sphygmomanometer and an appropriately sized cuff. Serum total and HDL cholesterol levels were determined with standardized enzymatic methods. Cigarette smoking status was ascertained by self-report. Diabetes was defined as history of physician-diagnosed diabetes and use of insulin or oral hypoglycaemic medications. Antihypertensive medication use was ascertained by the physician examiner at the heart study and based on self-report. Then, the study population was stratified into the following 5 categories according to the FRS: 0 to <10, 10 to <20, and ≥ 20 .

CACS was analysed from 45-65 images obtained using a 128-slice MSCT scan (GE Company). CAC was defined as a hyperattenuating lesion above a threshold of 130 Hounsfield units with an area of at least 3 adjacent pixels. It was calculated according

to Agatston-Janowitz's score based on the total amount of calcific lesions from five interrogated coronary arteries (left main, left anterior descending, left circumflex, right coronary, and posterior descending).⁶ For analytical purposes, we grouped the study population into the following 5 categories according to Agatston-Janowitz's score: 0, 0 to < 10, 11 to 100, 101 to 400, and >400.

The sample size was derived by calculating the correlation coefficient of 0.26 from a study by Sung, et al, 2008 and two-sided test size of 5% and statistical power of 80%.^{7,8} Data were expressed as mean \pm standard deviation or percentages. Spearman's correlation coefficient was used to investigate the relationship between CACS and FRS. The Mann-Whitney test was used to compare risk factor status to CACS. All statistical analyses were performed using SPSS, version 20.0. A *p* value of <0.05 was considered statistically significant.

Results

All the 110 participants had completed the study and had no missing data. Various clinical characteristics and risk factor profiles are shown in Table 1. The majority were non-diabetic and non-smoker participants. Almost half of participants was in the low-risk category of FRS and very low-risk category of CACS. A similar proportion was obtained in group of FRS and CACS for category above intermediate risk.

Table 1: General clinical characteristics of participants (n=110)

Risk Factors	Value
Age	54.1 \pm 10.7 years
Sex (Male: Female)	56.4:43.6%
BMI	25.85 \pm 4.44 kg/m ²
Smoker	18.2%
Hypertension Medication	30.9%
SBP	132.1 \pm 19.7 mmHg
Diabetes Mellitus	9.1%
Total Cholesterol	195.1 \pm 36.3 mg/dL
HDL	48.1 \pm 12.5 mg/dL
FRS Score	14.3 \pm 13.0
0-10 (low-risk)	45.5%
>10 - <20 (intermediate-risk)	35.5%
>20 (high-risk)	19.1%

Contd... Table 1: General clinical characteristics of participants (n=110)	
CACS Score	104.8±248.3
1 = 0 (very low risk)	45.5%
2 = 0-10 (low risk)	14.5%
3 = 11-100 (intermediate risk)	17.3%
4 = 101-400 (Moderately high risk)	13.6%
5= Over 400 (High risk)	9.1%

A significant positive correlation was observed between CACS and FRS (Spearman's correlation coefficient $r=0.51$, $P<0.0001$), as shown in Table 2. Age and systolic blood pressure were also positively correlated with CACS. Total cholesterol was the only parameter that showed a negative correlation with CACS. The BMI and HDL cholesterol were not correlated with the CACS.

Table 2: Correlation between various risk factors and CACS

Variable	Correlation Coefficient	P value
Age	0.446	0.000*
BMI	0.032	0.741
Systolic Blood Pressure	0.192	0.045*
Total Cholesterol	-0.234	0.014*
HDL	-0.175	0.067
FRS Value	0.507	0.000*
FRS Classification	0.532	0.000*

Remark: * = there is a significant relationship

As shown in table 3, no difference between male and female in terms of CACS value and CACS grading was demonstrated ($p = 0.078$). Smoking status also showed similar CACS. Participants who had hypertension medication or diabetes shown posed higher CACS ($p=0.035$ and $p=0.001$, respectively).

Table 3: Comparison of various risk factors and CACS

Variable	P value
Gender	0.078
Smoking	0.347
Hypertension Medication	0.037*
Diabetes Mellitus	0.001*

Remark: * = there is a significant relationship

Discussion

This study demonstrated strong correlation between FRS with CACS. The result was similar to other study in Korean population.⁹ However, discrepancy between CACS and FRS in our study of population was not evaluated in our study. Furthermore, our correlation coefficient was higher than value reported by Sung, et al.⁸ Zero CACS in their study was quite prevalent than our study population (70% vs 45.5%). Some studies have also suggested that CACS differs among different ethnic groups.¹⁰

CT usage put asymptomatic patients to cost burden, and radiation risk.⁴ Therefore the benefit of usage should be greater than the risk. ACCF / AHA 2010 recommends the use of CAC Score Measurement may be reasonable for cardiovascular risk assessment in low to intermediate risk patient (6% to 10% 10-year risk).¹¹ Otherwise, CACS of patient with low cardiovascular risk assessment ($< 6\%$ 10-year risk) cannot be treated.¹² Regardless the presence or absence of symptoms, the patient may have CAD even though he does not have an image of coronary calcification based on CT. CACS assessment along with the conventional risk stratification can improve the prediction of cardiovascular events.

In this study, age was positively correlated with CACS. Age is indeed one of the factors involved in calcification process of blood vessels either actively or passively.¹³ No correlation between BMI and CACS in this study was also shown by Roy et al.¹⁴ It reported that CACS was correlated with body surface area (BSA) but not BMI. A reversed correlation between total cholesterol with CACS in this study may be due to the tendency of non-calcified plaque formation in the high cholesterol levels.¹⁵

No difference CACS in male compared to female group in this study. However, men tend to have a greater atherosclerotic plaque and are more calcified than women.¹⁶ No difference CACS in smoking status group in our study may be because the smoking history was only submitted covertly. The result was similar with study by Yun-Ah Lee.¹⁷ Diabetic participants in our study had higher CACS compared to non-diabetic participants. High CACS prevalence was clearly found in patients with diabetes mellitus regardless their nephropathy status.¹⁸

Our study did not provide the outcome data or prognosis in the population included because this was a cross-sectional study. The findings in our study may not be applicable to other populations with different ethnic. Another limitation of our study is that the population was self-referred for regular health screening. This may be a source of selection bias.

Conclusion

In this study, there was strong positive correlation between FRS and CACS in asymptomatic participants. No difference of CACS value was shown in gender and smoking status.

Acknowledgment

None declared

Source of support: None declared

Presentation at a meeting: None declared

Conflict of interest: None declared

IEC approval: Ethical Committee of the Faculty of Medicine, Universitas Airlangga (No. 136-KE)

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A Cross Sectional Study to Estimate the Stature from Hand length in the Age Group of 18 to 25 Years in Telangana Population

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How to cite this article: K Srinivasulu, B. Shruthi Reddy, Sowmya Bhashini Popuri et al. A Cross Sectional Study to Estimate the Stature from Hand length in the Age Group of 18 to 25 Years in Telangana Population. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):218-221.

Abstract

A Cross sectional study on correlation of length of hand in relation to the height of an individual was conducted in medical students of Telangana region in the age group of 18 to 25yrs during the period of January 2018 to December 2019. A total of 150 individuals among 75 males and 75 females were taken as participants in this study. Measurement of the Body part hand length has taken as distance between the proximal wrist creases to the tip of middle finger, measured by using vernier calipers. The data was statistically analyzed by using SPSS (version-25) software.

The formula for linear regression for estimating the height is $y = a + (b x)$, y = dependent variable (height). a = constant. b = independent variable coefficient. x = independent variable i.e. length of the Hand. The linear regression formula for Right hand is $y = 41.25 + (6.89 x)$ and left hand is $y = 43.9 + (6.71 x)$. In case of male individuals the formula for Right hand is $y = 68.77 + (5.47 x)$ and for left hand is $y = 63.69 + (5.70 x)$. In case of female individuals the formula for Right hand is $y = 59.51 + (5.72 x)$ and for left hand is $y = 63.63 + (5.48 x)$, standard error was 0.3, R square of 0.7 and confidence interval was 6.17 - 7.60, the data results show the statistical significance the P Value less than 0.001.

Calculated stature from the equation $Y=a + (b x)$, is close to the actual height, only ± 5 cm difference was observed in most of the individuals. Height of an individual is approximately 9 times the height of the hand length. A separate linear regression formula for male and female is more accurate and reliable.

Keywords: Stature; hand length; linear regression.

Introduction:

Stature means standing height i.e., height of the person in upright position. It is usually measured from top of the vertex to the ground. Estimation of stature

holds a significant role in the field of anthropology. When a complete dead body is found, stature determination is rather an easy task; but in cases where only some parts of the body are available, the determination of stature of the individual is difficult.

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Vitruvius¹, says in his work on architecture that the measurements of human body are distributed by nature as follows, that is 4 fingers make 1 palm and 4 palms make 1 foot, 6 palms make 1 cubit; 4 cubits make a man's height. And 4 cubits make one pace and 24 palms make a man; and these measures he used in his building and he says the length of a man's outspread arms is equal to his height.

Stature is estimated from the length of long bones of both upper and lower limbs, skull, vertebra and metacarpal bones, etc. Estimation of stature from body parts also helps in estimating the height of the person and plays an important role in identifying the person. Forensic anthropologist depends on various anthropometric features to estimate sex, age, race and stature of an individual, among which stature is the most crucial aspect (Wilson²). Stature prediction occupies relatively a central position in anthropometric research.

Among the various parameters of identification, the individual stature is characteristic, the estimation of which is important when only mutilated remains of an unknown person are available. The length of certain long bones and appendages of the body represent certain proportion of relationship to the total stature. Hand length has documented as a significant predictor of stature^{3,4}. Study conducted by Amirshaybani HR³ and another study by Saxena, Thakur and Rai⁴, shown a great reliability of prediction of stature from hand length.

Estimation of stature is based on the principle that every body part bears more or less a constant relationship with height of an individual. Several studies show that the out of all mathematical methods used to estimate the stature, regression equations yield better results. Regression analysis is most widely used because of its ubiquity and wide availability in statistical methods. The regression formulae derived for one population does not always give accurate results for other populations, variations are because of nutritional, environmental and genetic factors. (Krogman & Iscan, 1986; Duyar & Pelin, 2010)⁵.

Stature, age and sex have key role in identification of unknown dead bodies.³ When a mutilation of body parts occurs in mass disasters like bomb blasts, fire accidents, building collapse, train accidents and plane crashes and even in natural disasters like cyclones, earthquakes, tsunamis and floods, where only parts of the body are available in such cases identification of

the person can be done by estimating the stature from the mutilated body parts. In criminal offences, where criminals usually conceal the identity of the dead body by mutilation to mislead investigation team, stature estimation plays a key role for identification.

Variations of stature observed in several situations like posture of the body, diurnal variation, malnutrition and age. The stature is 1 to 2 cm more on lying down position compared to standing due to relaxation of muscles; it is less in afternoon and evening than in morning due to reduced elasticity of inter vertebral discs, in both malnutrition and advancing age the stature reduced due to gradual atrophy and loss of elasticity of inter vertebral discs.⁶

The aim and objective of present study is to correlate the stature in relation to length of hand in the population of Telangana region. It is useful for estimation of stature of the individual indirectly. Our study is highly useful for forensic scientists and physical anthropologists to determine stature from fragmentary bodies of upper limb of hand, in turn useful in crime investigation.

Materials and Method

A Cross sectional study on correlation of length of hand in relation to the height of an individual was conducted in Medical students of Telangana region in the age group of 18 to 25 yrs, during the period of January 2018 to December 2019. A total of 150 individuals among 75 males and 75 females were taken as participants in this study. Hand length of both right and left hand measured separately in male and female individuals. We took informed consent from the participants and the names of the participants were kept anonymous.

Inclusion and exclusion criteria: A healthy individual of normal skeletal growth and without any deformities were included in this study. Individual with genetic or hormonal abnormalities, nutritional disorders and skeletal abnormalities were excluded.

The instruments used in this study are vernier calipers, divider, scale, Tape, stadio-meter (Height stand) and weighing machine.

Measurements of the Body part Hand length taken as distance between the proximal wrist creases to the tip of middle finger measured by using vernier calipers.

Statistical Analysis:

Analysis was done by descriptive statistics like mean standard deviation and range. Correlation coefficient and linear regression equations were used for estimating stature from hand dimensions. P value < 0.05 was considered as statistically significant. The data was statistically analyzed using SPSS (version-25) software. The formulae for linear regression for estimating the height is $y = a + (b x)$,

(y = dependent variable (height). a = constant. b = independent variable coefficient. x = independent variable i.e. length of the Hand).

Results

A cross sectional study on correlation of stature from hand length was conducted on 150 individuals, among 75 males and 75 females in the age group of 18 to 25 years, the following observations were found.

Table 1: Mean height and weight (mean ± SD)

Characteristics	Male	Female	Overall mean	P value
Ht (mean ± SD) in cm	169.74 ± 7.14	156.80 ± 6.80	163.40 ± 9.51	<0.001
Weight (mean ± SD) Kg	57.05 ± 10.49	50.42 ± 11.45	53.80 ± 11.43	<0.001

Table-1: The difference between mean stature of males and females in each group was statistically significant (p<0.001). Males are more in height than females due to hormonal and genetically variation.

Table 2: Mean value of hand length (mean ± SD)

Characteristics	Male	Female	Overall	P value
Rt hand (mean ± SD)	18.43 ± 0.94	16.98 ± 0.87	17.72 ± 1.16	<0.001
Lt hand (mean ± SD)	18.58 ± 0.92	16.99 ± 0.88	17.80 ± 1.20	<0.001

Table-2: Results reveal not much difference between the length of right and left hand of both the sex.

Table 3: Prediction of height by linear regression formula for total study population.

Independent Variables (Length) in cm	Formula: $y = a + (bx)$	R square	P value	Standard Error	95% Confidence Interval (CI)
Right Hand (cm)	$y = 41.25 + (6.89 x)$	0.71	<0.001	0.36	6.17 - 7.60
Left Hand (cm)	$y = 43.9 + (6.71 x)$	0.72	<0.001	0.34	6.03 - 7.38

Table 4: Prediction of height by linear regression formula in male population.

Independent Variables: (Length in cm)	Derived Formula: $y = a + (bx)$	R square	P value	Standard Error	Confidence Interval
Right Hand	$y = 68.77 + (5.47 x)$	0.52	<0.001	0.60	4.27 - 6.67
Left Hand	$y = 63.69 + (5.70 x)$	0.54	<0.001	0.60	4.50 - 6.90

Table 5: Prediction of height by linear regression formula in female population

Independent Variable Length in cm	Formula: $y = a + (bx)$	R square	P value	Slandered Error	Confidence Interval
Right Hand	$y = 59.51 + (5.72 x)$	0.53	<0.001	0.62	4.48 - 6.97
Left Hand	$y = 63.63 + (5.48 x)$	0.50	<0.001	0.64	4.20 - 6.76

Formula for linear regression: $y = a + b x$, (y = dependent variable (height), a = constant, b = independent variable coefficient, x = independent variable i.e. length of the Hand).

Discussion

The study results revealed that the dimension of the hand length is associated with height and can be

used in the estimation of stature. Linear regression equation showed a significant correlation with hand length and stature; hence these equations can be used by forensic anthropologist and law enforcement agents to determine the height of an individual from the mutilated, dismembered body part like Hand.

The formula for linear regression for estimating the height is arrived as $y = a + (b x)$. (y = dependent variable (height). a = constant. b = independent variable coefficient. x = independent variable i.e. length of the Hand). The linear regression formula for total population Right hand is $y = 41.25 + (6.89 x)$ and left hand is $y = 43.9 + (6.71 x)$.

The linear regression formula for male individuals Right hand is $y = 68.77 + (5.47 x)$ and for left hand is $y = 63.69 + (5.70 x)$. In case of female individuals the formula for Right hand is $y = 59.51 + (5.72 x)$ and for left hand is $y = 63.63 + (5.48 x)$.

As on today the most appropriate specimens for estimation of stature are long bones⁶ but our study results are revealed that the hand lengths also equally significant like any other long bones of the body. A separate linear regression formula derived for male and female population in our study gives more accurate results because hand lengths and heights of male and female population are different.

Calculated statures from the above equations are close to the actual height, only ± 5 cm difference in most of the individuals. Height of an individual in our study population is approximately 9 times the height of the hand length, which is obtained by the above equations.

Several similar studies conducted in India by Pratik R Varu⁷ at Rajkot Gujarat, study conducted by Apurva chowdary⁸ in Chennai Tamil Nadu and another study conducted by Amitava Pal⁹ and Sujaya de in Bengal population also revealed similar results, ± 5 cm variations are observed.

Study results R square 0.71, standard error of 0.36, confidence interval of 6.17 – 7.60 and the P value of <0.001 showing high accuracy of data. The linear regression formulae obtained in this study has got significant correlation with hand length and stature.

Conclusion

The results of the study revealed that hand length and height of the male and female individuals are slight difference, hence the linear regression formula designed separately for male and female individuals are more appropriate. Calculated statures from these equations are close to the actual height, only ± 5 cm difference in most of the individuals; this can be minimized if the study population is more. Height of an individual is approximately 9 times the height of the hand length; this is one of the best predictor for height estimation in mutilated bodies.

Conflict of interest: Nil

Ethical clearance: Yes

Source of funding: Self

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Study of Treatment Modalities for Blunt Abdominal Trauma and its Medicolegal Aspect

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How to cite this article: Keyur N. Surati, Jainam Shah, Rohit Zariwala et al. Study of Treatment Modalities for Blunt Abdominal Trauma and its Medicolegal Aspect. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):222-227.

Abstract

Background: Blunt abdominal trauma management has changed drastically from surgical to conservative. In our study we analyzed 100 patients visited hospital with blunt abdominal trauma and diagnostic and management modalities and medicolegal aspect are discussed.

Study evaluate the incidence and clinical presentation, various available investigations, the organs affected in blunt abdominal trauma and management of complications, morbidity and mortality following blunt abdominal injury. To handle such cases to save the patient and our self from litigation.

Material and methods: This study consists an analysis of randomly selected 100 cases with blunt abdominal trauma admitted during the period of May 2018 to August 2020. It is a prospective observational study.

Conclusion: Blunt abdominal trauma is on rise due to excessive use of motor vehicles. From our study we concluded that single solid organ injury patients with hemodynamic stability (specially Grade 1 to grade 3) managed conservatively more compared to multiple solid organ injury patients and best investigated by USG FAST and CT scan. Careful record keeping can assist to protect against bogus and false allegations and complaints, as in court of law documentation is considered as part of witness.

Keywords: Trauma; Injuries; Blunt Abdomen; Treatment.

Introduction

Trauma is a huge global health issue and is one of the leading causes of death worldwide among persons under the age of 45.¹ Abdomen is the third

most frequently injured region with injuries requiring surgery in civilian trauma victims.

Trauma is responsible for 8% of all deaths in India. In the city, 75 percent of abdominal trauma is caused by

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a blunt impact. The liver, spleen, and kidneys are the most often injured intra-abdominal organs. Majority of deaths occur shortly due to hemorrhage, while some occur later due to sepsis. Abdominal trauma is responsible for 15% to 20% of all trauma deaths.²

The treatment of abdominal trauma patients has shifted dramatically from operative to non-operative in recent years due to availability of improved imaging techniques. Conservative care is now considered first line of treatment for hemodynamically stable patients. ATLS (Advanced Trauma Life Support) uses standard methods to provide an organized approach to trauma patients.² It highlights the "Golden Hour" notion, which states that in order to avoid death, urgent, prioritized actions are required. They are not only bodily harm but also, they usually foretell sessions in courtroom or interrogation by lawyers. Thus, it is important about having the knowledge of the medicolegal work to protect the patient from harm and save ourselves from litigation or being caught.³

Medicolegal cases (MLC)

Medicolegal case is "a case of injury/illness where the attending doctor, after eliciting history and examining the patient, thinks that some investigation by law enforcement agencies is essential to establish and fix responsibility for the case in accordance with the law of the land. In all MLC apart from examination and treatment following details must be taken care of

- Police report of MLC
- Identification marks (minimum 2)
- Record of investigations- X-ray, USG, CT scan and MRI
- Certificate should be issued to police or court on demand.
- The medical officer should fill a 'injury certificate'.
- The report must be written clearly and accurately because these reports involve litigation, insurance claims, medical negligence claims and worker compensation issues.

There are certain medicolegal aspects in blunt trauma abdomen like table death, legal notice, summons and warrant.

Table death is an event of death on table, in such case postmortem is advised to ascertain the actual cause of death.

Legal Notice is a claim made by person by his advocate. After proper study of case and each question should be answered separately.

Summons are ordered by the Court and served by post or other modes. Warrant can be issued if summons is refused to accept.

Warrants are issued by a court and are delivered by Police Station. It can be bailable or non-bailable.

Police procedures:

Police procedure is that they come to hospital to take a statement. They can call health care worker to police station for statement. Such documents can be seized by police, if offence has been registered. Documents are preserved in form of panchnama and copy of panchnama is given to the doctor.

When to issue MLC?

- Wounded cases, irrespective of manner and nature of injury (treating and saving the life being the priority). This can be also done after patient is stabilized.
- In cases and major injuries.
- On the basis of Suspect of abuse.

Indemnity Insurance

To avoid toll on one's finance in case of actual negligence indemnity insurance comes into the picture. Insurance company can bear such finances with terms and conditions being applied which are mentioned in the contract.

Blunt abdominal injuries:

Two sets of factors, endogenous and exogenous, determine the types and degree of visceral damage sustained when blunt force traumatizes the abdomen.

Endogenous determinant in blunt abdominal trauma:

Significant intrinsic factors, which help to determine the outcome of blunt abdominal injury, reside in the viscera and their vasculature.

Organ Consistency:

- A firm and dense organ is more friable. Solid organs (e.g., liver and spleen) are more readily lacerated by blows than are such hollow organs as the (empty) stomach,

intestines, and (empty) urinary bladder.

Organ Mobility:

- Readily moveable or displaceable organs have considerable capacity to absorb the force of a blow, without serious injury, because of their ability to 'ride with the punch'.

Exogenous factors which influence severity and nature of visceral injuries resulting from trauma includes:

- Size and consistency of the traumatizing object, e.g., fist (punching), head (butting), foot (kicking), etc.
- Site of impact, e.g., epigastrium, inferior rib cage, suprapubic area, costovertebral angle, flank, etc.
- Speed and weight (force or energy) of the traumatizing mechanism.
- Nature of the traumatizing force, e.g., sharp impact or slow compression.
- Abdominal wall strength.
- Degree of abdominal "guarding" i.e., extent of protective contraction of abdominal musculature.
- Pre-existing visceral status.

Abdominal injuries:

Different varieties of blunt violence produce different kinds of abdominal injuries.

1. Contusion,
2. Tearing of parenchyma,
3. Rupture by a bursting force due to a rise of pressure inside the hollow organ
4. Tearing of attachments
5. Laceration of the organ by fractured bones.

The blunt force injuries of the abdominal organs are divided into

1. Injuries of the parenchymatous viscera and
2. Injuries of the hollow abdominal viscera and their attachments.

The Parenchymatous Viscera:

The most important organs in this group are the liver, spleen, kidneys, and pancreas. Since ordinarily their consistency is firm, they are not easily ruptured

by blunt force. The principal complication, which causes death in injuries of the parenchymatous organ is hemorrhage into the abdominal cavity.

The Hollow Abdominal Viscera:

The hollow abdominal viscera, including the gastrointestinal tract, the urinary bladder and the pregnant uterus, are injured by the same types of blunt forces as are the parenchymatous abdominal viscera. The structure of the hollow viscera is much more fragile and serious injury may be inflicted on them by a comparatively lower degree of violence.⁴⁻⁸

Materials and Methods

This study consists an analysis of randomly selected 100 cases with blunt abdominal trauma admitted during the period of May 2018 to August 2020. It is a prospective observational study.

Each patient was evaluated clinically taking into consideration history, general examination, abdominal examination, and investigations and analyzed according to data collected on a planned proforma.

Inclusion Criteria

Cases of blunt abdominal trauma managed at tertiary care Hospital from May 2018 to August 2020.

Patients presenting within 48 hours of trauma.

Cases of blunt abdominal trauma with age more than 6 years.

With or without associated other injuries (chest, head, peripheral extremity injury).

Exclusion Criteria

All patients less than 6 years of age.

Patients with blunt trauma abdomen without solid organ involvement.

All patients were managed in trauma center as per ATLS guidelines and underwent appropriate primary resuscitation and investigations as per ATLS protocols for management of abdominal trauma and underwent exploration accordingly:

Examination:

General examination:

The patient should be examined from head to toe for

Pulse, Blood pressure, Respiration, Consciousness, Pallor, Generalized/Localized, edema, Cyanosis, Abdominal girth, Abnormal posture and Limb deformity.

Revised trauma score:

The Revised Trauma Score (RTS) uses 3 specific physiological parameters.

1. The Glasgow Coma Scale.
2. Systemic Blood Pressure.
3. The Respiratory Rate

GCS score	RR score	SBP score
13-15 4	10-29 4	>90 4
9-12 3	>30 3	76-89 3
6-8 2	6-9 2	50-75 2
4-5 1	1-5 1	1-49 1
3 0	0 0	0 0

When used for triage, the RTS is determined by adding each of the coded values together. Thus, the RTS ranges from 0-12 and is calculated very easily. An RTS of less than 11 is used to indicate the need for transport to a trauma care center.

The patients were followed up for a varying period of 15 days to 3 months.

Discussion

In our study most common affected patients were male and between age group of 21-40 years as this age group people were more involved in working and due to socio cultural problem of our country.

Among them, 32% were less than 20 years of age, 48% were from 20-40 years of age and 20% above 40 years.

1. Age:

Age (years)	Total	Percentage
>6-20	32	32 %
21-40	48	48%
>40	20	20%
Total	100	100%

2. Sex:

Sex	No.	Percentage %
Male	74	74%
Female	26	26%
Total	100	100

Most common mode of blunt abdominal injury was Road Traffic Accident (68%) followed by falldown (22%), assault(08%), others (02%).

3. Mode of Injury:

Mode of Injury	No.	Percentage
RTA	68	68
Fall down	22	22
Assault	08	08
Others (trauma by animal)	02	02
Total	100	100

Blunt abdominal injury was commonly associated with other injuries due to force pattern. In our study chest injuries (20%) was more associated with blunt abdominal trauma. Most of the patient were presented to hospital within 5 hours after injury. This was similar to another study in America in which most of patient arrive in hospital in 1 hour. In western world patients reach tertiary trauma center early due to compatible transport services.

In this study USG FAST was commonly used to diagnose patients with abdominal injuries.⁹ Compared to CT scan it is easily available can be done in emergency at bed side. But it has false positive result also and it required minimum of 150-200 ml of fluid for detection. CT scan was done whenever required and compatible to patient condition [10].

4. USG/CT FINDINGS:

USG/CT FINDINGS	No.	Percentage %
Free Fluid	100	100%
Liver Injury	42	42%
Splenic Injury	58	58%
Renal Injury	28	28%
Pancreas Injury	08	08%
Total Patients	100	100

In our study most common injured organ was spleen (58%). And liver (42%), renal (28%) and pancreas (8%) were rest of solid organ involved. In our study most common single solid organ was involved (64%) compared to multiple solid organ involvement (36%). In our study 68.75% patients with single solid organ injury were managed conservatively while 50% patients with multiple solid organ injuries were managed conservatively. One patient who had single solid organ injury and put on conservative management ultimately converted to

operative intervention while two patients who had multiple solid organ injuries and put on conservative management ultimately converted to operative intervention due to deterioration. While 20 patients with single solid organ injury (31.25%) and 18 patients with multiple solid organ injuries (50%) undergone operative intervention.

5. Conservative Vs Operative Intervention in Solid Organ Involved(Soi):

SOI	Conservative	Operative	Total
Single	44(69%)	20(31%)	64
Multiple	18(50%)	18(50%)	36
Total	62	38	100

Recently change has shift toward nonoperative management. In operative intervention splenectomy was most commonly procedure performed. In our study ICU and hospital stay seen more in multiple solid organ injuries compared to single solid organ injury.

6. Organ Specific management

Grading of Injury	Management		
	Conservative	Operative	Total
Spleen			
1	06	00	6
2	14	00	14
3	20	02	22
4	02	10	12
5	0	04	04
Total	42	16	58
Liver			
1	04	00	04
2	14	02	16
3	10	02	12
4	08	00	08
5	02	00	02
Total	38	04	42
Renal			
1	20	00	20
2	06	00	06
3	00	00	00
4	00	02	02
5	00	00	00
Total	26	02	28

Contd... 6. Organ Specific management			
Pancreas			
1	0	0	00
2	0	02	02
3	02	02	04
4	0	02	02
5	0	0	00
Total	02	06	08

The chi-square statistic of above table was 6.43396. The P value was 0.0112. The result was significant as P<0.05. Most patients with multiple solid organ injuries have more complication compared to single solid organ injury. Complications which were most commonly seen were SSI, Septicemia, Respiratory complication, Acute renal failure.

For surgeon safety:

Following precautions could be taken

- A female patient should always be examined in presence of a female attendant.
- Proper General condition should be explained with no aspects hidden.
- Proper Functioning of the Operation Theatre and its Equipment like oxygen supply, surgical instruments, anaesthesia machine along with supply of emergent drugs should be kept in regular check.
- Informed and written consent should be taken prior to any procedure.
- For transferring patient to higher center proper consent and records of the procedure followed should be kept like calling of ambulance, presence of proper equipment etc.
- Complete name of patient should be written on every medical record and register.
- In complications follow standard protocol and documentation, refer if required and inform the patient of every protocol being followed. Maintain courtesy, empathy and respect for patient.
- Records should be maintained on regular basis with proper filing as per criteria.

Summary

Most common affected age group was 21-40yrs with male were mostly affected in it. RTA was commonest cause for blunt abdominal injury. 50% patients were able to get admitted within 5 hours of injury. Splenic injury found most common and affect 58% of patients. Most patient had single solid organ injury (64%). Most common surgery performed was splenectomy due to higher grade of injury and instability. Conservative management was more successful in single solid organ injury than multiple solid organ injury. Associated extraabdominal injuries were found in 46 cases in the present study. Patients with single solid organ injury had less ICU (5.34d) and Hospital (9.96d) stay compared to multiple solid organ injury 7.0d and 11.3d respectively. Patients with single solid organ injury had less chances of complication (9.37%) compared to multiple solid organ injuries (44.44%). AKI was most common complication seen in our study.

Conclusion

Blunt trauma to abdomen is on rise due to excessive use of motor vehicles. It poses a therapeutic and diagnostic dilemma for attending surgeon due to wide range of clinical manifestation ranging from no early physical findings to progression of shock. Hence trauma surgeon should rely on his physical findings in association with use of modalities such as X ray, USG FAST and if necessary, CT scan. From our study we concluded that single solid organ injury patients with hemodynamic stability (specially Grade 1 to grade 3) managed conservatively more compared to multiple solid organ injury patients and patients with single solid organ injury suffered less complication compared to multiple solid organ injury patients. Having proper and complete information about legal procedures and liabilities is the only way of safeguarding ourselves in case of medicolegal cases. Standard procedure

and protocols should be followed blunt abdomen management to prevent malpractice.

Ethical clearance: Taken from IEC

Source of Funding: Self

Conflict of Interest: Nil

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The Effects of Text Messaging for Increasing the Rate of long-acting Reversible Contraception use in Teenage Pregnancy: A Randomized Controlled Trial

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How to cite this article: Kusuma Meesin, Nirun Intarat, Nidhikul Temeiam et al. The Effects of Text Messaging for Increasing the Rate of long-acting Reversible Contraception use in Teenage Pregnancy: A Randomized Controlled Trial. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):228-233.

Abstract

Background: A teenage pregnancy rate is a one of SDGs goal. Thailand had a teenage pregnancy rate higher in Asia-Pacific from less developed regions. A one of cause is a sex when they was young. This study is aimed to study an effective of a text messaging of teenage pregnant to the long-acting reversible contraception.

Materials and Methods: This is a randomized controlled trial. The study was an effective of a text messaging of teenage pregnant to the long-acting reversible contraception. A samples had 2 group from teenage pregnant in Si SaKet hospital. The control group was 212 samples. The trail group was 212 samples.

Result: The control group had a standard care of postpartum. The trial group had a standard care of postpartum and a text message about long-acting reversible contraception. The researcher were following a both sample. They found a trial group had a reversible contraception higher than a control group at a significant level. (P-value<0.001)

Conclusion: Present, a communication is important onthe digital time especially in teenager. A one of measurement postpartum teenager in this time is a text message. It will be reducing a repeat pregnant among teenage.

Keywords: Teenage pregnancy, Repeat pregnant, Long-acting reversible contraception, Text message

Introduction

The United Nation (UN) was determine goal of teenage pregnancy. It is a one of goal for Millennium Development Goals "MDGs" and Sustainable Development Goals "SDGs".¹ The incidence rate of teenage pregnancy between 15 - 19 year old was 47 per 1,000 population in 2014. It's higher in Asia and Pacific region that was incidence rate

amount 35 per 1,000 population. A preterm birth in teenage pregnancy in Thailand was specified in less developed regions.² The problem is a repeated teenage pregnancy between 10 - 19 years old.³ A cause of repeated teenage pregnancy was a younger sex and un-contraception. They didn't had a good performance in a physical and mental.⁴ The teenager had a sex who had been pregnant within 1 year amount 90%. It's 78-85% of uncontrolled.⁵ A cause of

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repeated pregnancy of teenage is a teenage mother un-used a reversible contraception. They did not use because the attitude, believe and loss information. A teenage mother was receive a sex education and an experience from friend who did not known in a core knowledge.⁶ In 2017, Si Sa Ket province found a repeated pregnancy in teenage 14.88%. It's higher more over in Thailand. The teenage mother had a reversible contraception 56.52%. It's under a standard value in Thailand.⁷

Present, a text message by a mobile phone is a one of technology of communication. It can send information about health promotion knowledge and support an access a reversible contraception in teenage mother. It is a one of measurement to improvement a repeated pregnancy in teenage. A text message from a mobile phone can be read in every time, everywhere and comfortable for teenage mother.

This study conducted an effect of a text message to increase a reversible contraception. It was protect a repeated pregnancy in teenage in Si SaKet province. The study aimed to study an effective of a text messaging of teenage pregnant to the long-acting reversible contraception.

Materials and Methods

The study was a randomized controlled trial.

The study conducted an effective of a text messaging of teenage pregnant to the long-acting reversible contraception. A samples had 2 group from teenage mother who got a postpartum care within 24 hours at Si SaKet hospital. A sampling was a block randomization. The control group was 212 samples. The trail group was 212 samples. The sample in group was a block of 4. The study was prepare randomization list. Data were collect from May 2019 to April 2020.

The study used a questionnaire as a tool for research. It consisted of 2 parts. First, it is individual character covering age, a family, a contraception before pregnancy, family planning. Second, the data of teenage mother and her new born.

In analysis, this study used both descriptive statistic; frequency, percentage, mean, S.D., maximum and minimum. An inferential statistics were use a logistic regression at a significant level 0.05.

Result

Characteristic of teenage mother at Si SaKet province

The almost samples was 19 years old. (= 17.85, S.D. =1.46) More than half of sample was a student. (60.68%) The income was receive from a husband and a parent. As shown in a Table 1.

Table 1: A characteristic of teenage mother at Si SaKet province (n = 412)

Characteristic	Control group (n=206)		Trial group (n=206)	
	Numeric	Percentage	Numeric	Percentage
1. Age (years)				
Under 15	23	11.17	17	8.25
16	21	10.19	14	6.80
17	24	11.65	32	15.53
18	34	16.50	42	20.39
19	104	50.49	101	49.03
Mean (SD)	17.79 (±1.55)		17.92 (±1.36)	
Min : Max	13:19		13:19	
2. Occupation				
Student (Formal education)	126	61.17	124	60.19
Student (Unformal education)	4	1.94	7	3.40
House wife	24	11.65	27	13.11
Employee	44	21.36	38	18.45
Merchant	8	3.88	9	4.37

Contd... Table 1: A characteristic of teenage mother at Si SaKet province (n = 412)				
Officer	—	—	1	0.49
3. A source of income				
Working	30	14.56	19	9.22
Parent	75	36.41	93	45.15
Husband	100	48.54	87	42.23
Mean (SD)	5734 (±5043.22)		4762 (±3711.06)	
Min : Max	500:32000		500:20000	
4. Income (Baht)				
Under 5,000	24	17.52	16	12.5
5,001 - 10,000	68	49.63	76	59.37
10,001 - 15,000	32	23.36	22	17.19
More than 15,000	13	9.49	14	10.94
Mean (SD)	10316 (±5695.59)		10419 (±6336.47)	
Min : Max	1000:50000		1000:60000	

Information before a pregnancy

More than half of sample was not focus a pregnancy. (74.03%) They did not use a reversible contraception because they did not know a reversible

contraception method. (51.76%) The sample used an oral pill. (60.67%) Almost of sample was a first pregnancy. (88.11%) The sample was a first pregnancy in 19 years old. (39.32%). As shown in Table 2.

Table 2: An information before a pregnancy. (n=412)

Characteristic	Control group (n=206)		Trial group (n=206)	
	Numeric	Percentage	Numeric	Percentage
1. A focus to pregnancy				
Focus	51	24.76	51	24.76
Not focus	153	74.27	153	74.27
Did not answer	2	0.97	2	0.97
2. A contraception				
Condom	14	11.67	20	16.81
Oral contraception pill	80	66.67	65	54.62
Injectable contraception	2	1.67	2	1.68
Emergency contraception pill	16	13.33	20	16.81
Periodic abstinence	-	-	1	0.84
Coitus interruptus	8	6.67	11	9.24
3. A cause of pregnant				
Did not know a contraception	43	51.20	45	52.32
Complication of contraception	8	9.52	10	11.63
Focus a pregnancy	33	39.28	31	36.05
4. A frequency of pregnancy				
First pregnancy	181	87.86	182	88.35
Second pregnancy	25	12.14	24	11.65

Contd... Table 2: An information before a pregnancy. (n=412)				
5. Age of a first pregnancy (years)				
Under 15	32	15.53	22	10.68
16	28	13.59	24	11.65
17	29	14.08	35	16.99
18	40	19.42	40	19.42
19	77	37.38	85	41.26
Mean (SD)	17.41 (± 1.64)		17.64 (± 1.49)	
Min : Max	13:19		13:19	
6. A spontaneous abortion				
Yes	10	4.85	6	2.91
No	196	95.15	200	97.09

The effective of text message to a reversible contraception

The sample was evaluation of effective of a text message to a reversible contraception in teenage mother. The control group was teenage mother 206 samples. The trial group was a teenage mother from a simple random sampling 206 samples. The study found the control group used a reversible contraception 71.36%. The trial group used a reversible contraception 60.19%. As shown in Table 3. The rate of an access to a reversible contraception in teenage

mother was an effective to a reversible contraception to the sample 71.31% (P-value < 0.001). The trial group was access to a reversible contraception 49.03% (P-value < 0.015). As shown in Table 4. The control group was not a reversible contraception 8.74% and the trial group was not a reversible contraception 21.36%. The control group had a text message that affect to a reversible contraception was a different amount 1.45 at a significant level. (95%CI :1.23 to 1.71 ; p-value <0.001). As shown in Table 5.

Table 3: An effective of a text message to a reversible contraception (n = 412)

An effective of a reversible contraception of teenage mother	Control group (n=206)		Trial group (n=206)	
	Numeric	Percentage	Numeric	Percentage
1. A reversible contraception	147	71.36	101	49.03
2. An another contraception				
Condom	3	1.46	6	2.91
Oral contraception pill	8	3.88	9	4.37
Injectable contraception	30	14.56	46	22.33

Table 4: The rate of a reversible contraception of teenage mother (n = 412)

An effective of a reversible contraception of teenage mother	Control group (n=206)		Trial group (n=206)		P-value
	Numeric	Percentage	Numeric	Percentage	
1. A reversible contraception	147	71.36	101	49.03	
An another contraception	41	19.90	61	29.61	0.015
Did not used	18	8.74	44	21.36	

Table 5: A comparative of a text message to a reversible contraception of teenage mother after 3 month; binary logistic regression analysis.

A reversible contraception	Risk Ratio	95% CI	P-value
A control group	1.45	1.23-1.71	<0.001
A trial group	0.49	0.42-0.56	

Discussion

From the result of the effects of text messaging for increasing the rate of long-acting reversible contraception use in teenage pregnancy study found a pregnancy of teenage or teenage mother within 24 hours at Si Sa Ket hospital during December, 2020 – September, 2021 who was not focus a pregnancy, un-planning and un-contraception. It was a pregnancy among study. A school could be helping a teenage mother if they want to back to study again. It can help to decrease their quality of life.⁸ They had not an income. A teenage mother was rejected a study and became to take care their child. When a teenage mother was take care a new born who found a lot of problem such as they had not a hygienic, not a breast feeding etc.⁹ Their husband had not an occupation, no income and no working. A teenage mother was back to her family. The study found a problem from teenage mother. The problem was an abnormal of new born infant; low birth weight and weakness. The researcher was comparative a sample who got a text message and did not got a text message about a reversible contraception. It found a teenage mother who got a message, it can promotion to receive a reversible contraception. A counselling can help teenage mother to getting a contraception as soon as them want.¹⁰ Health service office can improve a health education method from a new technology especially a health education by mobile phone.¹¹ It can help teenage to understand in technical term of sex education.¹² The mobile application is a one of method which attraction a lot of people to interesting. It can help a people to knowing and understand about real knowledge and developing their skill.^{13,14}

In the present, people can access information from a mobile. Teenage were addicted a lot of information from a mobile phone. A health education such as sex, contraception, consumption or driving etc. should be adapting in a text message from mobile application.

Recommendation

1. A pregnancy in teenage is not a focus, planning, contraception. It is a pregnancy on the study. She did not have a family. The hospital should be change a name of a family planning office from “Family Planning clinic” to “A contraception clinic”. It could be helping teenage mother got a safe contraception.
2. A pregnancy in teenage affect to a study. They will be skip an education between studied in the school. The school should be preparing a sex education class in a primary subject in every level. An information of teenage pregnancy will be reported to health system. School should be support a teenage pregnancy to study.
3. A Government should be preparing care plan of a new born from teenage mother. It affect a quality of life of a new born.
4. Health service office should be preparing a technology to warning, communication, support and health promotion. It will be warning a right of health of teenage mother. It can be easily to use from mobile phone for a teenage.

Acknowledgement

This study were approved by the ethical committee in Mahasarakham University ethics committee for research involving human subject no. 168/2019.

The author would like to thank you Sisaket hospital to support case for this research.

Source of funding: Self

Conflict of interest: the authors declare no potential conflict of interests.

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Determinants of Incidence of Myofascial Pain Syndrome on Coffee Picker Farmers in Pulu-Pulu Village, North Toraja Regency

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How to cite this article: Loritma Lasarus, Yahya Thamrin, Atjo Wahyu et al. Determinants of Incidence of Myofascial Pain Syndrome on Coffee Picker Farmers in Pulu-Pulu Village, North Toraja Regency. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):234-239.

Abstract

Myofascial Pain Syndrome is a muscular pain that are often encountered in humans due to non-ergonomic positions during daily activities. In addition to the work position, Myofascial Pain Syndrome is also affected by individual factors and other occupational factors. This study aimed to determine the factors associated with the incidence of Myofascial Pain Syndrome on coffee picker farmers in Pulu-Pulu village, North Toraja Regency. This was a quantitative research with cross sectional study method. Interviews were conducted on 45 respondents who were coffee picker farmers which was obtained based on the total sampling method. Data regarding sex, age, working period, working duration were measured using questionnaire. Data regarding posture were measured using the RULA assessment sheet and data regarding the incidence of myofascial pain syndrome were obtained through doctor's diagnosis. The data were analyzed using SPSS 21 with the results of Chi Square test showed that there was a correlation between gender ($p = 0.011$), age ($p = 0.000$), working period ($p = 0.017$), working duration ($p = 0.010$), posture ($p = 0.019$) and the incidence of myofascial pain syndrome. While the Logistic Regression test showed that the most significant and positive correlation was age and working duration. Writer would kindly suggest farmers to take breaks periodically while working to relax their body.

Keywords: Myofascial Pain Syndrome; Coffee Picker Farmers; Posture; Ergonomic.

Background

Agricultural sector in Indonesia is still the main employment field. Back in February 2018 around 30.46% of the Indonesian population had a main job in agriculture.¹ Like any other job, working in agricultural sector also possessed risk

of encountering accidents as well as occupational diseases. Occupational disease is a disease caused by your occupation, tools used at work, unergonomic posture when working and working period. One of the diseases that might possibly be experienced by farmers is Myofascial Pain Syndrome or pain disorders that affect joints and muscles.²

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Due to unattended and inadequate ergonomic factors, existing hazards might lead to various complaints from workers related to musculoskeletal problems (Musculoskeletal Disorders) in many countries, one of which is the Republic of Korea. Korea reached 5,502 musculoskeletal problems in 2010. While in United Kingdom the proportion of musculoskeletal disorders (Musculoskeletal Disorders) reached 40% in all workers. Cases of low back pain and pneumoconiosis were common problems in Japan reaching 7,779 cases of work-related illnesses in 2011 (Haworth & Hughes, 2012). Overall musculoskeletal disorders in Indonesia in 2013 diagnosed by health workers reached 24.7%. The highest prevalence based on occupation was farmers, laborers and fishermen which reached 31.2% of it.³

Myofascial Pain Syndrome is pain in the muscles which is characterized by the appearance of one or more trigger points. This syndrome is caused by mechanical factors and medical factors. Conditions like this are commonly found among people who work with less ergonomic factors for a long time.⁴

Factors that affect Myofascial Pain Syndrome (MPS) are acute/chronic conditions of overworked muscles, heavy workloads, psychological stress, abnormal joint function, and motor control damage. The farmer's activity of picking coffee is often done in an incorrect or bad position, resulting in trauma to the muscles due to excessive loads. One of the muscles that often experience MPS is the upper trapezius muscle.

Data related to the prevalence of MPS cases in Indonesia were still very limited. In USA, MPS was the main cause of work disability and the second most common cause of disability.⁵ Generally, the incidence rate of MPS was 54% in women and 45% in men. In the age category, MPS was most often found in the productive age (27-50 years). Several factors that affected MPS were age, gender, smoking habits, physical fitness, muscle trauma, working period and working duration.⁶

One of the provinces with the biggest plantations and agriculture sector in Indonesia was the province of South Sulawesi with plantation commodities including coconut, rubber, cocoa (chocolate) and coffee. Coffee production (Robusta and Arabica) in South Sulawesi in 2015 was 9,862 tons with a land area of 24,526 Ha. North Toraja Regency was one of the coffee producers from South Sulawesi.⁷

Coffee picking farmers perform non-ergonomic work attitudes when picking coffee, including standing with the neck tilted up, bending when putting the picked coffee cherries into the bucket, squatting position and the position of the hands that was always raised upwards. These attitudes were carried out repeatedly and regularly. The most common occupational health problems experienced by coffee picker farmers, were fatigue and sore muscle.⁸ Musculoskeletal complaints in some parts of the body tend to refer to the occurrence of myofascial pain syndrome disorders.

Research Method

This research was an observational study using cross sectional study design. The purpose of this study was to determine the factors associated with the incidence of myofascial pain syndrome in coffee-picking farmers in Pulu-pulu' village, North Toraja Regency. This research was carried out in February-March 2021 in Pulu-pulu' village, North Toraja Regency.

The population in this study were coffee-picker farmers in Pulu-pulu' village, North Toraja Regency, totaling 45 farmers. The sampling technique used in this study was total sampling, where the entire population was taken as sample, hence amount of sample or respondents was 45.

Data about age, sex, working period, smoking habits were collected using questionnaires and direct interviews while for work posture variables using the RULA Assessment Observation Sheet (Rapid Upper Limb Assessment) where this sheet was filled out by direct observation on respondent's work posture while working and the complaint of Myofascial Pain Syndrome that was diagnosed with qualified doctor. Data were processed using SPSS 21 for Chi Square and Linear Logistics Regression tests.

Discussion

Based on the result of this study, there were several topics to discuss about:

1. Correlation between sex and the incidence of Myofascial Pain Syndrome

Both Men and women work within their physical abilities. The physical strength of women's bodies was on average 2/3 of men's. Poltrast stated that women had 65% of men lifting average power.

This gap existed because women experience biological cycles such as menstruation, pregnancy, postpartum, breastfeeding, and others.⁹

The result of this study showed that there was a significant correlation between sex and the incidence of myofascial pain syndrome (MPS) where the statistical test results obtained was $p(0.010) < 0.05$ and statistical tests showed a positive results positive or unidirectional meaning that women were more at risk for MPS complaints.

Based on direct field observations, it was found that there were more female coffee pickers than men. This was because women were considered to have more perseverance in choosing the quality of coffee berries that were suitable for harvesting compared to men. Based on interviews with female farmer respondents, more women complained of neck pain than male farmers. This was because assumably male farmers had more physical strength the job was more like a man's job compared to women. This was in line with research conducted on residents in Jeddah, Saudi Arabia which stated that women showed a significantly higher risk of exposure to MPS compared to men. The study explained because women had fluctuating hormones such as the time of menstruation, pregnancy and others.¹⁰

2. Correlation between age and the incidence of Myofascial Pain Syndrome

In general, complaints of myofascial pain syndrome (MPS) began to be felt at the age of 30 and increase at the age of 40 and more. This was because naturally at middle age, muscle strength and endurance began to decrease thus the risk experiencing pain in muscles increases.¹¹ As coffee pickers got older, the risk of bones elasticity being degraded would increase which then became one of the risk factors to make MPS getting more frequent.¹²

Based on the results of research conducted on coffee picking farmers in Pulu'-Pulu' village, it was found that there was a significant correlation between age and the incidence of myofascial pain syndrome where the statistical test results obtained was $p(0.000) < 0.05$ and statistical test showed a positive results or unidirectional meaning that that the older a person gets, the higher the risk of having MPS. This was also in line with research conducted by Lai et al., (2017) on patients at The Northern Region of Taiwan Hospital which stated that elderly patients had high sensitivity towards exposure to MPS due to changes in the body's systems because of aging.¹³

Most Coffee picking farmers in Pulu'-Pulu village were more than 30 years old, where at that age, gradually, the body's physiological functions such as muscle strength and muscle stability would decrease. This was multiplied by the way coffee pickers work when they had to look up higher frequently to pick the coffees. This posture where the neck last very long in abnormal position would cause the neck to be more tense which lead might lead to muscle strained.

3. Correlation between smoking habits and the incidence of Myofascial Pain Syndrome

Due to the presence of carbon monoxide within cigarette hence blood ability to consumed oxygen decreases. If the person performs a task that required high exertion, the person would easily get tired because the oxygen content in the blood was low, carbohydrate burning was inhibited, lactic acid would be accumulated and eventually muscle pain occurred.¹⁴

Based on the analysis results, it was found that there was no significant correlation between smoking habits and the incidence of myofascial pain syndrome where the statistical test results obtained was $p(0.529) < 0.05$ and the statistical test also showed a very weak correlation between smoking habit and myofascial pain syndrome. This was in line with research conducted by Sri Padmiswari B & Adiartha Griadhi, (2017) which stated that there was no correlation between smoking habits and musculoskeletal complaints in silver craftsmen.¹⁵

The results direct observation showed that the respondents were dominated by farmers who did not smoke. One of the supporting reasons according to the farmers at the time of the interview was that smoking can be harmful to their work environment and some of the farmers also realized that smoking was not good for health, especially in their age. In addition, the company where they work oblige them not to smoke at work. However, these farmers could still become passive smoker, especially when outside their work environment and they were likely to be exposed by active smokers. Thus people who did not smoke were still very likely to be exposed to cigarette smoke and experienced complaints of musculoskeletal disorders.

4. Correlation between working period and the incidence of Myofascial Pain Syndrome

Work period was the accumulation of a person's work activities carried out over a long period

of time. If these activities were carried out continuously, it would possibly cause some disturbances within our body. Physical stress over a long period of time resulted in reduced muscle performance. If the stresses accumulated every day over a long period of time it could worsen the health which was also called clinical or chronic fatigue. The longer the time of working, the more muscles and bones saturation found. Where it could affect how they work both physically and psychologically.

Based on the analysis results, it was found that there was a correlation between working period and the incidence of myofascial pain syndrome where the correlation was sufficient and positive. This means that the longer the working period, the higher the risk of myofascial pain syndrome complaints. According to the results of observations, it was found that there were more respondents categorized to have young working period, as much as 28 people (62.2%) compared to respondents with long working period. However, it was still found that there were farmers who were new and felt pain in the neck and the major criteria were found during the examination by the doctor. The farmers had to look up higher frequently to pick the coffee where this posture was when the neck last very long in abnormal position which might cause the neck to be more tense and might lead to muscle strained.

5. Correlation between working duration and the incidence of Myofascial Pain Syndrome

Working duration was the amount of time exposed to risk factors. Working duration implied the minutes of working hours/day of where the worker exposed to the risk. It was also could be seen as exposure/year to risk factors or job characteristics based on risk factors. Based on the analysis result, it was found that most of the farmers worked more than 8 hours which were 30 people (66.7%). Farmers who worked for more than 8 hours were the farmer with less side activities and those who were fit enough physically. While the farmers who worked less than 8 hours were the farmers who were still in school age or did not fit enough.

Based on the analysis result, it was found that there was a correlation between working duration and the incidence of myofascial pain syndrome (MPS) where the correlation was sufficient and positive. This meant that the longer the farmer's working duration, the higher the risk of MPS complaints. This was in line with research conducted by Mukherjee et al., (2020) which also

found that working duration had a significant correlation with complaints of muscle disorders upon several domestic workers were in Kolkata, India.¹⁶ One of the farmers complaint about having MPS related to poor working hours, with several complaints of muscle pain in some of their limbs, especially in the neck and shoulders after work. This was strongly affected by the working time of the farmer which was 06.30 to 16.00 or 10 hours/day where this exceeded the recommended working duration which was maximum 8 hours/day.

Based on the analysis result, it was shown that the farmers who extended their working duration more than usual, oftenly not followed by higher efficiency, in fact there was usually a decrease in productivity and a tendency to encounter fatigue, illness, and accidents. The maximum break time was 1 hour, while between working hours break time must be between 15-30% of the whole working time. If working hours exceeded these provisions, things such as a decrease in work speed and health problem might occurred which might lead to low levels of productivity.

6. Correlation between posture and the incidence of Myofascial Pain Syndrome

One aspect that was considered in ergonomics was work attitude. It was stated that work attitude was the various positions of the workers' posture during working. Natural work attitude was a work attitude that caused the position of body parts to move in a natural position. The farther the position of the body part from the centre of body gravity, the higher the risk of muscle complaints. Work attitude did not occur naturally just because of the characteristics of the task but work tools and work area which were not in accordance with the workers abilities would intervene as well.¹⁷

Based on the results of the study, it was found that there was a correlation between posture and the incidence of myofascial pain syndrome where the correlation was strong and unidirectional since it was positive result. In this study, it was found most of the farmers were at very high-risk posture which were 19 people (42.2%). This was due to how they worked. They stand in a static working position or stand in a long time without taking breaks or stretching whether it was leaning on something, sitting and others.

The results of field observations showed that found most of the farmers posture while working were not compatible to regular ergonomic

standard. There were many movements that constrained their body parts to lean out of the natural body positions with incorrect and awkward attitudes. These incorrect movement or attitudes would increase the risk of injury to the muscles. This was in line with the research of Qureshi et al., (2019) which stated that Myofascial pain syndrome was a disease that causes pain in the neck and shoulders and was mostly caused by incorrect posture. Therefore therapy and good ergonomic design were needed to prevent the MPS getting worse.¹⁸

7. Risk factors analysis of the incidence of Myofascial Pain Syndrome

Myofascial pain syndrome (MPS) was defined as musculoskeletal pain where the pain was located in the muscles. MPS could grow to acute or chronic, regional or generalized. This disorder could be a primary disorder that causes local or regional pain, or a secondary disorder that results from some other condition.¹⁹

Based on the results of the study, it was found that most of the farmers experienced complaints of MPS. In accordance with the results of observations, MPS complaints were motivated by the age of the farmers who were dominated by older farmers and they who worked for more than 8 hours with the head and neck looking up and arms and shoulders hanging outwards to pick coffee cherries. Sometimes also stand on tiptoe to reach higher.

Regression analysis used to analyze several risk factors simultaneously using binomial logistic regression with the backward LR model. The results of the regression test on the risk factors for MPS complaints when viewed from the significance value, the most dominant risk factor for the incidence of MPS was the variable of age and work duration. The older the farmer, the higher the risk of experiencing MPS hence the complaint would increase. In addition, the incidence of MPS was also due to the time or duration of work of farmers. The longer the working duration, the higher the risk of MPS. To overcome the problem of MPS in coffee pickers in Pulu'-Pulu' village, the employers should put more concern upon their workers regarding any health complaints especially MPS to maintain high productivity.

Conclusion and Suggestion

Based on the results of this study, it could be concluded that sex, age, working period, working

duration and posture have a significant correlation with the incidence of myofascial pain syndrome and after a deeper test using logistic regression it was found that age and length of work were the variable that has the closest and most positive relationship to the occurrence of myofascial pain syndrome. Based on this research, the researcher gives advice to the management to provide knowledge information to farmers about ergonomic work positions and perform muscle relaxation for at least 30 minutes at work to increase muscle flexibility.

Ethical Approval: Hasanuddin University

Conflict of Interest: None

Funding: Personal Fund

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Analysis of Motor Vehicle Accidents in Karnataka Between 2008-2017

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How to cite this article: Manashree Mane, Ambulu V R, Ajay Kumar. S et al. Analysis of Motor Vehicle Accidents in Karnataka Between 2008-2017. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):240-247.

Abstract

Background: Motor vehicle collisions usually result in consequences like property damage, injury and death. It causes financial losses to the victim himself, families of the victim and to the country. Traffic collision often results in injury, death or property damage.

Purpose: The objective of the research is to provide a better understanding on motor vehicle accidents that occurred in the time span of 2008-2017 in Karnataka.

Procedure: The data obtained was secondary data from report of Ministry of Road Transport and Highways and CEIC Data Global Database website. This data was then categorised under India Premium Database's Automobile Sector- table IN.RAF018: Road Accidents: Karnataka.

Conclusion: From the data obtained it is seen that the rate of Motor vehicle accidents in Karnataka is still growing at a rapid pace.

Keywords: Motor vehicle collision; Road Traffic Accidents; Injuries; Safety precautions.

Introduction

Motor vehicle accidents (MVA) happen when a motor vehicle strikes or collides another vehicle, a stationary object, a pedestrian, or an animal. Every year approximately 1.35 million lives are affected as a result of motor vehicle accidents. Somewhere around 20 to 50 million lives sustain injuries that are non-fatal. The accidents cause considerable economic loss to victims, the families of those victims and to the country. The financial loss or crisis mainly arise from

the treatment or consequence on productivity for those who died or have some disabilities by the injuries they sustain. Motor vehicle accidents cost many of them around 3% of their gross domestic product.¹

Common Causes of Car Accidents:

Weather and road conditions the most common factors that are correlated to the car accidents. Whilst the inability of the driver to keep awareness

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on the road and operating his vehicle are the most commonly associated factors, a few others related to the causes of car accidents are:

- a) Speeding –Negligence to adhere the legal speed limit.
- b) Using a Device – texting or talking on phone while driving or even turning on the music player or GPS device.
- c) Driver Fatigue – driving when extremely tired can lead to falling into a trans-state or even falling asleep.
- d) Drunk Driving – driving under the influence of drugs or alcohol.
- e) Defective Auto Parts – when a part on a vehicle is defective or goes bad,brake issues, defects in tires, defective air bags, top-heavy design and more.
- f) Rubbernecking – drivers looking along the roadway, traffic accidents, flashy billboards and other things cause distraction.
- g) Poor Weather Conditions – weather conditions like wet or icy roads, high winds, blowing dust, fog and torrential downpours.²

Who is at Risk?

- (a) Socioeconomic Status:

Majority of deaths occur in low-income countries and middle-income countries. Even in high-income countries, people from lower socioeconomic backgrounds are more likely to be involved.

- (b) Age:

Leading cause of death in children and young adults aged 5-29 years.

- (c) Sex:

About 73% of motor vehicle accidents occur among young males who are below the age of 25 years.³

In 225 autopsied of MVA victims, 55.11% victims fall between 21-30 years of age, with 78.22% as males of the total victims. Most of the MVAs occur during the daytime. Head injures account for 30.22% of the total injuries, followed by injury to the abdomen, thorax and limb. Hemorrhagic shock cause 63.11% of deaths, while head injury accounts to death in 30.22% of cases.⁴

Buses being major mode of public transportation, unfortunately involved in significant number of crashes in recent. Passenger's safety fully depends upon the bus driver's attitude and ability to comprehend traffic. The importance of driver behavior on road safety and the use of Zuckerman's sensation seeking scale (ZSSS) in behavioral studies of Indian drivers are crucial in understanding the effect of sensation behaviour on crash involvement rate of bus drivers.⁵

In 2015, total of 715 incidents were reported of traffic accidents, of which 499 convicted and in the year 2016, 699 cases were reported of traffic accidents and 439 were convicted.⁶

RTAs remains as one of the major reason for mortality and morbidity owing to the high usage of vehicles, change in lifestyle adapted by the individuals and the risk behaviors. In 879 autopsies conducted, 39% were accounted for RTAs. The head injuries were responsible for 3/4th of deaths followed by abdominal injuries. The most common victims of RTAs are mainly the occupants of motorized two wheelers (43%) and pedestrians (33%).⁷

Head injury is one of the key cause of mortality for individuals involved in fatal road traffic accidents. Most of the accidents occurred during afternoon and evening hours and the most vulnerable age groups was 21 and 30 years and mostly two-wheeler occupants. Skull fractures was reported in 88.88%, fractures of vault in 88%, base of the skull in 35.97%, intra-cranial hemorrhages in 52.63% and contusions and lacerations in 35% of the cases.⁸

Motorized two-wheeler accidents have been increasing in India. Most of the victims are males, and the accidents take place due to over speeding and negligence. A total of 630 external injuries, 56.5% of abrasions, 77.9% complications of head injury and linear fractures in skull. Obeying the traffic rules, wearing helmet, avoiding over speeding and negligence and taking necessary safety precautions can significantly reduce the two-wheeler vehicle accidents.⁹

Transport of petroleum products in India by road has a record of high fatality rate. Petroleum companies have as many as 1635 transport accidents in the last five years. The analysis reveals Hindustan Petroleum Corporation Limited records the highest number of transportation accidents in India. States

like Karnataka and Maharashtra have maximum number of transportation related accidents. Driver negligence, road conditions and equipment failure are the main reasons behind these accidents. The analysis of accident reports and inputs from experts may further strengthen the gap to reduce the number of accidents in future.¹⁰

The highways account for a major share of travel and transport in India. Road deaths and injuries are high on these highways. The burden, pattern, characteristics and outcomes of highway road crashes gives proper analysis using combined data from police and hospital sources. With the help of mixed method approaches, resource mapping, environmental scanning the high-risk crash locations are identified. The two national highways and five state highways contributed for 37% and 25% of total road deaths. Collision patterns indicated the involvement of heavy vehicles along with motor cars on the highways. Safety of all road users and vulnerable road users should be given greater importance on highways.¹¹

Road traffic accident is a public health problem. Globally 1.25 million people die each year due to road traffic crashes. A semi-structured questionnaire was used for the survey where it was found out that good knowledge was seen among 82.2%, good attitude in 88.9%, but good practice in 58.9% of the participants. It shows that the knowledge about road traffic accident is not brought into practice. Hence the laws should be made more stringent.¹²

Road Traffic Accident (RTA) is any vehicular accident occurring on the roadway this includes collision of an automobile with a pedestrian, another automobile or with a non-automobile on the roadway/ fall from a moving vehicle causing injuries or death of involved individuals. Victims in RTAs sustain injuries which are fatal to life. The study of injuries associated with fatal outcome helps in implementation of measures to prevent fatalities due to RTAs.¹³

Mandibular fractures are discontinuation that occur in from the mandibular bone which are commonly found in during road traffic accident. In

a study conducted, total numbers of autopsied cases during 2011 to 2014 were 453 where 21 cases had mandibular fracture. Maximum number of victims belongs to 21-30 years followed by 31-40 years. The maximum numbers of victims were found to be males. Not wearing helmet and seat belt may further lead to facial injuries and permanent disfigurement of face in all road traffic accident cases.¹⁴

Methodology

The data collected was secondary data from the report of Ministry of Road Transport and Highways and CEIC Data Global Database website. The records of the number of accidents, road traffic accidents, injuries and deaths were analysed. The data is categorized under the India Premium Database's Automobile Sector- Table IN.RAF018: Road Accidents: Karnataka. Data completely represents the entire number of road accidents, injuries and deaths which had occurred in the decade 2008-2017. This data includes all the vehicles like two wheelers, auto rickshaw, cars, buses and heavy motor vehicles (HMV).¹⁵

Discussion

The data obtained was analysed including the accidents, injuries, and death caused by two wheelers, cars, buses, auto rickshaws, and Heavy Motor Vehicles. According to data obtained the accidents due to two wheelers are high in the year 2013 when compared to others.¹⁶⁻¹⁸ (Refer Figure 1). According to data obtained the accidents due to autorickshaws are high in the year 2008.^[19-21] (Refer Figure 2). According to data obtained the accidents due to cars are high in the year 2008.^[22-24] (Refer Figure 3). According to data obtained the accidents due to bus are high in the year 2011.^[25-27] (Refer Figure 4). According to data obtained the accidents due to Heavy Motor Vehicles are high in the year 2008.²⁸⁻³⁰ (Refer Figure 5)

There was a significant reduction in mortality and morbidity from year 2014 which could possibly be due to introduction of National Road Safety Policy by Government of India and National Road Safety Council (NRSC).

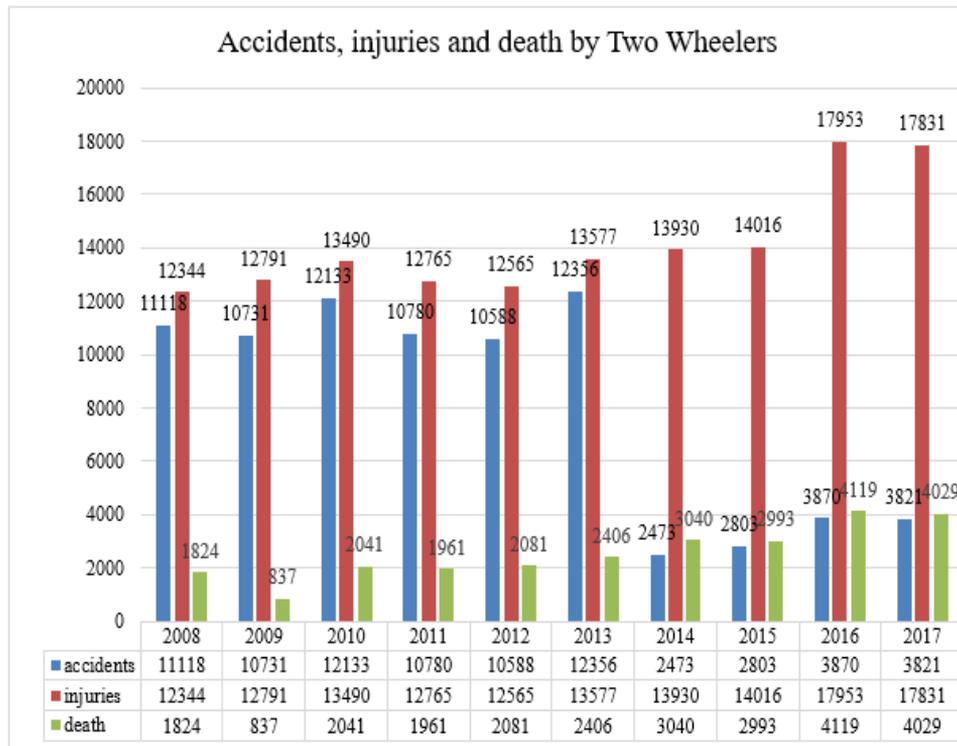


Fig. 1: Number of accidents, injuries and death caused due to two-wheelers in between the year 2008 to 2017.

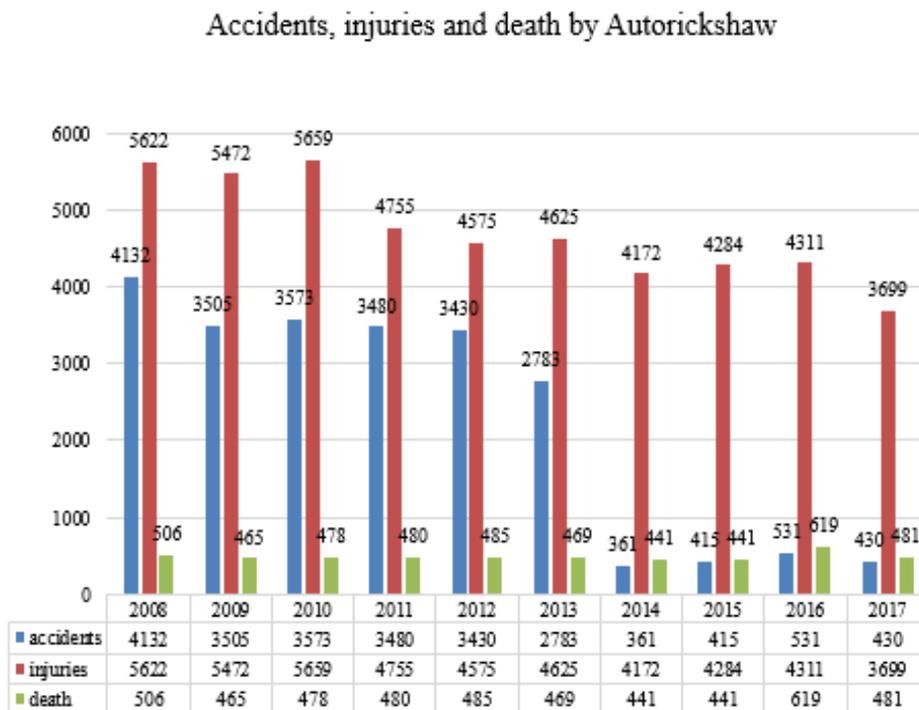


Fig. 2: Number of accidents, injuries and death caused due to autorickshaws in between the year 2008 to 2017.

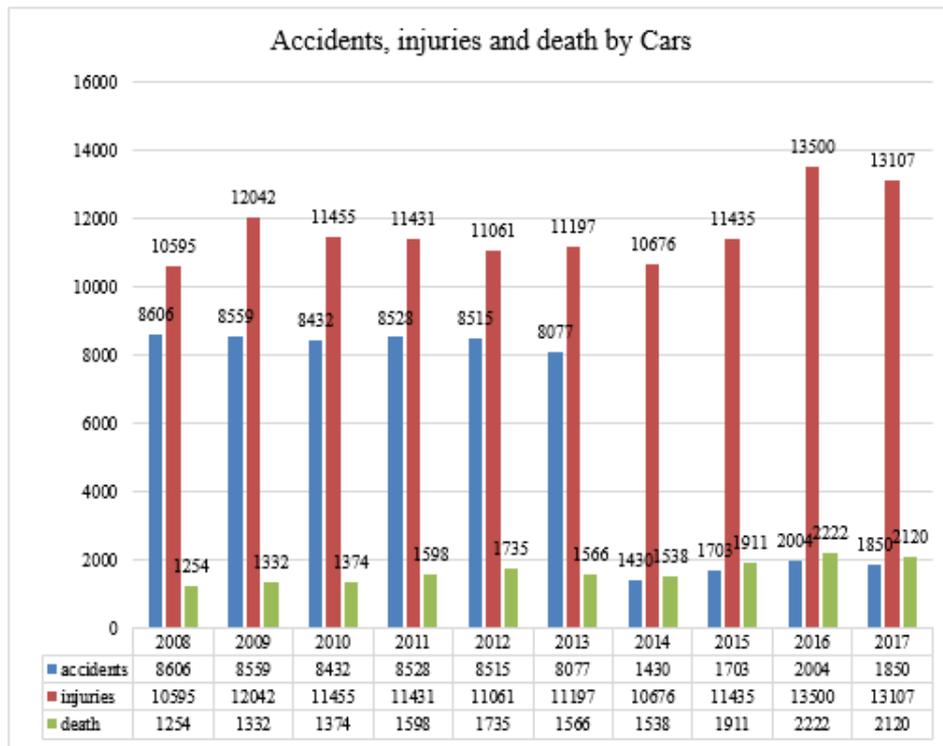


Fig. 3: Number of accidents, injuries and death caused due to car accidents in between the year 2008 to 2017.

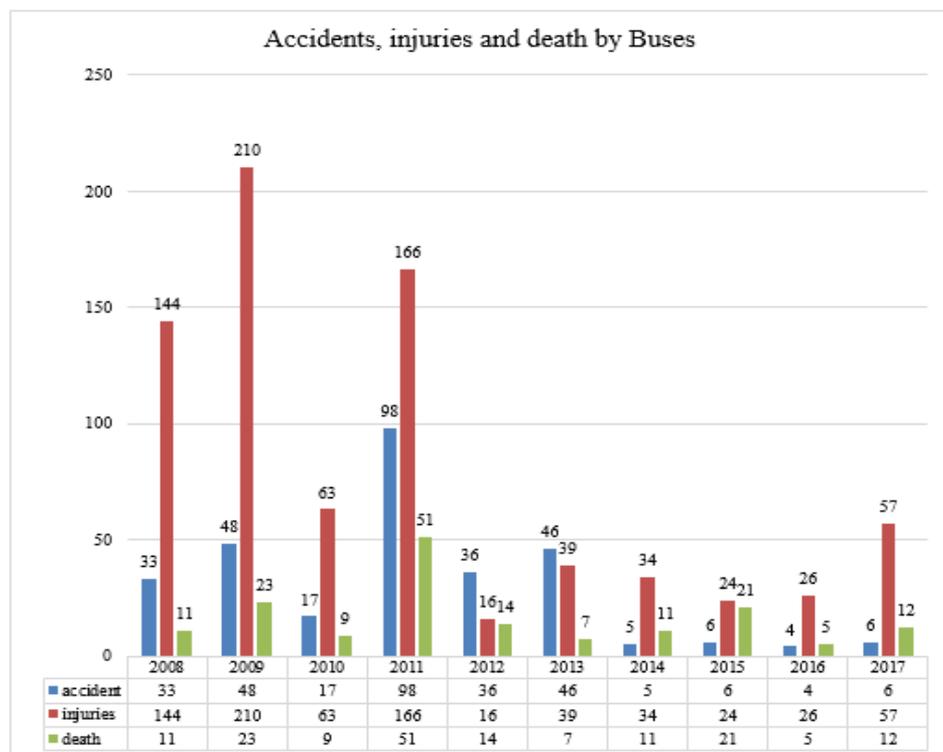


Fig. 4: Number of accidents, injuries and death caused due to Bus accidents in between the year 2008 to 2017.

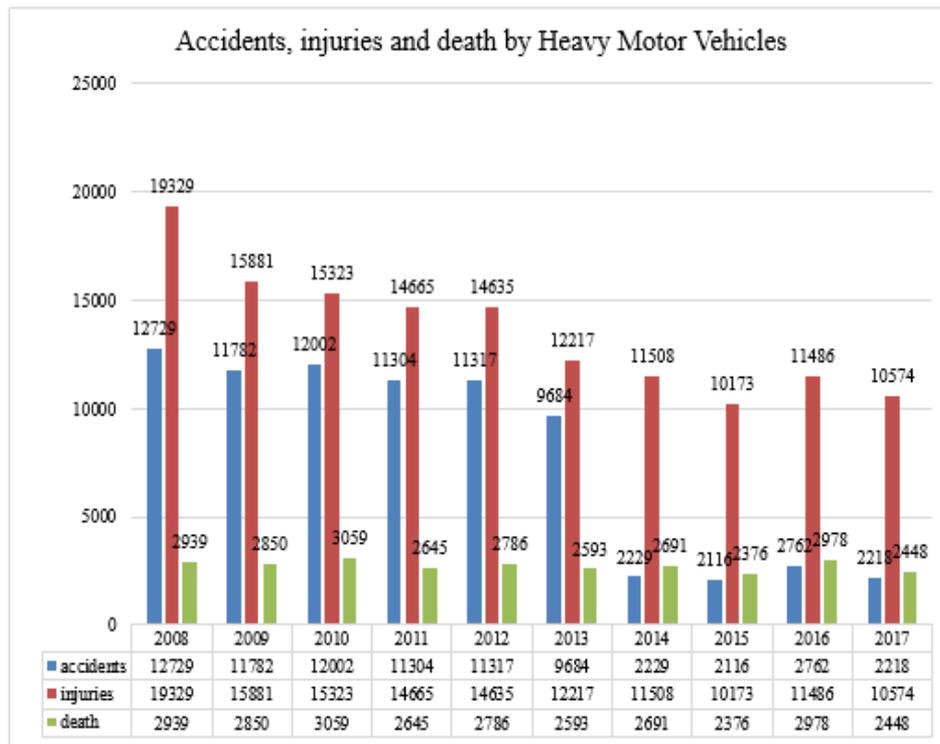


Fig. 5: Number of accidents, injuries and death caused due to Heavy Motor Vehicle accidents in between the year 2008 to 2017.

Engineering, enforcement, education and emergency care should be regarded as prime safety measures. Periodic assessment of accident-prone stretches by the zonal officers may help in reducing the accidents. A strict enforcement by Police and Transport officials for drunken driving will help set a benchmark for stricter implementation of the rules. Rules of the road, correct manner of crossing etc. by help of posters and electronic media exhibiting the serious results can be introduced as necessary instruction in the schools for the children.

Karnataka proposed a full-fledged Road Safety Authority (RSA) in 2016 on the lines of the one in Kerala to reduce the number of accidents. RSA will look after the improvement of road engineering and its design, increase the awareness related to the road-safety, provide emergency care facilities and strict enforcement on accident-prone roads. It will also oversee the development of an accident information system and coordinating with multiple departments.

Three main factors that are mainly associated to the cause of accidents are vehicle condition, road design and driver behaviour. The government should take steps to rectify road design flaws, especially if any particular stretch is witnessing more accidents.

Obedying the traffic rules, wearing helmet, keeping away from over speeding and by taking necessary safety precautions may result in a smaller number of accidents. Victims generally sustain injuries, which are fatal. Not using the helmet and seat belt may lead to permanent disfigurement of face which form the facial injuries. Collision patterns indicates the involvement of heavy vehicles along with motor cars on the highways. Excessive speeding is also a major cause of crashes. Safety of all road users and vulnerable zones should be given greater importance on highways.

Conclusion

The aim to improve and work on road engineering and design, road-safety awareness, emergency healthcare and medical facilities and strict enforcement on accident-prone roads are an integral part to accident information system. This also should be coordinated with multiple departments for an improved and enhanced way of managing the road traffic accidents. Steps to rectify road design flaws in the design or construction of road or a stretch witnessing more accidents can help in improving the system and avoiding the accidents.

Acknowledgment

The authors would like to acknowledge Dr. Asha Rajiv (Director, JAIN (Deemed-to-be University) and Dr. Reena Susan Philip (Head, Department of Forensic Science, JAIN (Deemed-to-be University) for their constant guidance and support.

Ethical Clearance: No ethical clearance required.

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interest: The authors declare there is no conflict of interest.

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Lead Induced Oxidative Stress and Affected the Expression of Steroidogenesis -related Genes in Testis of Male Mice

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How to cite this article: Nahla Abdalla Hassan Elsheikh, Nagmeldin Abd elwahid. Omer, Li-lian et al. Lead Induced Oxidative Stress and Affected the Expression of Steroidogenesis -related Genes in Testis of Male Mic. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):248-254.

Abstract

Background: Lead (Pb) is classified as a major risk factor affecting the male reproductive system; however, its precise mechanisms of action are poorly understood and inconsistent. This work aimed to investigate the effect of Pb toxicity on male reproductive function.

Methods: Accordingly, in this study, adult male mice treated with Lead acetate (PbAc) by gavage (200 mg/kg/day) for 28 days. We analyzed sperm count and morphology, oxidative stress, and the expression of antioxidant and steroidogenesis -related genes in the testes of male mice.

Results: Pb significantly ($P<0.05$) showed decreased body weight and sperm count, as well as significantly ($P<0.05$) increased the number of abnormal sperms and plasma testosterone level. The activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (Cat) were significantly decreased, whereas the level of malondialdehyde (MDA) was significantly increased in the testis of mice treated by Pb. The mRNA levels of antioxidant-related genes (SOD1, GPX1, and CAT) were significantly decreased following Pb treatment. Furthermore, the expression of genes involved in the steroidogenic pathway, including steroidogenic acute regulatory protein (Star), cytochromeP-450sc (Cyp11a1), 17 β -Hydroxysteroid dehydrogenase (17 β -HSD), 3 β -Hydroxysteroid dehydrogenase (3 β -HSD) and cytochrome P450, family 17 (Cyp17), were significantly ($P<0.05$) decreased after exposure to Pb.

Conclusion: In conclusion, Pb disrupts male reproductive function by inducing oxidative stress, negatively regulating the mRNA expressions of steroidogenesis and antioxidant -related genes, and ultimately reducing sperm quality and quantity.

Keywords: Lead; testis; spermatogenesis, oxidative stress; steroidogenesis.

Introduction

The environmental and occupational exposure to lead (Pb) has been well-documented and is considered as major public health issues.¹ The male reproductive system has been recognized as a primary target of Pb-induced toxicity.^{2,3} Previous studies have suggested that Pb can accumulate in the testis and alter its function by causing oxidative stress through excessive generation of reactive oxygen species (ROS) and disruption of the activities of antioxidant enzymes such as Catalase (Cat), Superoxide dismutase (SOD), and Glutathione peroxidase (GSH-Px).⁴ Oxidative stress results in apoptosis of spermatozoa, DNA damage, and the disruption of spermatogenesis events that lead to changes in sperm quality and function.⁵⁻⁸ However, the precise mechanisms underlying Pb toxicity to male reproductive function and spermatogenesis are unclear.

Pb is reported to affect the hypothalamic-pituitary testis axis,^{9,10} and alter the levels of luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone (T). The testis is responsible for the biosynthesis of steroid hormones in males and it requires continuous androgenic stimulation for normal growth and functions.¹¹ Testosterone is gonadal steroids produced by the Leydig cells and acts to maintain spermatogenic processes.^{11,12} The biosynthesis of testosterone is initiated by the transport of cholesterol from the outer to inner mitochondrial membrane; this step is mediated by steroidogenic acute regulatory (Star) protein.¹³ Cyp11a1 mediates the cleavage of the side chain of cholesterol to form pregnenolone. In the endoplasmic reticulum, pregnenolone is converted to progesterone by 3β -HSD. Finally, progesterone is metabolized into testosterone by Cyp17 and 17β -HSD (14). Pb can modify hormonal metabolism by altering the synthesis and breakdown of testosterone, FSH and LH.⁹ In albino rats, Pb affects testis steroidogenic activity, as well as plasma testosterone and gonadotropin levels.¹⁵ Nevertheless, data concerning the effect of Pb on testosterone production and steroidogenesis are scarce and inconstant.

Therefore, this study aimed to investigate the effect of Pb toxicity on male reproductive function. We analyzed sperm count and morphology, oxidative stress, and the expression of antioxidant, steroidogenesis and apoptosis-related genes in the testes of male mice.

Materials and methods

Animals and treatment

In this experiment, twenty adult male Kunming mice (8 weeks old) weighing 25 to 30g were purchased from Nanjing Qinglongshan Experimental Animal Factory (Nanjing, China), housed in the animal room, and provided with a standard diet and water *ad libitum*. Animals were maintained with a 12 to 12 light-dark cycle, $55 \pm 5\%$ humidity, good ventilation, and a temperature of $25 \pm 2^\circ\text{C}$. Mice were acclimatized for seven days before treatment. After acclimatization, mice were randomly divided into two groups and housed five per cage (10 mice per group). The control group was received 0.3 mL of water only, whereas the treatment group received the same volume of distilled water containing lead acetate (PbAc) obtained from Ding Si, Nanjing, China by gavage at a dose of 200 mg/kg/bw for 28 days. All mice were killed by euthanasia with ether, followed by cervical dislocation. Blood samples were collected and centrifuged at 3000 rpm at 4°C for 15 min to separate the plasma from the blood cells and stored at -20°C for later use. Testes were surgically obtained and frozen immediately in liquid nitrogen and stored at -80°C for further analysis.

Sperm morphology and count

Sperm analysis was performed at the Quality Supervision & Test Center of Cattle Frozen Sperm (Nanjing Agricultural University, Nanjing, China). Sperm morphology was assessed. After staining with Giemsa, a total of 600 spermatozoa were counted in random fields, and the percentage of abnormal sperm was calculated. Classification of sperm morphology was based on the criteria of abnormality described previously Wyrobek and Bruce.¹⁶ For sperm count, the number of sperm in five squares in the hemocytometer was counted as described previously.¹⁷

Determination of plasma testosterone

Plasma testosterone levels were measured using a mouse testosterone ELISA kit (Jiancheng, Nanjing, China), following the manufacturer's instructions.

Testicular antioxidant enzyme activity and MDA level

Testis samples (50 mg) from each mouse were homogenized in 10 volumes of precooled physiological saline; the homogenate was centrifuged at 3000 rpm for 15 minutes and the supernatant was used for

biochemical assays. The activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (Cat) and the content of malondialdehyde (MDA) in testis were detected using commercial Assay Kits (Jiancheng, Nanjing, China).

RNA extraction and real-time quantitative reverse transcription polymerase chain (qRT-PCR) analysis.

Total RNA from each sample was extracted from 50 mg testis tissue using TRIzol® reagent (Invitrogen, USA) according to the manufacturer's instruction. Purity and concentration of total RNA were determined by obtaining the absorbance values at the wavelength of 260/280 nm using NanoDrop™ (Thermo Fisher Scientific, Waltham, MA, USA) and the ratio between the absorbance values at 260 nm and at 280. Total RNA was reverse transcribed into cDNA using Prime Script™ RT Master Mix (TaKaRa, Tokyo, Japan). The reaction mix for qRT-PCR consisted of 2 µL of diluted cDNA, 0.3 µL of forward and reverses primers, 5 µL of SYBR green PCR master mix (Roche, Switzerland), and 2.4 µL of PCR grade water. qRT-PCR performed on Applied Biosystems 7500 HT Sequence (Thermo Fisher Scientific, USA). Mice-specific primers used were synthesized by Genscript® (Nanjing, China) and are listed in Table 1. The relative levels of gene expression were determined by the $2^{-\Delta\Delta Ct}$ method.¹⁸ GAPDH was used as housekeeping gene.

Statistical analysis

All data were expressed as means ± SEM. The differences between groups were analyzed using independent samples t-test with SPSS Statistical Package for Social Sciences software for Windows, version 20.0 (SPSS; Chicago, Illinois, USA). $P \leq 0.05$ was considered statistically significance.

Results and Discussion

Effect of Pb on sperm count and morphology

Pb-treated mice showed a significant reduction ($P < 0.05$) in sperm count as well as an increase in the percentage of sperm with abnormal morphology compared to the control group (Figure 1A, B). Several studies revealed defective spermatogenesis and altered production and maturation of sperm in the testis after Pb accumulation.^{2,3} Similarly, significant reductions in both the quantitative and qualitative characteristics of spermatozoa in the testes of albino rats exposed to PbAc have also been reported.¹⁹ Furthermore, an in vitro study suggested that Pb might inhibit testis spermatogenesis by disturbing the metabolic activities of the Sertoli cells.²⁰

Effect of Pb on the activity of antioxidant enzymes, MDA level, and the expression of antioxidant-related genes of testis tissues

The activities of SOD, GSH-Px and Cat in the testis of mice were significantly ($P < 0.05$) lower in the treatment group compared to those in the control. Furthermore, MDA levels were significantly ($P < 0.05$) higher in the treatment group than in the control (Table 2). These results were further confirmed by the decreased mRNA expression levels of SOD1, GPX1 and CAT after Pb exposure (Figure 2).

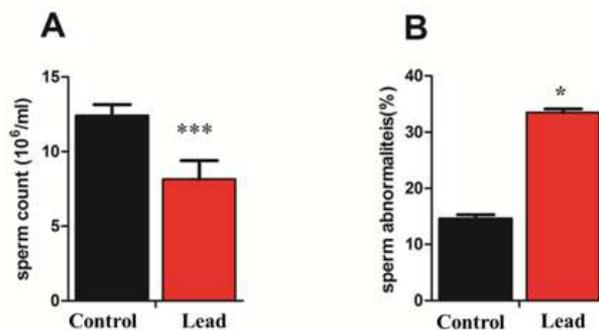


Figure 1 Sperm parameters. A) Sperm count. B) Sperm morphology. Data are represented as mean ± SEM, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ indicate significant difference from the control group.

Table 1: Primers sequences used for qRT-PCR analysis

Genes	Accession number	Primer sequence (5 to 3)	PCR products (bp)
Star	NM_011485.5	F:TCGCTACGTTCAAGCTGTGT R:ACGTCGAAGTGGACCCATCC	152
Cyp11a1	NM_019779.4	F:CTAAAGGACTTCCCTGCGCT R:CCCTCCAGAAGTGGTACAGG	186

Contd... Table 1: Primers sequences used for qRT-PCR analysis			
17 β -HSD	NM_008291.3	F: AGACCGCCGATGAGTTTGTT R: TCAGGAGGAATCGTTGAGCG	153
3 β -HSD	NM_001304800.1	F: GGCCTGTGTTCAAGCAAGTG R: TCTGTTCCCTCGTGGCCATTC	107
Cyp17	NM_007809.3	F:TGGAGGCCACTATCCGAGAA R: CACATGTGTGTCCTTCGGGA	119
SOD1	>NM_011434.2	F: GGAACCATCCACTTCGAGCA R: CGTCCTTCCAGCAGTCACA	232
GPX1	>NM_008160.6	F: CACAGTCCACCGTGTATGCC R: CTTGCCATTCTCCTGGTGTCC	230
CAT	>NM_009804.2	F: CACTGACGAGATGGCACACT R: TGTGGAGAATCGAACGGCAA	175
GAPDH	XM_001476707.5	F:AGAAACCTGCCAAGTATGATGAC R:CCTGTTGCTGTAGCCGTATTC	221

Table 2: Effects of Pb on superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), Catalase (CAT) and malondialdehyde (MDA) in testis of mice

Groups	SOD (U/mg prot)	GSH-Px (U/mg prot)	Cat (U/mg prot)	MDA (nmol/mg prot)
control	58.22 \pm 2.85	0.22 \pm 0.01	0.042 \pm 0.001	0.74 \pm 0.10
lead	38.56 \pm 2.00***	0.14 \pm 0.01**	0.018 \pm 0.00*	1.12 \pm 0.08*

Data are represented as mean \pm SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ indicate significance from the control group

Oxidative damage due to Pb administration was previously reported by Rao and coworkers.²¹ Pb could inhibit the activities of antioxidant enzyme by binding to the sulfhydryl groups of catalase and SOD with higher affinity.²² It is well-established that Pb induces cellular damage through generation of ROS and reduction of antioxidant enzymes, which might be responsible for disruption of testicular functions and spermatogenesis.²³

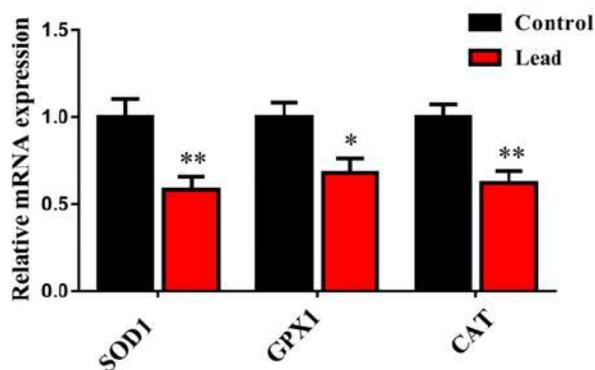


Figure 2: Effect of Pb treatment on mRNA expression of SOD1, GPX1, and CAT in the testis tissues of male mice. Data are represented as mean \pm SEM, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ indicate significant difference from the control group.

Effects of Pb on plasma testosterone levels and expression of steroidogenesis-related genes in testis tissues

In this study, a significant increase ($P < 0.05$) in the plasma testosterone levels of mice in the treatment group compared to those in the control group was observed (Figure 3A). qRT-PCR analysis revealed that the expression levels of Star, Cyp11a1, 17 β -HSD, and Cyp17 in the treatment group were significantly ($P < 0.05$) lower compared to those in the control group. However, no significant difference in the mRNA levels of 3 β -HSD between groups was observed (Figure 3B-D, F). Despite the reduction in the expression of genes responsible for testosterone biosynthesis, the plasma levels of testosterone were

increased by treatment with Pb. Moreover, in the treatment group, we detected lower mRNA level of Star, Cyp11a1, Cyp17 and 17-β HSD, which are genes that play a significant role in steroid hormone synthesis in the testis. Testosterone is synthesized by Leydig cells under the stimulation of LH, which binds to the androgen receptors (AR) found in Sertoli

cells to initiate the functional responses required to support spermatogenesis.^{11,24} It appears that Pb may accumulate in the testis and disrupt its function either through the abnormal metabolism of testosterone or through an abnormal interaction between androgens and their receptors in Sertoli cells.²⁵

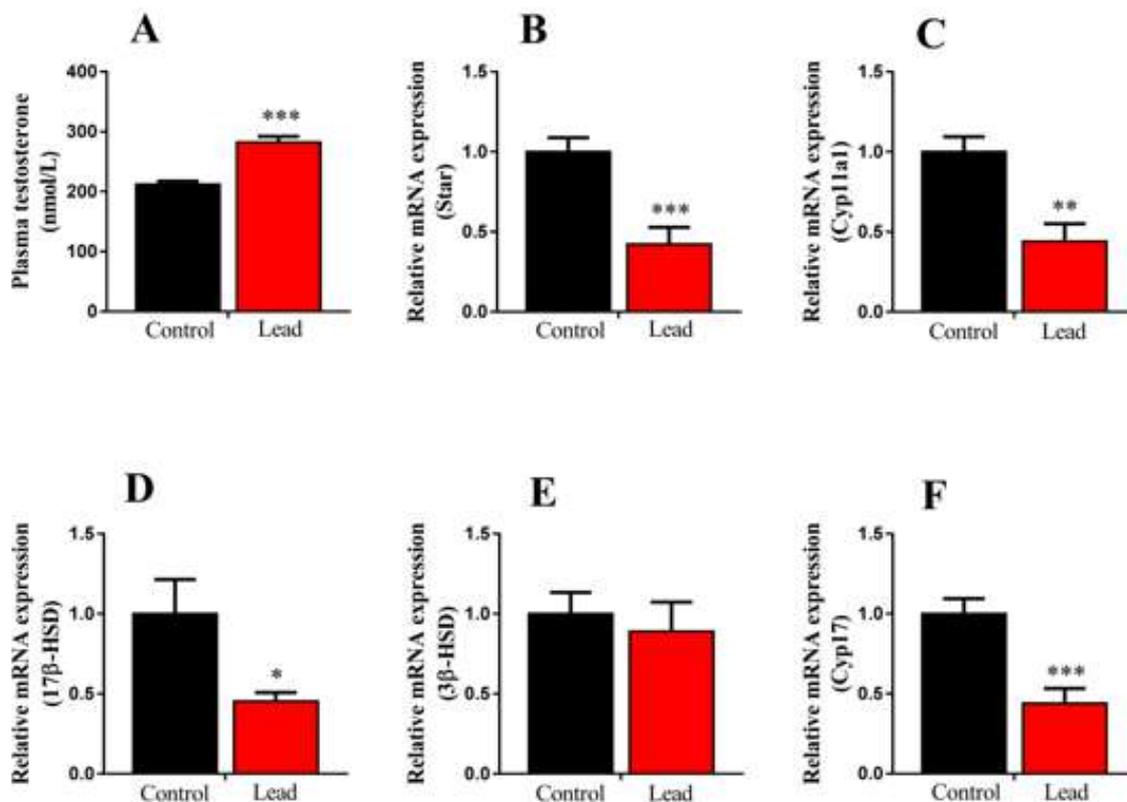


Figure 3: Effect of Pb treatment on plasma testosterone level and mRNA expression of Star, Cyp11a1, 17β-HSD, Cyp17, and 3β-HSD in the testis tissues of male mice. Data are represented as mean ± SEM, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ indicate significant difference from the control group

The stimulatory events include the enzymatic conversion of substrates to testosterone⁽²⁶⁾. Inhibition of testis steroidogenesis has previously been reported⁽³⁾, wherein the researchers described the reduced enzymatic activity of 3β-HSD and 17β-HSD in the testis of Pb-exposed rats. Furthermore, several studies reported the accumulation of plasma testosterone in rats that consumed Pb.²⁷ Conversely, other studies reported decreased plasma testosterone level after lead exposure in rats.^{2,23} However, the increase in plasma testosterone levels despite the reduced expression of steroidogenic enzymes requires further investigation. We hypothesized that Pb induced the aberrant metabolism of testosterone and caused its

accumulation in the plasma, followed by a feedback control of testis steroidogenesis. The oxidative stress induces after Pb administration might affect the expression of key enzymes involved in testicular steroidogenesis. Our result is line with Anjum who reported reduced the enzyme activity of 3β-HSD and 17β-HSD in the testis of Pb-exposed rats.^{3,23}

Conclusion

Taken together, our findings indicate that Pb administration reduces sperm quality by increasing oxidative stress and inducing apoptosis-related events in the testis, which is associated with reduction in the expression of testicular steroidogenesis genes.

Ethics statement

The experiments were approved by The Ethics Committee of Nanjing Agricultural University (Nanjing, China) according to the guidelines of the Care and Use of Laboratory Animals prepared by the Institutional Animal Care.

Funding: This work was supported by the National Natural Science Foundation of China (Grant No. 31772567). The Fundamental Research Funds for the Central Universities (Grant No. KJQN201607)

Conflict of Interest: The authors declare that they have no Conflict of Interest.

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The Correlation between Insulin Resistance and Urotensin II in Patients with Gestational Diabetes Mellitus

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How to cite this article: Najmah M. Meran, Farah Abdul Salam Hussein. The Correlation between Insulin Resistance and Urotensin II in Patients with Gestational Diabetes Mellitus. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):255-260.

Abstract

Gestational diabetes mellitus is glucose intolerance of varying degree with onset or first detection during pregnancy, it can cause long and short term morbidities in both the mother and the child, such as shoulder dystocia, preeclampsia, and high blood pressure. The most powerful endogenous vasoconstrictor peptide, urotensin II, and its receptor are involved in the etiology of gestational diabetes mellitus.

Aim of the study: The study's goal was to see if there is a link between Urotensin II levels and insulin resistance in pregnant women with gestational diabetes.

Patients and method: A case-control study that was conducted in obstetrics and gynecology department at Baghdad Teaching hospital from the first of January 2019 to the end of December 2019. A sample of 80 pregnant women participated in the study fulfilling inclusion criteria. 40 of them diagnosed with gestational diabetes mellitus by (2 hours 75 gm. Oral glucose tolerance test) and 40 women as control group.

Results: The mean age of the gestational diabetes mellitus group was 29.8 ± 6.9 years and control was 29.7 ± 6.6 years with no significant differences. The study showed highly significant increase in fasting Insulin, fasting blood glucose, Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), of the GDM group than that in the group without disease. Significant difference was found regarding high-sensitivity C-reactive protein hs-CRP ($p=0.004$). The level of Urotensin II in subjects with gestational diabetes was (109 ± 33.22) highly increased than that in healthy subjects (78 ± 22.6). There is a positive correlation between circulating Urotensin II levels with fasting insulin, and HOMA-IR. While negative correlation found with fasting blood glucose.

Conclusion: The level of UII was found to be raised in gestational diabetes pregnant women

Keywords: Diabetes; insulin resistance; urotensin II.

Introduction

"Gestational diabetes mellitus (GDM), is a state of carbohydrate intolerance, first diagnosed during

pregnancy, it is a glucose metabolism disorder which can cause long & short term morbidities in both mother & fetus as difficulty in delivery of shoulder & preeclampsia.¹ It is one of the common complication

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that can occur during pregnancy, in which increase in blood sugar occur during pregnancy.² "GDM affects about 14% of pregnancies worldwide, representing nearly 18 million births every year".³

"The American Diabetes Association (ADA) formally categorizes Gestational diabetes mellitus as diabetes diagnosed in the second or third trimester of pregnancy that was not clearly overt diabetes prior to gestation."² "The exact threshold for a diagnosis of GDM, however, is dependent on the criteria utilized, and there has been no unanimity among health specialists thus far".⁴

The considerable decline in insulin sensitivity that occurs late in pregnancy reveals the lower insulin sensitivity that occurs before to pregnancy. Furthermore, abnormalities in insulin production have been linked to the degree of glucose intolerance throughout pregnancy. Beta-cell dysfunction persisted after birth and is linked to the severity of glucose intolerance throughout pregnancy. Interleukin 6 (IL6) and circulating tumor necrosis factor alpha (TNF α) have been found to be inversely associated to insulin sensitivity in people with GDM, proposing that inflammatory factors play a role in the disease's etiology. Other cytokines, like as leptin, have been reported to be elevated in people with GDM.⁵

The endogenous vasoconstrictor peptide urotensin II (UII) and its receptor (UTR) are both implicated in the etiology of essential hypertension. This human receptor G-protein receptor (GPR14) was then renamed urotensin II (UII) and its receptor recovered the interest in this field. "The U-II gene is found on chromosome 1p36U-II, and the length of the peptide differs between species due to distinct cleaving sites positioned at different places. The length of U-II in humans is 11 amino acids. The core is the peptide sequence required for biological function in both U-II and urotensin II-Related Peptide (URP). It's a hexa peptide (-CYS-TYR-LYS-TRP-PHE-CYS-) with a disulfide link connecting the two ends. Similarly, to URP, the amino terminus can be changed with keeping its pharmacological action, implying that it is not required for receptor activation. In contrast to URP, U-II contains an acidic amino acid (Aspartic or Glutamic) before the core sequence. While the amino acid isn't required for urotensin II receptor activation, its conservation across species proposes it has a biological purpose which has yet to be uncovered".⁶

UII is found in the pancreas that blocks insulin from being released. Increased UII plasma levels and expression have been found in a variety of diseases, including hypertension, atherosclerosis, pulmonary hypertension, heart failure, pregnancy kidney failure, diabetes, and the metabolic syndrome.

"Type 2 diabetes mellitus (T2DM), insulin resistance, and diabetic complications as carotid atherosclerosis and diabetic retinopathy have all been linked to polymorphisms in the UTS2 gene. The UTS2 gene regulates skeletal muscle fat storage and fatty acid metabolism, two biological processes linked to T2DM in humans."⁷

Insulin resistance is defined as a failure of insulin signaling which lead to inadequate plasma membrane translocation of glucose transporter 4 (GLUT4), the primary transporter which transports glucose inside the cell to be used as energy. In GDM, glucose absorption which is stimulated by insulin is reduced by 54% in comparison to normal pregnancy.⁸

Aim of the study:

our goal was to see if there is a link between Urotensin II levels and insulin resistance in pregnant patients who have gestational diabetes.

Patients and Methods

Study design and setting: A case-control study that carried out in department of Obstetrics and Gynecology at Baghdad Teaching Hospital from the first of January 2019 to the end of December 2019. A sample of 80 pregnant women participated in the study after fulfilling inclusion criteria.

Ethical consideration: Approval of the Obstetrics and Gynecology Department of Baghdad Teaching Hospital/Medical city.

Inclusion criteria: (40 with gestational DM and 40 without) which is diagnosed by two hours 75gm oral glucose tolerance test at 24-28-week gestational age which they have risk factors for doing OGTT as a NICE guideline criterion Exclusion criterion:

1. Patients have history of DM 1 or 2
2. If they have impaired glucose tolerance (IGT) before pregnancy,
3. Previous complication of pregnancy (HT, PE, IUGR...)

4. Twin or triple pregnancy
5. Taking medication that affect the level of glucose (steroid, anti-inflammatory drugs, carbohydrate metabolism-regulating obesity)
6. Previous medical history: CA, acute or chronic systematic disease, hypo or hyperthyroidism.

Consent taken from all participants, history was taken and physical examination, Blood pressure and obstetric examination were performed. last menstrual period and/or early ultrasonography were used to determine their gestational age.

Method

5 cc sample of blood was taken from the participants in the morning who were fasting for at least ten hours. Then separation of the sample was done by 15 minutes centrifuging of the blood at 2000x, before the analysis of UII the serum sample were kept in aliquots at - 80 °C. Urotensin II were measured via the ELISA technique by using Ray

Biotech ELISA Kits. "Homeostasis model assessment: insulin resistance (HOMA-IR): $HOMA-IR = \text{fasting insulin } (\mu\text{U/ml}) \times \text{fasting glucose (mg/dl)}/405$ "

Statistical analysis: After the data entered in a table, the analysis done by using the Statistical Package for Social Science (SPSS) program, version 23 and for qualitative variables, we used frequencies & percentages, and for the quantitative variables, we used measures of central tendency and dispersion (standard deviation). For the inferential statistics, the tests used of chi-square test (with a significance of $P \leq 0.05$)

Results

Eighty pregnant women between 24 and 28 weeks' gestational age were enrolled in the study, 40 patients were taken as a study group with diagnosed of GDM and 40 women as control group. The mean age of the GDM group was 29.8 ± 6.9 years & control was 29.7 ± 6.6 years. All these findings were shown in table 1.

Table 1: Demographic characteristics of the studied groups

Variable	GDM (Cases) (n=40)		Normal (control) (n=40)	
	Number	%	No.	%
Age				
<20 years	9	22.5	2	5.0
20-29 years	14	35.0	18	45.0
≥ 30 years	17	42.5	20	50.0
Age (mean \pm SD)	29.8 \pm 6.9		29.7 \pm 6.6	

Fisher exact test

In Table 2, there is highly significant difference were found between the studied group among parity ($p < 0.001$) and there was no significant association

between other demographic criteria found between control group and GDM group ($p > 0.05$).

Table 2: Comparison between demographic criteria of the studied groups (mean \pm SD),

	GDM group (n=40)	Control group (n=40)	P value
Age	29.8 \pm 6.9	29.7 \pm 6.6	0.9
GA	26.87 \pm 1.09	26.69 \pm 1.24	0.5
parity	3 \pm 1	2 \pm 1	<0.001
SBP	114.3 \pm 12.2	112.1 \pm 9.6	0.3
DBP	72.4 \pm 4.2	71.2 \pm 4.5	0.2

Table 3 showed highly significant increase in FBG, fasting Insulin, HOMA-IR, of the GDM group than that in control group (P<0.001).

Table 3: Relationship between fasting blood glucose, fasting insulin and IR among studied group. (mean±SD)

	GDM group (n = 40) Mean±SD	Control group (n=40) Mean±SD	P value
FBG	83.0±13.9	71.7±9.2	<0.001
Fasting Insulin	14.82±7.9	9.78±4.3	<0.001
HOMA-IR	3.10±2.05	1.82±0.99	<0.001

As shown in table 4, the level of Urotensin II in subjects with GDM was (109±33.22) and in healthy subjects was (78±22.6) there was a highly significant

association between mean of Urotensin II than the women withno GDM (p<0.001).

Table 4: Distribution of Urotensin II mean in the studied group

Variable	GDM	Normal	P
	Mean±SD	Mean±SD	
Urotensin II (ng/mL)	109±33.22	78±22.6	<0.001* ^S

*Independent sample t-test, S=Significant.

As shown in Table 5 the marker has high sensitivity and lesser specificity So the accuracy about 87.5%.

Table 5: Validity test

Cutoff value of Urotensin II	Sensitivity	Specificity	PPV	NPV	Accuracy
92 ng/ml	95%	80%	90%	82%	87.5%

In table 6 No relation was established between the Urotensin II & Age, lipid profile and creatinine in GDM patients, while significant correlation were found regarding the hs-CRP. The correlation analysis between serum Urotensin II and various parameters showed that there is a positive relation between circulating Urotensin II levels with fasting insulin, and HOMA-IR. While negative correlation found with fasting blood glucose.

Table 6: Statistical correlation between many variables in GDM group with Urotensin II

	Urotensin II	
	GDM	
	r	P
Age	0.17	0.5
Cholesterol	0.441	0.7
hs-CRP	0.717	0.04
low-density lipoprotein	0.0941	0.08
High-density lipoprotein	0.0805	0.9

	Urotensin II	
	GDM	
	r	P
Triglycerides	-0.051	0.7
Creatinine	0.234	0.07
FBG	-0.086	0.6
Insulin	0.73	<0.001
HOMA-IR	0.820	<0.001

“Pearson’s correlation analysis was used. r: Pearson’s correlation coefficient. A P value of < 0.05 was considered significant”.

Discussion

Numerous polymorphic areas of the UII gene producing the UII peptide, including the Rs.228648 polymorphism, have been identified and linked to a variety of disorders, including diabetes, diabetic retinopathy⁹, breast cancer,¹⁰ Behcet’s disease,¹¹ and systemic sclerosis.¹² Yumrutas et al. discovered that the “Thr21Met (143G>A, rs228648) polymorphism in

the UII gene was linked to the likelihood of developing breast cancer, and that the variant genotype was linked to lower UII plasma expression, suggesting a putative mechanism for UII participation in disease risk".¹⁰

"Gestational diabetes mellitus is a metabolic condition linked to insulin resistance", according to Desoye G.¹³ Until date, the basic biological processes underlying the reduction of insulin resistance in pregnant individuals with GDM were unknown. Despite the fact that a variety of chemicals are expected to prevent the signaling pathway of insulin, there is no evidence of an association between insulin resistance and UII in pregnant patient with GDM.^{14,15}

"UII is a multi-functional peptide that plays a key role in metabolism of glucose and the occurrence of insulin resistance", according to Gruson D et al., and levels of circulating UII were observed to be greater in patients who had metabolic syndrome than in the control group.¹⁶

The current investigation demonstrated a negative connection between FBG and UII levels, which is consistent with Totsune et al.'s work which reported no link between HgbA1C or FBG and UII levels.¹⁷ "In a research by Totsune et al., UII levels were observed to be higher in type 2 diabetics with and without proteinuria in comparison to healthy patients".

The current study shows a positive correlation between HOMA-IR and UII in GSD patients group. Which is same that in Yilmaz Ö, et al, study and Suguro T et al., study, which confirmed that there was a positive relation, were found between HOMA-IR and UII in a variety of disorders.^{18,19}

Pregnant women with high UII levels are more prone to develop GDM. These data imply that UII may contribute to the occurrence of insulin resistance in women with GDM. Surprisingly, a small number of investigators have looked at "the consequences of the UII genetic polymorphism on the development of T2DM.²⁰ Furthermore, as compared to controls, the genetic polymorphism of UII was shown to be greater in diabetic retinopathy patients.⁹ GDM is linked to low-grade chronic inflammation, which causes insulin resistance.²¹

According to Yilmaz et al.¹⁸, "there is a link between UII and inflammatory indicators. In the current investigation, we discovered that levels of

the circulating inflammatory marker hs-CRP were greater in pregnant women with GDM than in controls".

In addition, UII demonstrated a favorable correlation with hs-CRP. These findings imply that UII may play a role in the development of insulin resistance in pregnant women with GDM via an indirect effector. In patients with essential hypertension, the most powerful vasoconstrictor peptide, UII, was shown to be raised.

We discovered no link between lipid parameters and UII in this study, which contradicts findings from a small number of preclinical investigations that held the concept that there is a linkage between lipid imbalance & UII.^{22,23}

We also discovered that circulating levels of UII were greater in women with GDM than in controls in the current investigation. Insulin resistance was observed to be greater in pregnant women with GDM in comparison with controls. Furthermore, UII levels were positively correlated with HOMA-IR or insulin, but not with lipid profile or age. UII had an independent relationship with HOMA-IR. In his study, Hursitoglu M discovered that levels of UII were greater in women with GDM.⁶

In this study, patients with GDM had considerably greater levels of circulating U II than those without the condition (healthy group). "This is in line with a Turkish study published in 2019 by Calan M et al., which demonstrated that elevated UII levels are linked to insulin resistance in patients with GDM".¹

Conclusion

Level of UII was found to be raised in GDM pregnant women and independently related with insulin resistance

Recommendations:

1. Urotensin II can be used as biomarker for screening of GDM women.
2. Further investigations were required to identify the role of Urotensin in women with gestational diabetes.

Conflict of Interest: There is no any conflict of interest.

Source of Funding: Self-funded study

Ethical Clearance: Ethical approval was obtained the form the Baghdad Teaching Hospital

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Incidences of Minor Sexual Abuse in Sahibzada Ajit Singh (SAS) Nagar District, India: A Retrospective Study of 5 Years

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How to cite this article: Natasha Thakur. Incidences of Minor Sexual Abuse in Sahibzada Ajit Singh (SAS) Nagar District, India: A Retrospective Study of 5 Years. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):261-266.

Abstract

Background: The incidences of child sexual abuse are increasing nowadays due to lack of awareness, child neglect, misuse of social sites, family violence, and much more. The present study aims to determine the socio-demographic profile of past five-year cases of child sexual abuse of SAS Nagar district.

Materials and Methods: The study was conducted on 114 cases of child sexual abuse registered under the POCSO Act, 2012. The period chosen was from 1st January 2013 to 31st December 2017, located in the SAS Nagar District, Punjab. The data was collected through RTI from the SSP office of SAS Nagar. The details were pertaining to socio-demographic parameters (age, sex, marital status, occupation, and profession), relationship with accused, place of incidence, the time interval between the date of incidence and date of reporting case noted in a self-designed Performa.

Results: There were a maximum number of child sexual abuse cases in 2017, 34 followed by 33 cases in 2015. The cases of female sexual assaults (92.98%) outnumbered male sexual assaults (2.76%). The commonplace offense was the accuser's house (22.80%). The most vulnerable age group was 11-15 years (46.49%). 51.75% of the alleged sexual assault victims were students. Unmarried victims were 97.36%. Most commonly, sexual crimes were committed by the person known to the victim (89.48%). Maximum numbers of cases were reported on the 2nd day of incidence (35.08%).

Conclusion: Developing moral values and ethics among people in the transitional phase of cultural devaluation and organizing minor sexual assault prevention programs in ways that contribute to the community's own capacity to prevent this heinous crime is crucial as the biggest threat to the minors is not from the strangers but from the known and close ones.

Keywords: Child sexual abuse, Minor abuse, Child crime, Sexual crime, CSA

Introduction

The impact of abuse and neglect of a child can lead to developing criminal tendencies in them.

Discovering the factors involved in child abuse plays a crucial role in forensic proceedings of a sexual abuse allegation. Forensic interviewers base their

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interview style on their beliefs that an abused child will disclose, deny or decant or recant allegation, and these interview styles can be linked and used for an investigation.¹ Even a scientific study on Child sexual abuse involves legal, moral, and ethical issues, which are pretty challenging for researchers. Further, another challenge encountered in unreported cases. Researchers estimate that only about 10-15% of CSA cases ever come to the attention of authorities.²

In the modern era, the internet has changed people's lives and has played a significant role in sex crimes. The new technologies and cyberstalking provide new ways of harassment and intimidation of children. The NCRB data of the year 2016 has reported a sharp spike in cases of minors, even as overall crimes against minors have increased. Incidences of child rape have increased by over 82% compared to 2015, according to the NCRB data.³ Molestation is an atrocious offense that has been exercised globally since time immemorial. Child molestation happens in all cultural, ethnic, and income groups. Abusers can be neighbors, friends, and family members. People who sexually abuse children can be found in families, schools, recreation centers, youth sports leagues, and any other place children gather.⁴

In a country like India, with its conservative and traditional society, copulation is not discussed freely, and sexual offense; especially in childhood concealed for fear of the victim's future. In cases close to home, abusers are frequently defended. About 90% of children who are victims of sexual abuse know their abuser. Only 10% of sexually abused children are abused by a stranger. Approximately 30% of children are sexually abused by their family members. The younger the victim, the more likely it is that the abuser is a family member. 70% were family members who molest a child less than six years of age. Family members also assumed for 23% of those molesting minors of age group 12- 17. About 60% of children are sexually assaulted by those people whom family trusts.⁵ Physical debilities like deafness, blindness, and mental retardation have been found to be associated with an increased risk of being sexually abused. Lesbian, gay, bisexual, transgender (LGBT) is more prone to sexual abuse.⁶ Children belonging to the lower socio-economic status are at higher risk. The absence of one or both biological parents, marital conflicts, and/or parental substance abuse increases the vulnerability.⁷ Children under the influence of alcohol or/and drugs are more prone to abuse. It is

not possible to pinpoint peculiar characteristics that are common to all the abusers who abuse children. Pedophilic perpetrators usually start offending at an early age, and often have many victims (frequently not family members).⁸

India is home to 19% of the world's total children population. A total of 33,098 cases of sexual abuse in children were reported in the nation during the year 2011 when compared to 26,694 reported in the year 2010, which has increased by 24%. A total of 7,112 cases of child rape were reported during 2011 as equated to 5,484 in 2010, depicting a growth of 29.7%.⁹ It is estimated that over 7,200 children, including infants, are raped every year, and it is believed that several cases go unreported. A recent study by the government proposes that 40% of India's children are susceptible to threats like trafficking, molestation, homeless, forced prostitution, forced labor, drug abuse, and crime.¹⁰ A survey by United Nations International Children Education Fund (UNICEF) on demographics and health was conducted in India from 2005 to 2013, which reported that 10% of Indian girls might have experienced sexual violence when they were 10-14 years of age, and 30% during 15-19 years of age.¹¹ Minor sexual abuse is the root cause of many social and health issues. Children who have been sexually abused may exhibit a range of physical, psychological, emotional, and behavioral reactions, many of which are characteristic of children who have experienced other types of traumas.

Law and Order associated: POCSO

Until 2012, the only sexual offenses against children recognized by the law were covered by three sections of the Indian Penal Code.¹² Child sexual assault is serious abuse, a pervasive social issue, and POCSO Act is formulated to effectively address the heinous crimes of sexual abuse and sexual exploitations of children. The Protection of Children from Sexual Offences (POCSO) Act, 2012 deals with sexual offenses against persons below 18 years of age who are deemed as children. This act is a comprehensive law to protect children from the offenses of sexual assault, sexual harassment, and pornography while safeguarding the interests of the child at every stage of the judicial process by incorporating child-friendly mechanisms for reporting, recording of evidence, investigation, and speedy trial of offenses through the appointment of special public prosecutors and designated special courts.¹³

Materials and Methods

Study Area:

The study was conducted on 114 cases of child sexual abuse registered under the POCSO Act, 2012, chosen was from 1st January 2013 to 31st December 2017, located in the SAS Nagar District, Punjab.

Study Setting and Data Collection

Local newspapers such as Punjab Kesari, Dainik Bhaskar, Punjab Bani, Punjabi Tribune, Ajit, Punjabi Jagran, Charhdikala, and some Punjabi - E-magazines such as Sada Punjab, Punjab Today, Mehram, Punjabi Digest, and Akas were gathered and studied to collect the news covering child abuse cases. The child abuse cases of District S.A.S Nagar were collected from the SSP Office though an RTI. The police station of Sohana, Zirakpur and NayaGaon visited for more data of respective places.

Inclusion and Exclusion Criteria:

Cases of child sexual abuse of SAS Nagar District reported under POCSO Act, 2012 were taken from the past five years (Jan 2012- Dec 2017). Details of victims and abusers are not disclosed, and cases other than the sexual abuse of minors are not taken.

Data Analysis

The analysis carried on 114 cases of child sexual abuse registered under the POCSO Act, 2012, at police stations under the S.A.S Nagar district. The data is sorted year-wise and selected parameters such as age, occupation, relation with the abuser, etc., were studied and analyzed. It is worth mentioning here that all the data provided was under FIR. Some cases successfully reached the conviction of the abuser and some were acquitted. A few of them are still under processing. Each case is analyzed and compared on various given parameters to determine the reason behind the perpetration of crime.

Findings

Observation Tables:

Table 1: Distribution of child sexual abuse case population according to the Occupation from 2013-2017 in SAS Nagar District (Punjab), India.

S.No.	Occupation	Frequency	Percentage (%)
1.	Student	59	51.75%
2.	School Dropout	38	33.33%
3.	Employed	3	2.63%
4.	Domestic Worker	4	3.50%
5.	Baby	10	8.77%
Total		114	100.0

Table 2: Distribution of child sexual abuse case population according to the Relation with Abuser from 2013-2017 in SAS Nagar District (Punjab), India.

S. No.	Relation with Abuser	Frequency	Percentage (%)
1.	Neighbor	36	31.57%
2.	Known	27	23.68%
3.	Father	4	3.50%
4.	Relative (Family)	5	4.38%
5.	Friend	11	9.64%
6.	Lover	9	7.89%
7.	Stranger	12	10.52%
8.	Shopkeeper	3	2.63%
9.	Teacher	2	1.75%
10.	Landlord	3	2.63%

Contd... Table 2: Distribution of child sexual abuse case population according to the Relation with Abuser from 2013-2017 in SAS Nagar District (Punjab), India.

11.	Grandfather	1	0.87%
12.	Servant	1	0.87%
Total		114	100.0

Table 3: Distribution of child sexual abuse case population according to the Place of Incidence from 2013-2017 in SAS Nagar District (Punjab), India.

S. No.	Place of Incidence	Frequency	Percentage (%)
1.	Victim's Home	23	20.17%
2.	Abuser's Home	26	22.80%
3.	Forest	5	4.38%
4.	Fields	15	13.15%
5.	Cowshed	1	0.87%
6.	Nearby Home place	12	10.82%
7.	Empty School	8	7.01%
8.	Some Shop	7	6.14%
9.	Unknown Place	9	7.89%
10.	Another City	6	5.26%
11.	Railway Line	1	0.87%
12.	Vehicle	1	0.87%
Total		114	100.0

Table 4: Distribution of child sexual abuse case population according to the Time Interval between the Date of Incidence and Date of Reporting (FIR) from 2013-2017 in SAS Nagar District (Punjab), India.

S. No.	Time Interval	Frequency	Percentage (%)
1.	Same Day	13	11.40%
2.	Second Day	40	35.08%
3.	Third Day	15	13.15%
4.	Fourth Day	4	3.50%
5.	Up to 7 Days	7	6.14%
6.	Up to 15 Days	12	10.52%
7.	After 1 Month	14	12.28%
8.	Data Not Available	9	7.89%
Total		114	100.0

Table 5: Distribution of child sexual abuse case population according to the Judgment from year 2013-2017 in SAS Nagar District (Punjab), India.

S. No.	Judgement	Frequency	Percentage (%)
1.	Conviction	15	13.15%
2.	Acquittal	35	30.70%
3.	Untraceable	6	5.26%
4.	Appearance In Court	25	21.92%
5.	Dismiss In Court	10	8.77%
6.	Under Interrogation / Investigation	23	20.17%
Total		114	100.0

Discussion

The study carried out on 114 child sexual abuse cases reported under the POCSO Act, 2012; at the police stations comes under Sahibzada Ajit Singh Nagar District. Cases selected for the study all occurred from 1 Jan 2013 to 31 Dec 2017. The cases were segregated based on different parameters to extract observations and to draw a conclusion.

After studying all the cases; based on parameters selected, it has been observed that out of 114 cases, the maximum number of child sexual abuse cases occurred in the year 2017 with (22.80%), 2014 with 14 in number (12.28%) and 2013 with 07 in number (6.14%).

Study: Based on Age group and Gender:

The distribution of child sexual abuse case population over age was done in 5 years gap. Out of 114 cases, maximum incidences of sexual abuse were found at the age group of 11-15 years with 53 in number (46.49%), which is followed by 30 cases in the age group of 16-18 years (26.31%), 20 cases in the age group of 6-10 years (17.54%), and least number of cases were found in the age group of 0- 5 years with 11 in number (9.64%). The female child number retrieved is 106 (92.98%), and the male child number recorded is 4 (3.50%). 4 cases fall in the category of not mentioning gender.

The leading causes behind the sexual abuse observed were poor socio-economic status, money crisis, family violence, neglect of parents towards their offspring, alcoholism and substance abuse, lack of education and awareness, and love affair. Heterosexual cases are more prominent than homosexuals. It is observed that misuse of social sites plays a significant role in the sexual abuse crime, as in many cases, the abuser gained the trust and get closed to the victim through the chatting sites and lately abused the victim sexually.

Based on Marital Status and Religion:

The majority of sexual abuse was observed among the unmarried population with 111 cases in number (97.36%), as compared to the married ones with 3 in number (2.63%). In the case of religion status, Hindu children were molested more with 66 (57.89%) out of 114 cases, followed by Sikhs with 32 cases (28.07%), Muslims with 12 cases (10.52%). In 4 cases, religion was not mentioned (Table 4). The leading causes observed were poverty, love affair, lack of

education and awareness, battering, substance abuse and alcoholism, etc.

Based on Occupation:

It has been observed that out of 114 cases of child sexual abuse cases; most were amongst the students (59) 51.75%, 38 cases were amongst school dropouts (33.33%), 10 cases were seen among babies 98.77%), 4 cases were among domestic workers (3.50%), and 3 cases were among employed children (2.63%) (Table 1).

Based on Relation with Abuser:

In 102 cases, the victim or/and victim's family already knew the abuser. The abuser was neighbor of the victim in 36 cases (31.57%), known in 27 cases (23.68%), a friend in 11 cases (9.64%), lover in 9 cases (7.89%), a family member in 5 cases (4.38%), father in 4 (3.50%), grandfather in 1 case (0.87%), followed by 1 more case (0.87%) in which the abuser was the servant. (Table 2). The main causes observed were alcoholism and substance abuse, family violence, lack of awareness, money crisis, personal grudges, love affairs, etc.

Based on Place of Incidence:

The maximum incidences were perpetrated in the abuser's home with 26 in number (22.80%), followed by 23 (20.17%) in the victim's home, 15 cases (13.15%) in fields, 12 cases (10.52%) taken place nearby victim's home, 9 cases (7.89%) at unknown places, 8 cases (7.01%) in an empty school, 7 cases (6.14%) at some shop, 6 cases (5.26%) in another city, and 1 case (0.87%) in a cowshed. (Table 3).

Based on Time Interval between Date of Incidence and Date of Reporting (FIR):

The study shows that out of 114 cases, 13 cases (11.40%) reported on the same day of incidence, 40 cases (35.08%) on the second day, 15 cases (13.15%) on the third day, 4 cases (3.50%) on the fourth day, 7 cases (6.14%) reported up to 7 days, 12 cases (10.52%) were up to 15 days, 14 cases (12.28%) reported after one month and in 9 cases (7.89%) the date of incidence was not mentioned. (Table 4).

On the basis of Judgment by Court:

Maximum number of reported cases were got acquittal by the court with 35 in number (30.70%), 25 cases (21.92%) judged as appearing in court, 23 cases (20.17%) were under interrogation, the court got 15 cases (13.15%) conviction, 10 cases (8.77%)

dismissed in court, 6 cases (5.26%) were untraceable. (Table 5). The figures of convictions were deficient.

Conclusion

The main reasons for the abuse are lack of education, alcoholism and substance abuse, lack of awareness, poverty, neglect of parents towards their offspring, love affair, money crisis, personal grudges, misuse of social sites, family violence, and child battering, etc. Child sexual assault is a beastly crime and no age group can be considered safe, though young girls and adolescents are at a greater risk. Hence, the POCSO Act, 2012, made a symbolic contribution to dealing with India's child sexual assault cases. An increase in the numbers of reported cases shows that law is vital in educating the people and sharpening the criminal justice system.

The Government and Non-Governmental organizations (NGOs) should promote awareness and widespread educational programs to educate the general public about child sexual abuse. Violence and minor sexual assault prevention programs should be organized in ways that contribute to the community's capacity to prevent this heinous crime.

Parents should talk openly and listen calmly to their children about all the matters as children have a hard time telling parents about troubling issues. The development of moral values and ethics among people in the transitional phase of cultural devaluation is a crucial step to take as the study has revealed that the biggest threat to minors is not from strangers but the known and close ones. Finally, healthy facets of social control, social resources, social coherence, and social supports are salient to capacity building and collective efficacy, all of which have an optimistic impression on the well-being of children and youth.

Conflict of Interest: The author declares that there is no conflict of interest.

Funding: This study received no funds from any agency in the public, commercial, or not-for-profit sectors.

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Deaden talks through Bones about their Final Fate: A Case Report

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How to cite this article: Naveen Sharma, Kuldeep Kumar, Priti Singh et al. Deaden talks through Bones about their Final Fate: A Case Report. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):267-269.

Abstract

Background: Skeletonization of body is a complex process. Solving a suspected crime is multidisciplinary approach, which requires experts from the various specialities. Various methods are employed to commit crimes throughout the world. Many cases of homicide remain unsolved due to misleading, lack of suspicion, incomplete or inadequate investigations.

Methods: In present case post mortem examination of a skeletonised body was conducted by the authors, identification and apparent cause of which after inquest was impossible for investigating agencies. Usually, it is difficult to pinpoint cause, manner and time since death with certainty in the such corpse when body devoid of soft tissue. In present case authors opined the cause, manner and time since death in a skeletonised and partially burnt body. Which will help the investigators to find out the perpetrator.

Conclusion: A post mortem examination of a corpse by the experts is mandatory for the identification, collection and preservation of trace evidences to correlate the victim, accused, scene of incidence and suspected weapon of offence.

Keywords: Skeletal remains; cause of death; identification and charring.

Introduction

Incidence of crimes are increasing throughout the world in a modern era.¹ A critical phase of the death investigation will be a preliminary reconstruction of events that preceded the onset of death.² In India, an unconfined buried body is reduced to a skeleton

within about a year.³ Decomposition rate shows geographical variations. In hot climates, bones on the ground surface may decay in 5 to 10 years.³ Only a careful and detailed analysis of human remains allows collection of all the data necessary to define the biological profile for identification and to establish the cause of death.³ When the object of forensic

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investigation is constituted by skeletal remains, obtaining information is more difficult because of the absence of soft tissues because many injuries that involves only soft tissues without involving bones. Human bones are resistant to environmental insult and made with organic and inorganic components. The cause of death from the bones could be made with certainty in the cases like burns, deep cuts, fracture of the bones, metallic poisons can be detected in bones even after burning.⁴ After about 25, ageing becomes much more difficult as all bone growth has ceased. Despite the fact that changes do still occur in the skeleton, none is of practical use. Additional data can be obtained by radiology of the trabecular pattern in the head of the humerus and femur, which re-models with age.⁵ If all soft tissues are absent, identity depends solely on osteological examination and measurements and the recognition of any pathological or anatomical abnormalities in bone.⁶

Case Report

Authors reporting a case of homicide, which was committed in 2019, where the body was burnt after committing the crime in order to obscure the corpus delicti. A corpse was brought to the authors for post mortem examination and expert opinion regarding the identification, cause, manner and time since death. The human body, almost skeletonized, was recovered from sidewalk of highway in suspicious circumstances.



Figure 1: The skeleton gives information about the circumstances of death and the body's position.

The police was unable to find out the clear apparent cause of death after their inquest and

thorough investigation. On examination, remnants of soft tissue which were adhered firmly and few fibres of remnants muscles were found black and burnt off at places over the bones without any sign of vital reaction.



Figure 2: Bones and body parts least protected by soft tissues.

Skull and face were devoid of skin and soft tissue and underlying bones were visible. Anterior wall of the trunk was missing along with organs of thoracic and abdomen cavity. Mandible, anterior ends of the ribs, right iliac crest and right tibia and fibula bone were burnt off at places and rest were showed gnawing effects. Intercostal muscles showed greenish discoloration as depicted in.



Figure 3: Evidence of charring especially at mandible bone.

On meticulous examination, a depressed comminuted fracture of size 15 x 12 cm was present over the right fronto-parieto-temporal region of skull with pieces of bones impinging the underlying dura matter and brain matter.



Figure 4: Depressed comminuted fracture of skull bones

On exploration, a linear fracture of length 8 cm present horizontally over the left parietal and left frontal bones of the skull situated 8 cm left to midline and 11.5 cm above to left external auditory canal as seen in Figure 5: Linear radiating fracture over right of skull bones.

On opening the skull vault, extradural haematoma without any cherry red or bright red discoloration was appreciable over bilateral temporal region. The hematoma was friable, loosely present over the dura mater suggestive of heat hematoma. The subdural haemorrhage and subarachnoid haemorrhage were present over cerebral hemisphere of the brain. On further dissection and exploration, right frontal and parieto-temporal lobes of the brain were found contused. Facial bones were deformed and found fractured. On exploration, nasal and left zygomatic bones were found fractured through and through at places. All the fractured ends of bones showed infiltration of blood in their bony trabeculae. After the post mortem examination, opinion was formulated after reconstructing the sequence of events and scene of recovery of corpse "it was the dead body of a young adult male individual. The cause of death was cranio-cerebro-facial injuries and their complications which are antemortem in nature, recent in duration, homicidal in manner, caused by hard blunt object and sufficient to cause death in ordinary course of nature. The burns which were found over the remnants of soft tissues adhered to the skeleton were post mortem in nature. The probable duration between death and post mortem examination was opined to be about 3 days.

Discussion

The challenge of a forensic evaluation of burned skeletal remains is not only a methodological one. The fire in fact erases much of the evidence, if not even the entirety of that, when burning is complete.⁷ Most murders remain unsolved due to many reasons like advanced decomposition, skeletonization, inadequate or partial investigation, medicolegal examination by unexperienced hands and unestablished offence, disturbances at the scene of crime and evidences surrounding the deaths. Homicide is one of the crimes by taking one's life. It is usual crime in anywhere but when murder unrevealed and to be continued will

become crime extraordinary. Many such types of bodies were examined by Ali RızaTümer et al. and they found postmortem burning of corpses to cover homicide.⁸

Conclusion

From the above case report and discussion, it is crystal clear that the role of forensic experts become more pivotal, responsible and prudent to solve the crime mystery in cases where the main investigating agencies failed to do so. In this contemporary era of technology, crime become more advanced in term of its methods, and it can only be prevented by the same ways, i.e., field investigations, proper history, meticulous and complete autopsy, reconstruction of scene as well as dead body and their simulation in respect to manner and infliction.

Acknowledgment of Funding Sources: NA

Disclosure and Conflicts of Interest: NA

Ethical clearance: This data is taken from the post mortem examination which was conducted by the authors so no ethical clearance is required in this case.

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Esthetic and Prosthetic Rehabilitation of Maxillary Lateral Incisor: A Case Report

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How to cite this article: Neetha J Shetty, Sweta Pradhan. Esthetic and Prosthetic Rehabilitation of Maxillary Lateral Incisor: A Case Report. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):270-274.

Abstract

Introduction: The maxillary anterior region poses the highest number of aesthetic challenges in implant dentistry as any tooth loss in the region leads to bone resorption and collapse of gingival architecture leaving inadequate bone for implant placement. However, immediate implant placement and provisionalization has been a viable option for replacing failing maxillary anterior teeth as it preserves the vertical existing osseous and gingival architecture. A proper case selection guided by an appropriate step-by-step treatment plan can ensure the success of any immediate implant placement.

Case Summary: This case report describes atraumatic extraction of a fractured right maxillary lateral incisor, followed by immediate placement of a dental implant in the prepared socket. Implant was successfully loaded and was functional during 24 months follow up period. The patient exhibited no clinical or radiologic complications post operatively. Recall visits exhibited patient's high-level confidence of smile with optimum satisfaction and successful aesthetic outcome.

Conclusion: Immediate implant placement into fresh extraction socket reduces the treatment time, cost, preserves the gingival aesthetic and increases the comfort of the patient.

Keywords: 1. Atraumatic Extraction; 2. Immediate Implants; 3. Maxillary lateral incisor.

Background

Implant dentistry has progressed considerably since its introduction, with modifications and enhancements being made to the surgical techniques, restorative protocols, and the implants themselves.

Traditional guidelines as suggested, that following tooth extraction, 2 to 3 months of alveolar

ridge remodeling occurs. If there is loss of the buccal alveolar plate following tooth extraction which mostly is seen in cases of maxillary anteriors, may lead to palatal implant positioning with esthetic complication. The initial delayed loading and placement protocols have been modified to expedite the treatment process. These modified protocols (immediate placement and immediate loading) have

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been successfully applied in both fully and partially edentulous patients. Immediate implants have become widely accepted and the available literature consistently cites high levels of success (ranging from 94-100% on average), immediate implants provide clinically recognizable benefits. Broadly speaking, these benefits include reduction of morbidity, reduction of alveolar bone resorption, preservation of gingival tissues, preservation of the papilla in the esthetic zone, and reduction of treatment cost and time. However, it is challenging to achieve esthetic results with anterior teeth having soft and hard tissue discrepancies.

This case report illustrates a therapeutic treatment modality consisting of atraumatic extraction followed by immediate implant placement and provisionalization with 24 months follow up.

Case presentation

In 2017, a healthy 25-year-old male reported for evaluation of a fractured upper right lateral incisor (Figure 1). Patient was in good systemic as well as periodontal health. On extra oral and intra oral examination, there was no abnormality detected. Clinical examination revealed fractured tooth w.r.t 12.



Figure 1: PRE-OP Picture

The patient was referred to the Department of Endodontics for further evaluation and development of a treatment plan.

On consultation with an endodontist, it was decided to extract the tooth and go for an immediate implant placement as the patient was very much concerned about esthetics.

Investigations

- Complete hemogram was performed to rule out any systemic problems

- CBCT of the area of interest was done
- IOPA of the area of interest was taken

A thorough clinical evaluation and a CBCT was recorded (Figure 2) and analyzed, after which the patient was posted for implant placement as he did not have any facial plate deformity. The patient was explained about the procedure and a consent form was duly signed by the patient.



Figure 2: Cone beam computed tomography (CBCT) was done to rule to facial bone deformities.

Surgical Procedure

The area of interest was anaesthetized with 1:80,000 lignocaine hydrochloride. With a no. 15 scalpel blade, the supracrestal fibers were dissected. This was followed by the use of a periosteal elevator to sever the periodontal ligament fibers from the alveolar bone so that the tooth becomes loose, thereby extracting the tooth atraumatically. The walls of the alveolar socket were found to be intact after extraction (Figure 3). The socket was degranulated and curetted for any remnant of periapical lesion. Osteotomy was performed in a palatal position so as to not perforate the labial cortical bone, about 2mm apical to the apex (Figure 4). An implant of length 13mm and diameter 4.5mm

was placed and primary stability was achieved with a torque of 20Ncm/2 was achieved. Since the torque achieved was less, it was decided to go for delayed loading and a gingival former was placed (Figure 5).

Post-operative instructions were given.



Figure 3: Extraction of upper right lateral incisor was done as atraumatically as possible.



Figure 4: Osteotomy done in the deepest part of the socket with care not to perforate the labial cortical bone.



Figure 5: After Implant Placement

Post-operative instructions

Post-op instructions were given to the patient. Antibiotics (Cap Amoxicillin 200mg, thrice daily for 5 days) and analgesics (Tab Divon plus twice daily for two days) were prescribed.

Outcome and follow-up

After 5 months, an IOPA was taken to evaluate the implant and bone interface. The gingival former was removed revealing a good soft tissue contour. Impressions were taken for prosthetic rehabilitation using putty with light body for the maxillary arch and an alginate impression for the mandibular arch. The final ceramic crown was cemented after 1 week. After 1 year, radiographic and clinical follow up demonstrated successful result, meeting aesthetic and functional requirements (Figure 6 and 7).



Figure 6: Pre-treatment and Post-treatment IOPAR



Figure 7: After complete rehabilitation

Discussion

Atraumatic extraction is the mandatory requirement when placing an immediate implant. The best healing conditions is achieved by attempting it atraumatically.¹ Despite all the efforts, extracting a tooth results in severing the collagen fibers and blood vessels to the periodontal ligament causing trauma to the bundle bone. Therefore, one more important surgical consideration is flap elevation or without flap

elevation. Covani et al affirmed that flap elevation may cause alveolar bone resorption in the exposed area, whereas a flapless technique reduces patient discomfort, alterations in alveolar crest dimensional alterations, and better soft tissue quality around implant.²This also reduces post-surgical trauma, and by preserving the vascular supply integrity, as the periosteum is maintained.³The stages of alveolar healing are initiated as soon as the tooth is extracted.⁴ It has been postulated that there is a horizontal resorption of bone dimension that amounts to 56% immediately after extraction.^{5,6}

Also, when an implant is inserted immediately after an extraction, there is a void created between the buccal wall and the implant. Some authors suggest the filling of this void with a biomaterial in order to maintain hard tissue contour.⁷The clinician also has to consider that soft tissue will also go through the process of remodeling following implant placement.⁸

Immediate implants require a complicated and precise soft tissue management.

The maxillary anterior region poses the highest amount of aesthetic challenges in implant dentistry as any tooth loss in the region leads to bone resorption and collapse of gingival architecture leaving inadequate bone for implant placement.

Many authors demonstrated that using single immediate implants with instant provisionalization, can help optimize esthetics. It was concluded that this can limit the amount of midfacial soft tissue loss, being this area the most critical in aesthetic implant dentistry.^{9,10} Nevertheless, if the primary stability is not achieved, or the patient's case does not fit the ideal requirements for immediate provisionalization, this should not be done, and therefore, a different type of treatment should be considered.¹¹ However, immediate implant placement and provisionalization has been a viable option for replacing failing maxillary anterior teeth as it preserves the vertical existing osseous and gingival architecture.

Conclusion

Based on the outcomes of the present report, it can be concluded that immediate implant placement may be a viable treatment option for cases requiring earliest restoration of teeth to be extracted. However, this approach is considered highly technique sensitive and requires expert dental implant team

for its execution. Careful selection of cases, proper treatment plan and follow-up of surgical and prosthetic protocols are the keys to success.

Patient's perspective

Patient was very satisfied with the esthetic and functional outcome after the prosthesis on the implant.

Acknowledgements: None

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

Ethics Statement: The procedure has been assessed by the Institute Ethical committee, MCOOS, Mangalore and is in accordance with the Declaration of Helsinki.

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Metformin Versus Insulin in the Management of Gestational Diabetes Mellitus

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How to cite this article: Nuha Muhsen, Sajda Al-Rubai, Huda Qahtan. Metformin Versus Insulin in the Management of Gestational Diabetes Mellitus. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):275-279.

Abstract

Background: Insulin and metformin have been used extensively in the management of gestational diabetes mellitus (GDM). Insulin has been the primary medical treatment if maternal glucose targets are not achieved by dietary therapy. Insulin is safe for the fetus, because it does not normally cross the placenta. oral antidiabetic agents, glibenclamide and metformin are the most studied agents to treat GDM patients.

Objective: To examine If oral metformin is as effective as insulin in the prevention of fetal macrosomy in pregnancies complicated with gestational diabetes mellitus.

Method: This study is an open -labeled prospective randomized controlled study that was carried out in Basra maternity-outpatient clinics in the tertiary level hospital in Basra. One hundred women with GDM who did not attain euglycaemia with diet participated. Women were randomized to therapy with insulin n= 50 or oral metformin n=50. Incidence of macrosomia in infants and neonatal morbidity was measured.

Results: There were no statistically significant differences in the incidence of macrosomia (16% versus 20%) , and neonatal morbidity between insulin and metformin group. Around 15 (30%) of the metformin treated women needed supplemental insulin. They were more obese,(36.2 versus 30.6) kg/m² had higher fasting blood glucose level (7.4mmol/L versus 6.1 mmol/L) and needed medical treatment for GDM earlier (27 versus 32 wks) than women who were normoglycemic with metformin alone. There was a tendency to a higher rate of caesarean sections in the metformin group than in the insulin group.

Conclusion: Metformin seems to be suitable for the prevention of fetal macrosomia , especially in lean or moderately overweight women developing GDM in late pregnancy Women with considerable obesity, high fasting blood glucose and an early need for pharmacological treatment may be more suitable for insulin therapy.

Keywords: Metformin; Insulin; Gestational Diabetes.

Introduction

Basal and postprandial glucose metabolism is altered in pregnancy. During pregnancy eating causes

stronger insulin secretion, but postprandial glucose concentrations are still higher than in non-pregnant individuals. Although fasting glucose is decreased,

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basal hepatic glucose production is increased, because hepatic insulin sensitivity and glucose suppression are reduced. This, in turn, leads to increased insulin production. Gestational diabetes mellitus (GDM) is classically defined as "a state of impaired glucose tolerance recognized during pregnancy in women not known to have had impaired glucose tolerance before pregnancy."¹

Fasting glucose concentrations are higher in pregnancies complicated by GDM than in normal pregnancies, while basal hepatic glucose production is similar. Insulin sensitivity is lower in pregnancies of lean and obese GDM patients compared with normal pregnancies. Insulin resistance is increased by 40% in late pregnancy in patients with severe GDM compared with normal pregnancies.² GDM occurs when the pancreatic b-cells do not produce enough insulin to combat the increased insulin resistance. Obesity and chronic insulin resistance are the most common factors that predispose to b-cell dysfunction during pregnancy.

Insulin has been the primary medical treatment if maternal glucose targets are not achieved by dietary therapy. Insulin has several disadvantages since its use needs training, it is administered by subcutaneous injections, it can cause hypoglycemia and it increases appetite and weight³ However, oral antidiabetic agents, glibenclamide and metformin are the most studied agents to treat GDM patients. A meta-analysis of 6 studies with 395 GDM patients on metformin, 291 on glibenclamide and 702 on insulin reported no differences between the groups in terms of maternal fasting and postprandial glycemic control. The use of metformin or glibenclamide compared to insulin did not increase the rate of neonatal hypoglycemia, birth weight, incidence of LGA-babies or cesarean deliveries.⁴

According to the literatures, identification of high risk group (GDM) women and offering them oral metformin treatment could improve both the morbidity and mortality for the pregnant women and their fetuses in our community because most of our diabetic pregnant mothers are relactating for using subcutaneous insulin injections during their pregnancies. So our goal or our aim of the study was to investigate the efficacy of metformin in the prevention of fetal macrosomy and its influence on neonatal and maternal morbidity in women with GDM in comparison with insulin therapy.

Methodology

A target number of 100 women with GDM was obtained in the study from Basra maternity and children hospital. The gestational diabetes was diagnosed by measuring the concentration of serum blood glucose before breakfast and 1.5 hour of the main meals. The target concentration was < 5.3 mmol/L for fasting and > 6.7 mmol/L for postprandial glucose. The women with singleton pregnancies diagnosed with (GDM) between 12:34 week of gestation were asked to participate. The study were randomized to treatment with either metformin (n=50)or insulin (n=50).

Randomizations was achieved using numbered selected envelopes containing a randomization on e-generated manually in blocks of ten. Exclusion criteria were pre-eclampsia, essential hypertension requiring antihypertensive drugs. Metformin 850mg once daily for the first week, twice daily for the second week and three times daily from the third week onward. Medication was discontinued if significant side effect achieved or if normoglycemia was not achieved within 1-2 weeks and supplement. Insulin was added. While in second group, along acting insulin was used to normalize fasting and rapid-acting was used to normalize postprandial glucose concentration.

The women continue to measure the daily profiles of capillary glucose concentration twice a week. The women were followed at the outpatient maternity clinics of the hospital at 4 weeks intervals between 12-32 weeks of gestation, at 2 weeks intervals between 32-36 weeks of gestation and once or twice weekly after 36 weeks of gestation. The primary outcome was the incidence of macrosomia, and secondary outcome included neonatal complication such as admission to the neonatal intensive care unit, neonatal hypoglycemia requiring intravenous glucose treatment, hyperbillirubinaemia treated with phototherapy and birth injuries, the Apgar score in 1 and 5 minutes were recorded .

Maternal outcome included a need for supplemental insulin in the metformin group, incidence of premature delivery before 37 weeks of gestation, hypertensive complication of pregnancy, weight gain during pregnancy and mode of delivery. The significance of the difference between the groups studied was assessed by Chi-square test and t - tests as appropriate, statistical significant was defined as $p < 0.05$, $p < 0.01$, $p < 0.001$.

Result

During the study period, 239 women were referred to the out patient clinics of the study hospital for the consideration of pharmacological treatment for GDM, 128 were eligible for the study and follow up till birth and only 100 of them can be followed and agreed to participate and were randomized in two equal group~ each of 50 patient. Table 1 shows the maternal baseline characteristics. The mean gestational age at delivery did not differ between the study group. There were no significant differences in the mean birth weight of the newborn, or in the macrosomia and neonatal complications as shown in Table 2.

There were no perinatal deaths in this trial, also

the incidence of pregnancy complications did not differ between the two study groups, five women in both study groups had mild pre-eclampsia, while the incidence of caesarean section were significantly higher in the metformin group compared with the insulin group (P=0.04) Table 2, Around 15 out of 50 (30%) women randomized to metformin therapy did not reach normoglycemia and needed supplemental insulin. After starting supplemental insulin three of the women discontinued metformin because of its gastrointestinal side effects. The women needing supplemental insulin had greater BMI, high fasting glucose concentration and needed pharmacological treatment of earlier gestational age than women who were normoglycemic with metformin (Table 3).

Table 1: Maternal Baseline characteristics

Character	N=50	N=50	P
Age in year	33.1 ± 5.1	33.6 ± 5.4	N.S
Parity	2.4 ± 1.2	2.1 ± 1.8	N.S
Nulliparous	16(32.0)	18(36)	N.S
BMI at the first antenatal visit	30.8 ± 1.2	32.2 ± 6.5	N.S
Fasting glucose serum level mmol/L	5.7 ± 0.6	6.2 ± 0.9	N.S
Length of gestational enrollment-WK	30.1 ± 3.2	30.2 ± 3.3	N.S
Education No%	6(122)%	5(10)%	N.S
HBA1c% at randomization	5.8±0.2	5.9±0.5	N.S

Data or means ± SDs or n (%)

Table 2: Neonatal Data and the Mode of Delivery

Character	N=50	N=50	P
Gestational age at delivery in WK	39.3 ± 1.1	38.4 ± 1.6	N.S
Macrosomia	8(16)%	10(20)%	N.S
Apgar score at			
IM)	7.2 ± 0.3	7.6 ± 0.7	N.S
SM)	8.9 ± 0.7	9.0 ± 0.6	N.S
Neonatal transferral to NICU	11(22)%	8(16)%	N.S
Neonatal hypoglycemia	8(16)%	6(12)%	N.S
Neonatal hyperbillirubinaemia	16(32)%	14(28)%	N.S
Spontaneous V.O	21(42)%	34(68)%	(S)
Labour induction	25(50)%	22(44)%	N.S
c.s	11(22)%	19(38)%	0.001

Data or mean ± SDs or n (%)

Table 3: The Baseline Characteristics and Neonatal Outcomes in the Metformin Group

Character	M alone=35	M+I=15	P
BMI at the first antinatal visit	30.6 ± 1.4	36.2 ± 3.4	0.002
Fasting glucose serum level	6.1 ± 0.5	7.4 ± 1.2	0.001
G-estational age at randomization	32 ± 3.4	27 ± 6.2	0,001
Birth weight (g)ms	3923 ± 412	4179 ± 600	N.S
Macrosomic	8(16)%	10(20)%	N.S
Apgar score at			
1M	7.4 + 1.2	7.6 + 0.9	N.S
SM	9.3 + 0.7	9.1 + 1.2	N.S

Date or mean ± SD or n (%)

Relative risk in the metformin with supplemental insulin group compared with the metformin - only group.

Discussion

The prevalence of GDM has considerably increased in recent years. At the same time, the number of patients for whom lifestyle modification alone failed in achieving adequate postprandial glucose targets and, therefore, requiring drug therapy for GDM, also rose.⁵ This development awakens interest in gaining more information on antidiabetic drug therapy in pregnancy, which was the focus of the present study. Glucose levels directly influence maternal and neonatal outcomes and even glucose values lower than normally diagnostic for diabetes seem to have an adverse effect, such that strict glycemic control is necessary. Several studies have been conducted comparing efficiency and tolerability of different antidiabetic agents, such as insulin and metformin.

Historically, insulin was used for GDM as it does not cross the placenta (from maternal to fetal circulation)⁶, and due to the fact that only limited data on oral antidiabetics were available. Insulin was the most often prescribed agent in GDM. However insulin holds several disadvantages, such as the requirement of intensive educational instruction at the beginning of therapy, its subcutaneous application, the necessity of ideal storage conditions, close and frequent stringent blood glucose monitoring as well as the fact that it is much costlier than oral metformin, therefore, patients prefer metformin to insulin⁷, moreover oral metformin have a good compatibility

with pregnancy, for example, in various studies the metformin discontinuation rate was found to range between just 2 and 6% and this was mainly due to intolerable gastrointestinal side effect.^{5,7,8}

Our study was randomized controlled study showed that metformin is a safe and clinically relevant medical alternative for treating GDM, the incidence of adverse pregnancy or neonate outcomes was not increased with metformin compared with women treated with insulin. Metformin was found to be especially suitable for lean and moderately overweight women with postprandial hyperglycemia in the later half of pregnancy. The mean birth weight of the newborns did not differ significantly between the metformin and insulin groups, which is in line with both earlier cohort studies and a prospective study (MIG trail)⁹, in our study the incidence of birth weight over 4000GM was 20% in the metformin and 16% in insulin group which was less than the figures of 26.8% in study in Finland 2008.¹⁰

In our study the frequency of neonatal hypoglycemia, neonatal hyperbilirubinaemia and the need for treatment in NICU was slightly but not significantly higher in the insulin group, which is in line with one but not all previous studies in the MIG trial which found that the incidence of severe neonatal hypoglycemia was significantly higher in the insulin treated group than in the metformin treated group.¹¹ Although metformin crosses the placenta leading to concentration similar to those present in maternal circulation, it neither increase the rate of congenital malformations nor harms fetal or neonatal growth, however it has advantages such as significantly lower incidence of neonatal hypoglycemia and maternal pre-eclampsia and fewer neonatal admissions to the NICU than in the insulin group.¹²

Other benefits appear to include a reduced rate of macrosomia which is probably responsible for a reduction in the number of cesarean deliveries. While in our study the cesarean section rate (38%) was more than that in the insulin group, and it may be attributed to increase incidence of macrosomia. In this study 30% of the women on metformin required additional insulin to reach normoglycemia. In the MIG trial¹², the proportion of the metformin treated women requiring supplemental insulin was even higher 46.3% by contrast, in the two retrospective studies only 18 and 13% of the women needed additional insulin¹³, this may have resulted from patient selection.

Women with a need for supplement insulin in our study were more obese, had higher fasting blood glucose concentrations and needed medical treatment earlier than women who reached normoglycemia with metformin suggesting that they exhibited a more severe insulin resistance. They also needed higher insulin doses to reach normoglycemia than the women in the insulin group. Newborns in the supplemental insulin group had significantly higher mean birth weight when compared with the metformin only group, but the differences did not reach statistical significance probably because of the small sample size. It is possible that women needing supplemental insulin had more severe disturbance in their glucose metabolism.

The glycemic level in those women was also unsatisfactory over a longer period, which is supposed to accelerate the growth of the fetus before reaching normoglycemia. So in summary, our study was the first that confirmed in Basra that GDM treated with /' metformin can be safe and effective alternative to insulin and that it is especially suitable for women with mild GDM. In cases with severe disease determined by early diagnosis, fasting hyperglycemia and significant obesity promptly initiated insulin treatment seems to be a more optimal choice. Although it will be the task of future follow up studies to assess the possible differences in childhood between children exposed to metformin and those exposed to insulin in utero, which might lead to further changes in prescription manner in GDM.

Ethical clearance: Taken from Basrah Teaching Hospital, Basrah, Iraq

Source of funding: Self

Conflict of Interest: None

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Role of Calcium Supplementation on Pregnancy Induced Hypertension Outcomes

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How to cite this article: Ola Hussein Jasim, Waleed Ibraheem Ali, Alaa Moyasser Sadiq. Role of Calcium Supplementation on Pregnancy Induced Hypertension Outcomes. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):180-286.

Abstract

Background: Pregnancy induced hypertension (PIH), is an important cause of both maternal and perinatal morbidity and mortality. Low dietary calcium (Ca) intake represents a factor associated with an increased incidence of hypertensive disease in general and in pregnant population in most low and middle income countries. Ca supplementation has the potential to reduce adverse gestational outcomes of PIH.

Aim: To assess the effects of low-dose Ca supplementation on PIH outcomes.

Method: A single- blind, randomized controlled trial was conducted in January through March, 2021. A total number of 66 pregnant women with PIH at their 20th week of gestation attended maternal care outpatient in Baghdad Medical City were assigned randomly in two groups those received Ca supplementation 500 mg/day vs none and follow up both groups till delivery for outcomes.

Results: A significant reduction in diastolic blood pressure mean (93.28 mmHg vs. 88.62 mmHg, $P=0.015$) and almost significant reduction in systolic blood pressure mean (149.65 mmHg vs. 142.58 mmHg, $P=0.051$) in the participants after receiving Ca supplements. Pre eclampsia (10.3% vs. 45.9%, $P=0.002$), Low birth weight (6.9% vs. 40.5%, $P=0.002$), Pre term delivery (10.3% vs. 43.2%, $P=0.003$), and admissions to neonatal intensive care unit (10.3% vs. 45.9%, $P=0.002$) were significantly lower in the intervention group.

Conclusion: Ca supplementation of 500 mg daily started at 20th week of gestation associated with reduction of maternal blood pressure, pre-eclampsia, preterm delivery, low birth weight and admission to neonatal intensive care unit especially in a low dietary Ca intake mothers.

Keywords: Calcium supplementation; pre-eclampsia; preterm; low birth weight.

Introduction

Pregnancy induced hypertension (PIH), is a form of high blood pressure in pregnancy, characterized by

systolic blood pressure (sBP) ≥ 140 mmHg or diastolic blood pressure (dBp) ≥ 90 mmHg,¹ an important cause of both maternal and perinatal morbidity and

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mortality,^{2,3} complicated 6 to 10% of pregnancies,⁴ responsible for approximately 14% of global maternal deaths.⁵ Although overall maternal mortality has been decreased, the percentage of maternal deaths resulting from hypertension has remained stagnant.⁶ Low dietary calcium (Ca) intake represents a factor associated with an increased incidence of hypertensive disease in general population,^{7,8} and with hypertensive pregnancy disorders in most low and middle income countries (LMIC).^{9,10} Ca supplementation has the potential to reduce adverse gestational outcomes, by decreasing the risk of developing hypertensive disorders during pregnancy, which have been associated with low birth weight (LBW), preterm delivery and maternal mortality.^{11,12}

Pregnancy is a state of high Ca demand, as ~ 30g of it is transferred to the fetus during gestation.¹³ Although intestinal Ca absorption normally increases during pregnancy and there is no need for additional intake to supply sufficient Ca to the fetus, but such increase in the absorption is directly related to maternal Ca intake.¹⁴

Recommended dietary allowance of Ca for pregnant women established by Food and Nutrition Board is 1300 – 1000 mg/day depending on age. Ca intake could be a useful indicator of status at the population level. Serum Ca concentrations are maintained within narrow limits in the body and thus have limited use for assessment of Ca nutritional status at both individual and population levels.¹⁵

Several Ca salt formulations are recently available in a variety of doses. Ca carbonate is cheaper, provides 40% elemental Ca, better absorbed, well tolerated especially when taken with a meal and the very cost-effective choice in most settings. The percentage absorbed influenced by Ca dose; as the Ca content of the dose increases, the fractional absorption of Ca decreases. Doses of ≤ 500 mg per administration are recommended for the best absorption.¹⁵⁻¹⁶ This implies that the World Health Organization (WHO) recommendations of 1.5 to 2 gm will involve ≥ 3 pill-taking events daily. Moreover there is a concern that Ca might interfere with iron-folic acid (IFA) absorption and the mother should not take Ca tablets with them.^[17] Research on other drugs and supplements has found that adherence decreases as the number of pill-taking events increases.¹⁸ It is likely that the benefits of a feasible lower-dose regimen may be greater than a higher-dose regimen with low uptake and adherence.¹⁹

Aim of the study:

To assess the effects of low-dose Ca supplementation on both maternal and fetal outcomes of PIH.

Patients and method:

A single-blind, randomized controlled trial (RCT) was conducted from January through March, 2021. A total number of 66 pregnant women with PIH attended maternal care outpatient in Baghdad Medical City were involved and assigned randomly in two groups, 29 of them were received Ca carbonate supplements of 500 mg (elemental Ca) once daily from the start of their 20th week of gestation till delivery, and the other 37 were receive nothing, follow up both groups for the outcomes, compliance with Ca intake were assessed by counting the remaining tablets, Contact was maintained by telephone calls.

Exclusion criteria:

Women weren't eligible for the trial if they were already taking Ca supplementation before 20th week of gestation; had chronic hypertension or other medical conditions; had a history or symptoms of urolithiasis, renal disease or parathyroid disease; and those with known hazardous condition (multifetal gestation or hydatidiform mole); were not on antihypertensive medication; were adequately intake of dietary Ca or were unwilling to enroll in the trial.

Ca supplementation was the primary exposure variable. Women were instructed to take one Ca tablet (500 mg) daily in the evening with dinner meal separately from IFA until pregnancy termination and were encouraged not to take any additional Ca supplements as analgesic or antacid or pregnancy multivitamins that contain Ca.

The maternal outcomes assessed were blood pressure (BP), pre-eclampsia (PE), eclampsia (E) and admission to maternal intensive care unit (MICU). While **the neonatal outcomes** assessed were LBW, preterm delivery, admission to neonatal intensive care unit (NICU) and perinatal mortality. The outcomes variables validated through cross-checking with medical professionals and their documentation in patient's card.

Statistical analysis and data management:

IBM SPSS version 26 was used for this study statistical analysis. Participants' non-parametric data were presented as frequencies, percentages and

diagrams. Independent samples t test was used to compare BP mean change between the two study groups. Paired sample t test was used to compare BP mean before and after Ca supplement period for both study groups. Chi square test was used to assess the association between Ca supplement and maternal and fetal outcomes (significant P-value < 0.05).

Results

Ca supplementation: About 44% of participants received Ca supplement and 56% of them didn't (Figure 1). **Socio-demographic characteristics of participants:** There was no significant difference between the intervention vs none groups regarding age, parity, body mass index, socioeconomic status and educational level.

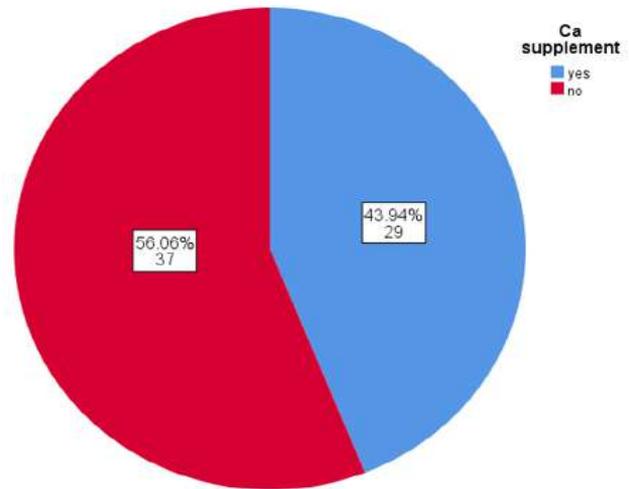


Figure 1: Distribution of study groups according to Ca supplement

Maternal BP: The dBP mean was significantly decreased and almost significant decrease in sBP mean in participants after receiving Ca supplementation (Table 1).

Table 1: Comparison of BP before and after Ca supplement

Diastolic BP (mmHg)	Mean ±SD		Pre Ca	Post Ca	P value
			Mean ±SD		
Ca Supplement	Yes		93.28 ±4.28	88.62 ±8.75	0.015
	No		96.49 ±5.88	95.27 ±8.97	0.525
Systolic BP (mmHg)	Mean ±SD		Pre Ca	Post Ca	P value
			Mean ±SD		
Ca Supplement	Yes		149.65±7.31	142.58±18.54	0.051
	No		153.64±14.27	154.45 ±14.32	0.808

Significant P-Value < 0.05

Maternal outcomes: PE was significantly lower in participants received Ca supplements (10.3% vs.

45.9%, $P = 0.002$). There was no **maternal death** in our study.

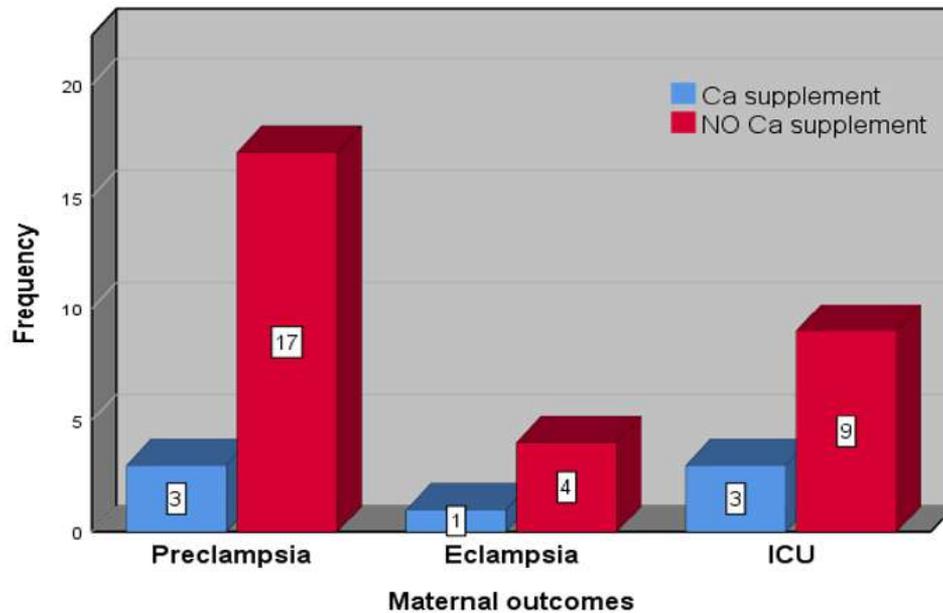


Figure 2: Distributions of study groups according to maternal outcomes

Fetal outcomes: LBW, Preterm delivery and admission to NICU were significantly lower in participants received Ca supplements (6.9% vs.

40.5%, $P = 0.002$), (10.3% vs. 43.2%, $P = 0.003$) and (10.3% vs. 45.9%, $P = 0.002$) respectively.

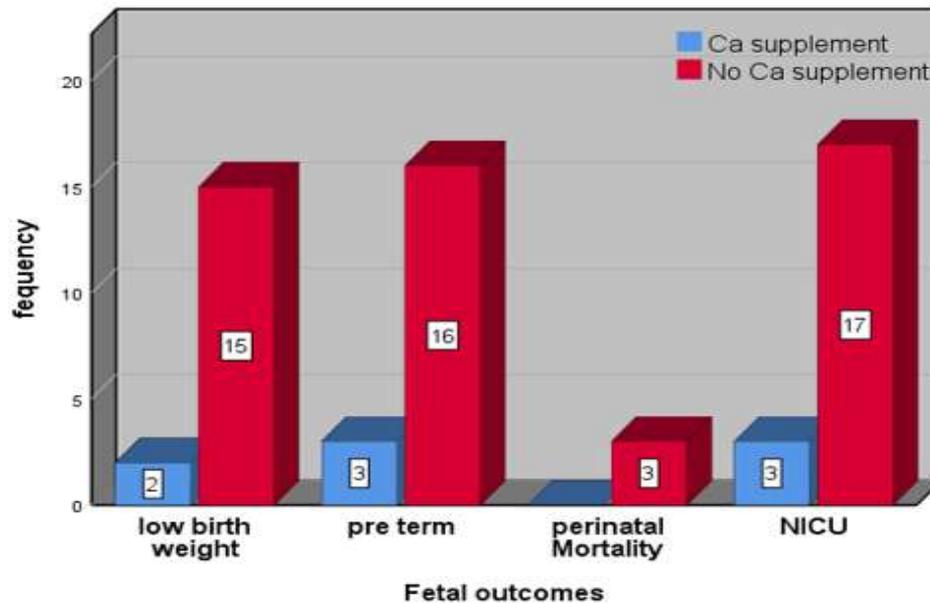


Figure 3: Distributions of study groups according to fetal outcomes.

Discussion

Daily supplementation of 1.5 to 2 gm of Ca during pregnancy reduces PIH outcomes. However, the efficacy of low-dose Ca supplementation on PIH is limited. We try to illustrate the effectiveness of use of low dose Ca supplement on both maternal and fetal outcomes.

In our study, there was significant decrease in dBP mean and almost significant decrease in sBP mean in participants after receiving Ca supplements. While there was non-significant decrease in dBP mean and non-significant increase in sBP mean in participants didn't receive Ca supplements. In Kumar et al. study the mean sBP and dBP were different significantly in the Ca and placebo group

($P = 0.007$ and $P = 0.02$).^[20] And WHO found that fewer women on Ca supplementation had high BP compared with those receiving placebo.²¹ Fouzia et al. presented an absolute change in mean sBP and DBP from baseline to the last follow up visit, they observed a slower increase over time among those who consumed 500 mg/day Ca tablets, the slowness more obviously for those received Ca supplement for > 6 months during pregnancy than those consumed it for < 6 months.²² On the other hand Goldberg et al. reported that no significant reduction in sBP ($P = 0.3$) or DBP ($P = 0.8$) between Ca compared with placebo after 1.5gm/day of Ca supplementation.²³

PE was significantly lower in participants received Ca supplements. This is consistent with Imdad et al.²⁴ and Hofmeyr et al.²⁵ Pooled analysis where they show that Ca supplementation during pregnancy was associated with a significant reduction in the risk of pre-eclampsia. Although, imdad et al. showed that the effect was more statistically significant with 2 gm/day compared with < 2 gm/day.²⁴ While Hofmeyr et al. found a reduction in the risk of PE in both high and low dose of calcium supplementation.²⁵ WHO found that for all women, irrespective of the baseline risk of developing hypertension and Ca intake status, Ca supplementation more than halved the risk of PE when compared with a placebo.^{4,21} Sun et al. found that Ca supplementation reduced incidence of PE and their sub-groups analysis showed that high, moderate and low-doses of Ca supplementation could reduce the risk.²⁶ While Wanchu et al. said that Ca supplementation didn't lower the incidence of PE, it did reduce its severity.²⁷ On the other hand, Trumbo et al. concluded that the relationship between Ca and risk of PIH and PE was highly unlikely.²⁸

In present study there was no effect of Ca supplementation on incidence of E, similar to that founded by WHO²¹ and Imdad et al.²⁴

Also we found that Ca supplementation not affects MICU admission consistent with WHO²¹ and Villar et al.²⁹ finding.

There was no maternal mortality in our study. Although Imdad et al.²⁴ and Villar et al.²⁹ reported that Ca supplemented women had a statistically significant lower risk for maternal mortality. While WHO found that Ca supplementation had no effect on risk of maternal death.²¹

About fetal outcomes; LBW was significantly lower in participants received Ca supplements.

Similarly Imdad et al. showed a reduction of LBW by 15% and there was an extra gain of 85 gm following Ca supplementation; however the results were statistically non-significant.²⁴ Also Niromanesh et al. stated that infants born to the Ca group, on average, were 552 g heavier than infants born to the placebo group but the dose of Ca used was 2 gm/day.³⁰ On the other hand Pranom Buppasiri et al.³¹ and WHO²¹ found that there was no significant difference in LBW between two groups. However, Pranom Buppasiri et al found that compared to the control group, women in the Ca supplementation group gave birth to slightly heavier birth weight infants.

The preterm delivery was significantly lower in participant received Ca supplements. This is go with what Kumar et al.²⁰ found in their study, that the risk of preterm delivery was less in the Ca (7.0%) than in the placebo (12.7%) group. Imdad et al.²⁴ showed a significant reduction of 24% in the intervention group compared with the control group. While Hofmeyr et al.²⁵ found that the average risk was reduced in high dose of Ca supplements (≥ 1 gm/day) but not in low dose (< 1 gm/day). WHO stated that there was no effect of Ca supplementation on preterm birth although a subgroup analysis by supplemental dose suggested that among pregnant women consuming ≥ 1.5 gm/day of Ca fewer babies were born before 37 weeks' gestation than among those receiving < 1.5 gm/day.²¹

We found that admission to NICU was significantly lower in participant received Ca supplements. This is consistent with Hofmeyr et al.²⁵ who noticed that supplementation with low dose of Ca reduced the risk of admission to NICU, while not affected by high dose supplementation. This finding is inconsistent with what WHO found.²¹

About the neonatal mortality, reduction in Ca supplemented group weren't statistically significant which is consistent with WHO²¹ and Kumar et al.²⁰ who stated that there were no significant differences between two groups in risk of having a stillbirth or neonatal death before hospital discharge. Whereas Villar et al. found that the neonatal mortality rate was lower in the Ca group, but they use higher dose of Ca supplements (1.5 gm/day) than in our study.²⁹ Analysis in Mehnaz et al. has shown that Ca supplementation during pregnancy reduced stillbirths by 19 %; however results weren't statistically significant.³²

Conclusion

The dilemma facing health policy-makers is often whether supplementation with a lower-dose would be better than no supplementation at all especially in LMIC. The findings of this trial are a step toward addressing this issue. Our trial demonstrated that maternal BP, pre eclampsia, LBW, preterm delivery and admission to NICU were significantly lower in participants received Ca carbonate supplements of 500 mg/day at 20th week of gestation. Other maternal and fetal adverse outcomes may be reduced when higher dose or earlier than 20th week are implemented and this should be considered when making decisions about the use of Ca supplementation during pregnancy, particularly for those with very low dietary Ca intake or high risk pregnancies. Further larger well designed RCTs are still required to clarify issues related to dose, timing, barrier, acceptability, adherence, feasibility, side effects and cost-effectiveness of full-scale implementation is urgently needed. Pending such results, in settings of low dietary Ca intake where high-dose supplementation is not feasible, the option of lower-dose supplements might be considered rather than no supplementation.

Conflict of interest: None.

Source of funding: None.

Ethical Considerations: Permission to conduct the study was obtained from health authorities. Oral consent was obtained from participants. Entering and leaving the study was completely voluntary and free of charge.

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Gender Differences in Pain Intensity and Functional Performance among Older Adults with Knee Pain living in Suburban of Bangkok, Thailand

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How to cite this article: Pattaraporn Piwong, Tiwaporn Junkhaw, Ratana Somrongthong. Gender Differences in Pain Intensity and Functional Performance among Older Adults with Knee Pain living in Suburban of Bangkok, Thailand. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):287-291.

Abstract

Purpose of study: To investigate gender-related differences in pain intensity functional performance among older adults with knee pain who live in the suburban area of Bangkok, Thailand.

Method: This cross-sectional study recruited 220 older adults who suffering from knee pain, aged between 50-65 years (male=79, female =141). A convenience sampling method was used to select the participants in this study, each participant underwent an actual functional performance test such as TUG, 30CST, and completed self-report questionnaires consist of demographic characteristics, NPRS, and KOSADLS. An independent samples t-test was used as appropriate to determine gender differences.

Results: Males and females had a difference in two aspects, females have significantly more pain intensity (NPRS scores of female: 4.88 ± 1.20 ; male: 3.99 ± 1.27 , $p < 0.001$), and poorer functional performance also more impairment on a specific functional task such as KOSADLS scores (female: 71.74 ± 7.49 ; male: 74.67 ± 5.01 , $p < 0.01$), TUG scores (female: 11.66 ± 1.11 ; male: 10.81 ± 1.06 , $p < 0.001$) and 30CST scores (females: 11.23 ± 1.40 ; male: 12.50 ± 1.44 , $p < 0.001$).

Conclusion: The differences between genders regarding pain intensity and functional performance which are assessed by self-report measure and actual functional performance test, these two methods provide valuable information. The difference evaluation and preventive health care strategies based on gender differences would be considered in Thai older adults with knee pain to improve their knee function and reduce the pain.

Keywords: Gender; Older adults; Knee pain; Functional performance.

Introduction

Knee pain is a major public health issue causing disability in older adults worldwide¹, with individuals often report difficulty walking or climbing stairs. One of the known influencing

factors of knee pain is female gender which is more affected and leading to a higher prevalence of knee pain.² Complaints of knee joint pain are common in female older adults.³ Nearly 50% of older adults aged 50 years and over report knee pain during one year period and of these approximately half of them (50%)

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had some restrictions of their activities of daily living and reduction in quality of life.⁴

Gender difference in the demographic characteristics of older adults with knee pain is probable affect their health-seeking behavior, access to health care for their musculoskeletal problem.⁵ Previous studies have reported that gender differences play a vital role in the level of knee pain and knee functional performance.⁶ Although females have a higher prevalence of knee pain⁷, gender-related differences among older adults with knee pain have not been adequately studied also received scant attention. Consequently, there were a few studies related to gender differences in pain intensity levels among knee pain older.^{8,9} To date, no study has examined gender-related differences regarding pain intensity levels and functional performance in these older adults group, especially in a suburban area of Bangkok, Thailand. Consequently, it is essential to explore gender-related differences among Thai older adults who had knee pain. Although the assessment of functional performance in knee pain older adults is often based on self-reported measures rather than actual functional performance measures.¹⁰ On the other hand, an inclusive assessment of the functional performance of older adults with knee pain should apply not only self-report measures but also, actual functional performance measures. Therefore, the purpose of this study was to assess gender differences in pain intensity and functional performance among older adults with knee pain who live in suburban areas of Bangkok, Thailand which assess by both self-reported measurement and actual functional performance tests.

Materials and Method

A descriptive cross-sectional study was used. The older adults both males and females, aged between 50 to 65 years, living in suburban Bangkok also had been diagnosed with knee pain by physicians at the selected health center of Bangkok were eligible for the study. In total, 220 older adults were recruited using the convenience sampling method. Data were collected from August to October 2020. The inclusion criteria were as follows: (1) having knee pain either right, left, or both sides of the knee during movement, knee pain at least on most days in a week or more within the past 12 months. (2) Able to read and understand Thai language. (3) Can perform activities of daily living without assistance. (4) Willingness to

participate in the study. Regarding older adults who diagnosed osteoarthritis knee with radiologically confirmed or had previous knee replacement surgery, cognitive impairment, vision, and hearing impairment were excluded from this study.

Measurements

Demographic characteristics data

Demographic characteristics data of each participant were collected by self-reported questionnaires such as gender, age. For height and body weight were obtained without shoes or heavy clothing on a suitable calibrated weighing machine via standard techniques. Body mass index (BMI) was calculated by dividing the weight in kilogram by the square of height in meters (kg/m^2). In this study, BMI was categorized by using BMI categories in Asian populations as the following: underweight (BMI <18.5), normal weight (BMI 18.5-22.9), overweight (BMI 23-24.9), and obese (BMI > 25).¹¹

Pain intensity

The numeric pain rating scale (NPRS) is valuable in describing even the most severe levels of knee pain, and the number of levels makes it sensitive to clinically applicable changes in knee pain which is a valid and reliable measurement tool to assess knee pain intensity for older adults in this study.¹² Participants were requested to indicate the knee pain experienced over the past week, The NPRS has a range from 0-10, which is 0 for no pain and 10 for the most severe pain, and cut-off point of NPRS scores ≤ 3 correspond to mild pain, scores 4-6 to moderate pain and scores ≥ 7 to severe pain.¹³

Functional performance test

1. Knee Outcome Survey Activities of Daily Living Scale (KOS-ADLS)

The KOS-ADLS is a self-reported questionnaire consists of 14 items that are reliable and valid measurement tool for assessing symptoms of the knee and limitation of the knee joint in older adults with daily activities of life. The first 6 items (subscale "symptoms") to measure symptoms involved pain, stiffness, swelling, instability, weakness, and limping. Then follow by 8 items (as subscale "activities of daily living") to assess participants' functional limitation of the knee through their daily activities for example walking, climbing and descending stairs, standing, squatting, kneeling, and stand from a chair. Each item

was scored on a six-point Likert scale (ranging from 0 to 5 per item), the maximum score is 70 and the total score can be expressed as 0 to 100% (the lower score defined as worse symptoms and more serious about functional disability).¹⁴

2. Timed up and Go (TUG)

The Timed Up and Go test was selected to evaluate older's function mobility in this study, this test has been suggested as a simple screening tool for the older population. Testing was implemented by researchers following the original protocol.¹⁵ A standard chair with an armrest is used as equipment for the test. The participants were asked to stand up from an armchair, walk 3 meters at their normal pace, turn then walk back again, and sit down on the chair. To completed the test, participants were performed twice consecutively, an average score in seconds as the TUG test score was recorded. A TUG score of participants ≤ 10 seconds refers to good functional performance, a score ≥ 13.5 seconds indicates a high risk of fall.¹⁵⁻¹⁷

3. 30-Second Chair Stand (30CST)

The 30-second chair stand test is widely used to measure the strength of lower limbs in older adults, this test is easy to complete and score.¹⁸ To perform the test by researchers asked participants to stand up with arms crossed on the chest from a standard chair (with a 40 centimeters of seat height, without armrests), and then return to sitting down as many times as safely within 30 seconds. The participants

suggested to practice 1-2 times before completing the test, if they use their arms to stand, the score is recorded "0". All older adults are instructed to complete the test within 30 seconds by performed many full stands as possible and fully sit between each stand. Researchers count the total number of times as fully stands within 30 seconds and record it. The cut-off point score defines as lower than 12 times meaning that older adults have a low functional performance.¹⁹

Data collection procedure

The researchers contacted and visited all participants at their houses initially to recruit them into the study. The data collection process was accomplished in 12 weeks at a selected area in Saimai district, suburban of Bangkok. Participants completed a face to face interviews to answered self-reported questionnaires included demographic data, NPRS, KOS-ADLS. Moreover, the actual functional performance tests consist of TUG and 30CST were instructed by researchers under the standard protocol.

Data analysis

All data were analyzed by IBM SPSS version 22 for windows. Descriptive statistics involved frequency, mean and standard deviation (SD) were calculated for all outcomes in this study. A t-test for independent samples was conducted to assess gender differences. Statistical significance was considered at *P*-value less than 0.05.

Results

Table 1: Demographic characteristics (mean \pm SD) of participants by gender and comparison between genders, n=220

Variables	Male (n= 79)	Female (n= 141)	P value
Age (years)	59.77 \pm 2.73	58.47 \pm 3.31	< 0.01*
Weight (kg)	64.62 \pm 4.32	63.64 \pm 4.52	0.11
Height (cm)	164.29 \pm 4.74	163.42 \pm 4.17	0.15
BMI ^t (kg/m ²)	23.99 \pm 1.00	23.86 \pm 1.14	0.39

* *P*-value < 0.01; ^tRef WHO, BMI for Asian populations⁽¹¹⁾

There were 220 participants (79 male and 141

female) comply with the study's inclusion and exclusion criteria. Demographic characteristics of participants by gender are shown in Table1.

Table 2: Functional performance variables (mean ± SD) of participants by gender and comparison between genders, n= 220

Variables	Male (n=79)	Female (n=141)	P value
NPRS	3.99 ±1.27	4.88 ±1.20	< 0.001**
KOSADLS	74.67 ±5.01	71.74 ±7.49	< 0.01*
TUG (sec)	10.81 ±1.06	11.66 ±1.11	< 0.001**
30CST [†]	12.50 ±1.44	11.23±1.40	< 0.001**

*P-value < 0.01, **P-value < 0.001; NPRS: Numeric pain rating scale; KOS-ADLS: Knee Outcome Survey Activities of Daily Living Scale; 30CST: 30-second chair stand test; TUG: Timed up and go test; [†] score are number of chair stand in 30 second

Table 2 shows the function performance results of participants, the statistical analysis revealed significant gender differences for NPRS, KOSADLS, TUG, and 30CST. Furthermore, the female reported greater knee pain levels, lower KOSADLS scores, lower levels of 30CST, and poorer TUG performance when compared to the male.

Discussion

The present study found that female older adults represented more than half of all participants (64.1%), which is consistent with the previous study as the higher prevalence of knee pain among females.⁽²⁾ Furthermore, we found that female participants had a higher knee pain intensity in comparison with male participants, which is showed the same result of Peat et al.,²⁰ who also demonstrated there was a difference between males and females in an analysis of pain intensity related to gender differences among older adults in the community. A current study related to musculoskeletal pain, in concurrence with the prior literature, revealed clear gender differences in pain intensity with the female having a greater pain intensity and it's linked to hormonal change and psychological factors.²¹ The differences in the results of this study might be associated with the differences in older adults' background characteristics such as age.

Among both gender, we also found females had significantly lower functional performance in every aspect (eg. KOSADLS, TUG, and 30CST). These findings are in accordance with most previous studies.^{14,22} The results of this study revealed that knee pain had a greater impact on functional performance in females compared to males. This indicates that knee pain had more adversely affect lower limbs function among females. One particular interest, there are conflicting findings from the literature

about the reported functional performance test in gender differences of those who had knee pain, while some have found that females tend to have worse functional performance than males even measure by self-report and actual functional performance test²² same as this study, others have not found the differences between gender.²³ From their conflicting reported might stem from different background characteristics of older participants and/or pain catastrophizing of the study population compared to others. Our findings highlight the importance of using a variety of measurement tools when assessing knee pain among older people.

Conclusion

In comparison with males, females reported greater knee pain intensity, lower functional performance as evaluated by the self-report and actual functional performance test. Accordingly, a disparity of knee pain in Thai older adults between males and females was observed and exits prior to the onset of knee pain in this age group. Our study results contribute to the literature that self-report measures and actual functional performance tests could be used in combination with each other to gain potentially greater benefit to developing an effective health prevention program for the specifics needs among knee pain older adults.

Source of Funding: Supported by the 90th Anniversary of Chulalongkorn University Scholarship under the Ratchadaphisek Somphot fund, research project no. (GCUGR1125621049D)

Conflict of Interest: None

Ethical Clearance: The study was approved by The Research Ethics Review Committee for Research Involving Human Research Participant, Health Science Group, Chulalongkorn University, Bangkok, Thailand (COA No.166/2020).

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Management of Airway Foreign Body Aspiration with Atelectasis Complication

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How to cite this article: Puji Utami, Rizka Fathoni Perdana. Management of Airway Foreign Body Aspiration with Atelectasis Complication. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):292-296.

Abstract

Background: A sharp foreign body aspiration is a problem that is usually found in teenage women who wear hijab. Straight-pins are used in wearing hijab to make a model and to hold it tight in place. Straight-pin aspiration has the potential to become a serious problem if it is inhaled in the airway and must be removed immediately in optimal conditions by using complete equipment to prevent complications.

Case Report: A 14 year old girl with straight-pin aspiration in the airway had come to emergency room of Dr Soetomo General Academic Hospital, Surabaya, Indonesia. The initial location of the straight-pin was in the right main bronchus and a rigid bronchoscopy was planned. Rigid bronchoscopy was performed but the foreign object failed to be removed and the foreign object moved further down and difficult to reach. A flexible bronchoscopy was performed and the foreign body was successfully removed. However, there was a complication of left lung atelectasis after the procedure. It was treated conservatively and healed.

Conclusion: Straight-pin aspiration in the airway is treated by rigid and flexible bronchoscopy with atelectasis as a complication. Flexible bronchoscopy is an alternative therapy for inhaled foreign bodies that are located distally and cannot be reached by rigid instruments.

Keywords: flexible bronchoscopy; foreign body; rigid bronchoscopy; straight-pin aspiration; child neglect

Introduction

Foreign body aspiration is the inhalation of an object into the respiratory tract and a severe incident that could potentially cause a fatal complication.¹ Straight-pin aspiration is common in muslim countries that occur a lot in young women.² Straight-

pins are used to hold and make a model on the hijab. In the process of putting on the hijab, both hands are used to shape the hijab while the straight-pins, before they are used to hold the hijab tight in place, are temporarily bitten with the front teeth, so they are easily inhaled when speaking and coughing.³

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Usually, the blunt position, in the shape of pearl, is in the mouth so that the sharp part is proximal. The incidence of foreign bodies aspiration at Dr. Soetomo General Academic Hospital Surabaya, Indonesia, in the 2010-2015 period was recorded as many as 87 cases, consisting of 27 cases of organic foreign bodies and 60 cases of inorganic foreign bodies with cases of needle foreign bodies as many as 50 cases. Complications of tracheobronchial foreign bodies can be due to foreign bodies themselves or due to the bronchoscopy procedure. Atelectasis is one of the complications that occur due to bronchoscopy⁴. We presented a case on foreign body aspiration treated with bronchoscopy with atelectasis as complication. The purpose of this case was to discuss the clinical experience of handling a patient with a needle airway foreign body, in which the removal of the foreign body at the first attempt failed, and the presence of complications that arose as the result of the procedure.

CASE

A 14 years-old girl came to the Emergency Room (ER) of Dr. Soetomo Hospital, Surabaya, Indonesia, on November 6, 2017, with a diagnosis of straight-pin aspiration in the airway that called foreign body aspiration. The patient inhaled a straight-pin about 8 hours before. When she was doing her hijab, she bit the pin with her front teeth, but she had suddenly coughed and the pin was accidentally inhaled. She felt pain in the neck, without any complaints of breath shortness. Physical examination of the patient showed she was within normal limits. Chest x-ray examination showed a metal density in thoracic 3-4 left vertebrae (Figure 1)

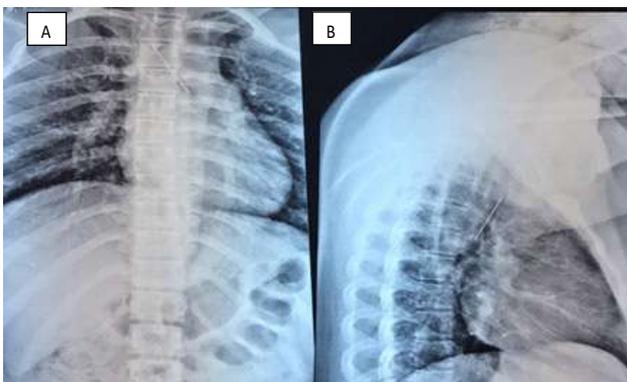


Figure 1: Chest radiograph before bronchoscopy. A. Anteroposterior, B. Lateral

The patient was diagnosed with a straight-pin aspiration in the left main bronchus. Bronchoscopy was performed with general anesthesia. The foreign

body of a straight-pin was found in the left main bronchus, but failed to be extracted. It was dislodged and moved to the distal airway. Re-evaluation obtained a foreign bodies in the left superior lobe of bronchial lingua. Several attempts was done to remove the foreign objects but failed. The airway mucosa became edematous. It was decided to stop the bronchoscopy and scheduled to do flexible bronchoscopy three days later.

One day after the bronchoscopy, chest X-ray showed atelectasis of the superior lobe in the lower left lung (Figure 2). Atelectasis was treated conservatively with intravenous antibiotics and corticosteroids before the procedure.



Figure 2: Plain chest radiograph after rigid bronchoscopy. A. Anteroposterior, B. Lateral. Arrows indicate foreign needle objects.

Flexible bronchoscopy was performed three days later under general anesthesia. The objects were found after 90 minutes of action. The foreign body was found in the left superior lingula of the bronchus and was embedded in the mucosa with a proximal sharp edge. The object was successfully removed within 2 hours of action.

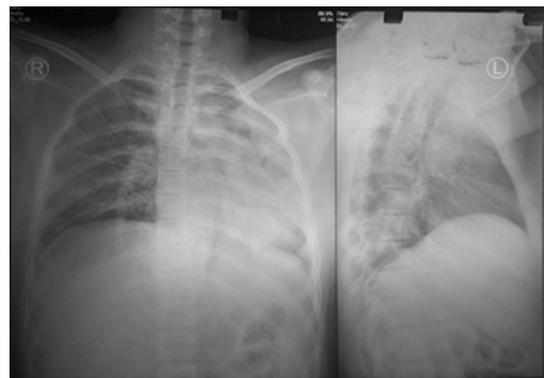


Figure 3: Plain chest radiograph after flexible bronchoscopy. A. Anteroposterior, B. Lateral



Figure 4: A. Needle foreign body in the mouth of the superior lobe, showing the sharp tip buried in the mucosa, B. The foreign body of the pin that was successfully removed.

Eleven hours after the procedure, there was no breathless, but there was a cough with blood spots. Vital signs of the patients showed oxygen saturation of 93% without supplemental oxygen. Examination of the chest revealed asymmetrical chest motion with left side trailing and decreased breath sounds in the lower left lung. The patient was given with supplemental oxygen with a nasal cannula 3 liters/minute and was given with chest physiotherapy. There was a suspicion of lung atelectasis in the left superior lobe.

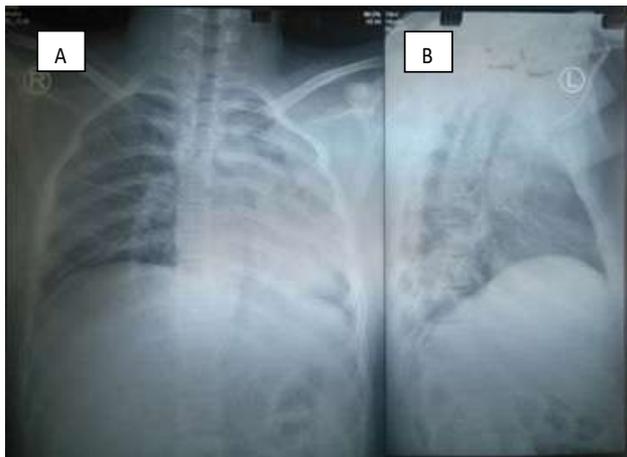


Figure 5: Chest plain photo after flexible bronchoscopy. A. Anteroposterior, B. Lateral.

Consolidation is seen on the lobe of the bottom of the superior left lung.

The patient did not have difficulty to breathe and the cough was minimal one day later. The oxygen saturation improved 97% without additional oxygen. Chest motion was symmetrical, vesicular lower left lung breath sound improved compared to the previous day, and there were additional crackling breath sounds in the lower left lung. The treatment was continued with additional nebulization therapy with salbutamol every 8 hours. On the second day, the crackles improved and thoracic plain photo was taken, showing normal results.

The patient was discharged with cefixime 2x100 mg home medicine. The patient was scheduled for check-up after 3 days without any complaints.



Figure 6: Chest plain photo before discharge

Discussion

The patient aspirated a foreign body, a straight-pin, which she bit with her front teeth before being used for fixing her hijab. She suddenly coughed, so the straight-pin was inhaled into the airway. Straight-pin aspiration into the tracheobronchial system rarely causes symptoms because the needle does not cause obstruction, but causes infection or granulation around the needle which can cause discomfort.³ The patient experienced a persistent cough in this case when the initial foreign object was inhaled and then the cough disappeared. The patient came to the hospital in asymptomatic phase so that there were no complaints and physical examination was within normal limits.

Airway foreign bodies may not cause acute airway obstruction, but may cause other reactions, depending on the type of the bodies, whether they are organic or inorganic. Inorganic foreign bodies, such as needles, are not hygroscopic, so that there is less tissue reaction than organic foreign bodies, but sharp foreign bodies are more dangerous because they have potential to cause airway perforation.⁵ In this case, the foreign body did not cause obstruction nor showed clinical symptoms. The small size of the straight-pin did not obstruct the lung segment because the proximal or distal end diameter of the foreign body was smaller than the left bronchial lumen's size so that the airflow in lung was not disturbed.

Straight-pin airway foreign-body is mostly located in the main branch of the left bronchi. Some experts attributed this finding to the Bernoulli

phenomenon. Coughing, laughing or talking would cause greater negative pressure on the left bronchus, which is narrower than the right bronchus, causing the movement of small and long shaped needles to the left side.¹ A straight-pin that was inhaled in this case located in the main branch of the left bronchus, in accordance to the Bernoulli phenomenon.

The difficulty of diagnosis is a common problem when faced with cases of airway foreign-bodies. The straight-pin airway foreign-bodies diagnosis is more straightforward because the straight-pin is made of metal and is easily recognizable from its shape on radiological examination.¹ Chest x-ray of the patient showed a metal-density object in the left thoracic 3-4 vertebra so that the diagnosis of straight-pin foreign body could be made.

Sharp radiopaque foreign bodies seen on plain chest radiographs should be removed immediately by rigid and flexible bronchoscopy. Foreign-bodies could be taken by rotating to reduce the risk of trauma and could be covered by advancing the bronchoscope or pulled into the bronchoscope lumen.⁶ When performed rigid bronchoscopy, the straight-pin should be grasped and brought into the bronchoscope.³ In this case, taking a straight-pin was done with a rigid bronchoscope because it was the standard for taking out foreign-bodies on the airway. Due to the the shape of the straight-pins, when taken, the straight-pin came off and moved to the lingula position of the left superior lobe of the bronchus.

The foreign body was hard to remove since the needle pointed into the bronchial mucosa which created a resistance when it was pulled. The mucosa of the bronchi was edged and made it difficult to take foreign objects so it was decided to stop the operation. Plain chest radiographs were performed primarily for repeated bronchoscopy which could show the distal migration of foreign bodies.

Airway foreign bodies located in the main tracheobronchial tract are frequently displaced and moved during the retrieval procedure. Objects of irregular shape and objects with sharp edges tend to puncture the tracheobronchial mucosa and become fixed.⁷In this case, the bronchoscopy procedure caused the straight-pin to shift distally. Flexible bronchoscopy is used to remove foreign objects located in the lingula with a sharp tip that makes it difficult to remove using rigid bronchoscopy.

Complications that may occur in the aspiration of a foreign body in the tracheobronchial can be due to from the foreign body itself or due to the bronchoscopy.⁵ In this case, complications occurred as a result of bronchoscopy in the form of atelectasis of the inferior lobe of the left lung and edema on the bronchial mucosa. The diagnosis of atelectasis is based on clinical symptoms, physical examination, laboratory examination, radiological examination and bronchoscopy. The patient complained tightness and cough with spotting blood.

Physical examination showed asymmetrical chest motion with left side trailing and decreased breath sounds in the lower left lung. Plain chest radiograph of the patient after bronchoscopy showed opacification in the lower part of the left lung. The patient was suspected of having atelectasis as a complication of bronchoscopy and was given with systemic corticosteroid therapy, systemic antibiotics, nebulization and chest physiotherapy.

The patient's condition had gradually improved. Her complaints had improved, and physical examination and support had also improved. The patient was discharged and scheduled for a visit in the outpatient unit. At the time of control, three days later, the patient had no complaints and was advised to return to control if there were complaints.

Conclusion

We reported straight-pin aspiration in the airway treated by rigid and flexible bronchoscopy with atelectasis as a complication. Flexible bronchoscopy is an alternative therapy for foreign bodies that are located distally and cannot be reached by rigid instruments.

Conflict of Interest: The authors report no conflict of interest related to this manuscript.

Ethical Approval: This study received a certificate of ethical clearance from the ethical commission of Dr. Soetomo General Academic Hospital number 0202/LOE/301.4.2/XI/2020.

Source of Funding: Self Funding

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The Effect of Air Pollutants on liver Enzymes and Pituitary Gland Hormones of Smokers and non-smokers of Oil Refinery and Gas Station Workers in Basra/Iraq

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How to cite this article: Rafad A. Al-Hulfi¹, Bushra Abdul Mohsin Al Salem, Ibrahim M. Al-Naiema. The Effect of Air Pollutants on liver Enzymes and Pituitary Gland Hormones of Smokers and non-smokers of Oil Refinery and Gas Station Workers in Basra/Iraq. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):297-301.

Abstract

Air pollutants that originated from oil refineries and gas pump stations can have a negative effect on human organs, especially liver and brain. This study focuses on evaluating the pollutants from oil refinery and gas station on workers' health by measuring three hepatic enzymes GOT (Glutamic Oxaloacetic Transaminase), GPT (Glutamic Pyruvic Transaminase), ALP (Alkaline phosphatase) and three pituitary gland hormones LH (Luteinizing hormone), FSH (Follicle-stimulating hormone), TSH (Thyroid-stimulating hormone). Study groups were (i) 33 males from Basra oil refinery workers including 16 smoker and 17 non-smoker (ii) 23 male from Gas station, 11 of them were smoker while the other 12 are non-smoker (iii) 24 male local volunteers from Basra (all had non-oil related job) as a control group, 12 smoker and 12 non-smokers. All three groups' subjects had neither family history of respiratory problems nor diabetic. The results indicated a significance decrease in FSH, LH, TSH hormones and GPT, ALP enzymes of oil refinery workers, comparing to the control group. The same trend was also observed among gas station workers, suggesting hepatotoxicity and alternation in pituitary hormones. Pollutants that circulating around oil industry sites can negatively impact human body especially for those who work near these locations.

Keywords: Pituitary gland; hormones; liver enzymes; oil; air pollution.

Introduction

During oil processing, natural gas is usually burned in open air leaving particulate matter, polyaromatic hydrocarbons, volatile organic compound. Gas flaring can be very dangerous not only to human but to animals and to the environments.¹ Organs such as heart, lung, kidney, and skin can be negatively affected by oil industry by-products.²

The hypothalamic-pituitary-gonadal (HPG) axis is a system that combined the effect of hypothalamus and pituitary gland and gonadal glands. This system achieve many tasks because it includes many important hormones and affecters. Any change or dysfunction in one of these hormones can change or effect HPG.³ One of factors that can interrupt the functions of HPG are the anthropogenic disrupting chemicals (EDCs), which are outside chemicals that

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can cause damage to the endocrine system. EDCs are found in outdoor air that have been mixed with different pollutants including polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs), and diesel exhaust (DE). Pollutant like PAH can damage the estrogenic and the aryl hydrocarbon receptors.⁴

The levels of some infertility hormones such as Follicle stimulating hormone (FSH) and Luteinizing hormone(LH) have also found to be associated with exposure to fossil fuel emissions, especially in the petroleum sites.⁵ Since the pituitary gland also produce thyroid stimulated hormones which act on thyroid gland⁶, it will also be affected by EDCs. Liver main catalytic enzymes are glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), and alkaline phosphatase (ALP). These enzymes help moving the amino groups of aspartate and alanine to a-keto groups of ketoglutaric acid.⁷ Previous studies have found that PAHs in the air can alter the levels of these enzymes.⁸ In this study, we evaluate the impact of air pollutants on workers in petroleum industry via analysing the levels of the above mentioned compounds in the blood serum samples.

Methods

Sampling:

Blood withdrawal as well as centrifuging were achieved inside Basra oil refinery clinic. Similarly, the gas station worker blood samples were locally collected and centrifuged. The sampling of the third group was conducted in a medical laboratory in Basra city which is a city located in the southern part of Iraq. The process of sample collection lasted from February 15 to January 16, 2020.

Sampling notes:

- All workers have been working for at least one year.
- All haemolysis samples were discarded during measurements.
- All test were done in a certified medical laboratory in Basra city.
- Only male participants were selected to this study.

Hormones Measurements

MINI VIDAS is an autoanalyzer using solid phase fluorescence Immunoassay. It is the smaller version of VIDAS (Biomerieux, Marcy-l'Étoile, France). This analyser is widely used in Middle East and Europe due to the high sensitivity and multisampling technology. Every test has its own kit, which was purchased from the analyser company. The kits contain solid phase receptacle (SPR), control and a calibration reagent, and a sealed multigap reagent strip. The kit master lots (MLE) were scanned by the analyser barcode reader before the analysis. Every kit has a unique (MLE) to identify test relative fluorescence values and calibration ranges. If a calibration were out of the master lot ranges, system will not run. For FSH, LH and TSH, a 200 µl were pipetted in the sample gape in the reagent stripes and inserted in the analyser, along with the receptacle solid-phase (SPR). Each reagent strip contains dilutant, washing buffer, second dilutant, second washing buffer, and substrate. The measurement started when the solid phase interacts with every gap in reagent strip. All tests reports were printed in mini Vidas build-in printer.

Liver enzymes measurements

Measurements of liver enzymes were performed using Chem 200 instrument from Gesan production (Campobello Di Mazara, Italy). Calibration standards and controls were prepared and measured immediately before the actual samples. Levels of GOT, GPT, and ALP enzymes were determined in all samples using the analyser computed system.

• Statistical analysis

SPSS (version 26) was used for statistical analysis such as mean, average standard deviation, and t-test. Paired-sampled t-test was used to compare between control group and workers of oil Refinery. The difference considered to be significant when *p*-value was less than 0.005.

• Results findings and discussion

The results of the study showed a significant decrease in the level of TSH, FSH and LH hormones for each of the workers in oil refinery and gas stations, compared to the control group, as shown in Table 1. These results may indicate a minor defunction of hypothalamic-pituitary gland, which can be attributed to the change in the tropic hormone, downregulation

of gonadotropin-releasing hormones (GnRH).⁵ The detected lower levels of TSH among the oil refinery

workers in study groups can be used as a sign for the exposure to a high concentrations of pollutions.⁹

Table 1: FSH, LH, TSH hormones levels in (Mean±SD) along with P-values.

	Oil Refinery	P ¹ value	control	Gas station	P ² value
FSH (mlu/ml)	3.48±2.30	P <0.001	8.48±1.32	3.43±1.80	P <0.001
LH (mlu/ml)	5.08±3.13	P <0.001	5.93±0.60	4.65±1.89	P <0.001
TSH (μU/ml)	1.86±0.50	P <0.001	4.84±2.60	1.90±0.61	P <0.001

A comparison between smokers and non-smokers in the oil refinery and gas stations with the control group has been performed. The results showed a significant decrease of FSH, LH, and TSH among smokers workers, compared to the smokers from control group, Table 2. The results suggest that smoking is a vital factors because smokers can more be harmed by toxins than non-smokers.¹⁰ Table 2 indicate that the mean concentration of FSH among

the smokers in oil refinery is 4 mlu/ml, compared to 2.9 mlu/ml among non-smoker of the same group. When comparing the FSH levels of smokers (8.5 mlu/ml) from control group to the non-smokers of oil refinery workers (2.9 mlu/ml), we can easily conclude that a person who is non-smoker and work in petroleum industry can have a more damage to the hypothalamic-pituitary gland hormones than a typical smoker who does not work in oil relating job.

Table 2: FSH, LH and, TSH hormones as in smokers and non-smokers samples.

	FSH (mlu/ml)	LH (mlu/ml)	TSH (μU/ml)	FSH (mlu/ml)	LH (mlu/ml)	TSH (μU/ml)
	Non-Smoker			Smoker		
Oil Refinery workers	2.90±1.19**	4.70±2.20	2.04±0.51**	4.00±3.10**	5.40±3.90**	1.60±0.59**
Gas station workers	3.60±1.75**	4.50±2.20	1.95±0.60**	3.10 ±1.90**	4.70±1.50*	1.90±0.59**
Control group	8.30±1.36	5.70±0.66	7.03±2.00	8.50±1.30	6.10±0.50	2.60±0.61

P values when compared with control: *<0.05, **<0.001

The study also evaluated the effectiveness of liver enzymes for each of the workers in the oil refinery and gas station. The results of the study showed that GOT enzyme level was significantly increased among the samples of the workers in oil refinery compared to the control group, while no significance change in GOT was observed among the gas stations workers. Meanwhile, the levels of both GPT and ALP enzymes decreased significantly in each of the workers in the oil refinery and gas stations compared to the control group, Table 3. GOT and GPT are liver enzymes and also exist in various tissues, and a change in the levels of these enzymes indicates a liver disease. However, GOT considered a better

indicator compared to GPT, because many health issues in the human body increase the serum GPT.¹¹ For this reason, the elevation in GOT in oil refinery group is likely related to the destruction of liver cells and releasing GOT enzyme in the blood. The results also showed a lower serum ALP levels for both of oil refinery and gas station, compared to the control group. The lower ALP levels indicates a deficiency of zinc or magnesium in the blood serum of the workers in both groups, as ALP function is directly linked to levels of these two metals.¹²

1 A significant difference between Oil refinery workers and control group (P <0.05).

2 A significant difference between Gas station workers and control group (P <0.05)

Table 3: Liver enzymes GOT, GPT, ALP in (Mean±SD) with P-values.

	Oil Refinery	P ¹ value	Gas station	P ² value	control
GOT (U/L)	31.45±11.91	P <0.001	16.87±9.50	0.71	17.33±4.44
GPT (U/L)	17.95±11.00	P <0.05	11.70±8.10	P <0.001	25.46±5.33
ALP (U/L)	141.58±52.30	P <0.001	141.17±38.60	P <0.001	210.50±11.98

Table 4 list the results of the liver hormones levels for smokers and non-smokers . That data indicated a significant decrease in GPT and ALT enzymes among smokers in the refinery workers, while GOT levels increase within the same group. For the smokers in gas station, the results showed a decrease in ALP and GPT enzymes compared to the smokers of the control group. Similar trend is observed with liver enzymes

when comparing a non-smoker from oil refinery to a smoker from control group. The mean serum GOT is significantly higher (27.30 U/L) than the control group(17.33 U/L), while the levels of serum ALP was less (151.35 U/L) than the values found in the control group(215.50 U/L), and for both of oil refinery and gas station workers.

Table 4: GOT, GPT and ALP enzymes for smokers and non-smokers in the three study groups.

	GOT (U/L)	GPT (U/L)	ALP(U/L)	GOT (U/L)	GPT (U/L)	ALP (U/L)
	Non-Smoker			Smoker		
Oil refinery workers	27.30±9.80**	19.80±11.90	151.35±64.00	35.80±12.60**	15.90 ±10.00**	131.10±35.20**
Gas station workers	16.10±3.80	10.70±6.30**	143.00±40.20**	17.60±13.50	12.70±9.90**	139.10±38.70**
Control group	17.33±4.59	24.50±6.15	205.50± 9.94	17.33±4.40	26.41±4.40	215.50±12.10

P values when compared with control: *<0.05, **<0.001

Conclusion

The study conclude that direct and indirect exposure to pollutants resulting from petroleum products causes a clear effect on the level of pituitary gland hormones, as well as an effect on liver enzymes. These findings are important and can be used to improve the work environment for the people work in the oil industry.

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

Funding: This study was self-funding.

Ethical clearance: This study was approved by the committee of Ethical research in University of Basra, Department of chemistry. And later on, a paper was sent to the oil refinery and Al-jazaer gas station to get blood samples from workers and use it for this research.

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Clinical Course and Outcome of COVID-19 Patients in a Tertiary Care Hospital: A Retrospective Study

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How to cite this article: Rizwan Yusaf Aziz A, Kisan Khade, Swati Sonawane et al. Clinical Course and Outcome of COVID-19 Patients in a Tertiary Care Hospital: A Retrospective Study. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):302-308.

Abstract

Introduction: COVID-19 the deadly virus, was declared a pandemic by WHO in March, 2020 because of its virulent nature. It has been a piece of work to understand the mechanism of action and the disease pathology of the virus, due to its novel origin. The quality of healthcare is seen to be severely degrading during these times. The two different types of COVID tests that are commonly available in the facility are RTPCR and Rapid Antigen Test or RAT. These help to identify whether the person is infected with the virus or not. With the current management being successful in majority of the cases, we should also consider strengthening the existing modalities.

Objective: To find the prevalence of COVID-19 patients and study their clinical course and outcome

Material and methods: Data regarding covid-19 patients was collected on basis of demographic profile by using google forms questionnaire at Dr. D.Y. Patil Hospital, Navi Mumbai.

Results: A total of 500 patients were included in the present study with male preponderance with 61.4%. 99% patients were tested COVID positive, 73.2% had a history of exposure to COVID patients, 80% had a positive travel history, 99% lab tests were positive. 50.4% duration of the treatment lasted for 7-14 days. 67.6% were treated in the wards, whereas, the remaining 32.4% were treated in the ICU. 19.6% patients required mechanical ventilator support. 51% patients required oxygen therapy. 80.2% did not require intubation. All the patients were on antibiotics, and majority of the patients, i.e. 99.8% were on immuno-boosters as well. Antivirals were administered in 80.6%. 82.8% were discharged with a negative swab. Out of the 17.2% patients with positive swab, mortality was seen in 6.2% patients. 3.4% patients were referred to different centers, while the treatment of the remaining 7.6% patients was continued for a longer period of time.

Conclusion: A stepwise perspective of non-pharmaceutical interventions, screening and testing procedures, implementation and compliance to distancing, hygiene measures and use of masks at airports, railway stations, other public places with pragmatic testing and tracing are effective measures that can be implemented. Worldwide

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numerous clinical trials are taking place for the treatment and prevention of COVID-19. Although, there is a rapid comprehensive expansion in regard to COVID-19 and few agents appear to be promising, there are no definitely proven effective therapies at this time. Evaluation of several agents by Scientists and researchers are progressive and commendable.

Keywords: Antiviral; Comorbidity; COVID-19; HRCT; Nasopharyngeal Swab; Negative Swab; Mortality; PCR antigen test.

Introduction

COVID-19 the deadly virus, was first discovered in Wuhan, China in the year 2019. COVID 19 popularly known as Coronavirus has swamped the entire world like a termite and created a ruckus in the modern medicine. It was declared a pandemic by WHO in March, 2020 because of its virulent nature. In the past, multiple health concerns in relation to the various coronavirus strains have been identified, namely, The Middle Eastern Respiratory Syndrome (MERS), Severe Acute Respiratory Syndrome (SARS) and the COVID -19. This ongoing pandemic caused by the COVID-19 virus, has held the medical fraternity in the conjectures of the challenges seen and yet to come. It has been a piece of work to understand the mechanism of action and the disease pathology of the virus, due to its novel origin. Another considerable reason for non substantial data availability can be comparatively lesser autopsies.¹ The global pandemic, has adversely affected the health of the population in every sphere- physically, psychologically and economically. Grueling times were made worse by the global COVID 19 outbreak. The psychological and financial set back due the pandemic has summed up an arena of psychiatric problems leading to mental instability like anxiety and depression.² A vast number of patients in home quarantine or isolation have developed negative outlook towards their health and other worldly affairs. Main concerns expressed by the COVID 19 patients were Stigma and uncertainty of viral disease progression. Patients who are hospitalized have recorded to experience significant psychological distress, studies have correlated this with the presence of higher inflammatory markers, which are said to have depressive qualities.³

The quality of healthcare is seen to be severely degrading during these times. Out of many reasons, the reasons that can be held most accountable for the same are; One, with the known risk of getting infected with virus, people are terrified to visit a clinic or hospital believing that they might come in contact with an infected patient or contaminated objects.

Secondly, because of the lock-down and restrictions imposed on travel, majority of them are unable to reach a healthcare practitioner. Patients with minor ailments or long term health conditions who require constant follow up, are either terrified or barred to travel because of the imposed restrictions. In some parts, health workers and doctors have minimized their practice, mainly to avoid the spread of infection. Healthcare services in some areas may have been hampered because of quarantine of the healthcare workers. In the fear and desperation of protecting self, it is plausible that people may consume medicines with unknown effect for prevention against COVID-19.^{4,5}

It is not news that HCWs, especially the front-line workers in the battle against COVID-19, are at the highest risk.⁶ Non-healthcare occupations at the highest risk of contacting the infection are dominated by workers who are either in direct contact with high risk environment, such as flight attendants, teachers, barbers, jailers, and transportation security screeners, or may be directly exposed to SARS-CoV-2 (ambulance drivers, morticians, embalmers). These workers do not have an option of working remotely, hence, specific on the job interventions are required to prevent them from getting infected, these mainly involve the precautionary measures.⁷

The country had a humongous rise in the number of cases of COVID-19. The disease spread like a forest fire, increasing number of people started getting infected with the novel disease which lead to the rise in the number of people getting tested for the same. However, getting an RTPCR or RAT test done became a tedious job. From the booking of test slots, to the availability of testing kits and maintenance of necessary precaution during testing was extremely difficult. The two different types of COVID tests that are commonly available in the facility are RTPCR and Rapid Antigen Test or RAT. These help to identify whether the person is infected with the virus or not. RTPCR is considered the gold standard for testing COVID-19, while the latter that is RAT is used to determine SARS-CoV-2 antigens in

the patient. Despite the availability of tests, a good amount of false-negative results have been reported. The main reasons found to be responsible are related to the sampling procedures, sources of samples, and the sensitivity/specificity of the nucleic acid test kit. As a consequence, when asymptomatic subjects are tested it is quite impossible to distinguish between recurrence of COVID-19 infection or intermittent shedding of RNA fragments or new-onset infections. However, it could be possible in cases with persistent infections, reactivation or reinfection of the virus with another strain, especially if comorbidities are present.⁸

There is a grave exigency for effectual and specific antiviral treatment. The rapid genomic sequencing of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) laid out a noteworthy number of therapeutic targets. Worldwide numerous clinical trials are taking place, calculating and evaluating multiple investigational agents and other known drugs which are used for other diseases for the treatment and prevention of COVID-19. Although, there is a rapid comprehensive expansion in regard to COVID-19 and only a few agents appear to be promising in these difficult times, there are no definitely proven effective therapies that are known. Evaluation of several agents by Scientists and researchers are progressive and commendable. The known management followed currently include, supportive care measures such as ventilation, oxygenation, fluid management and immuno-boosters. With the current management being successful in majority of the cases, we should also consider strengthening the existing modalities.

Objectives

1. To find the prevalence of COVID-19 patients
2. To study the clinical course and outcome of COVID-19 patients

Material Methods

Study Subjects: COVID-19 suspects admitted in Dr. D.Y. Patil Hospital, Navi Mumbai

Study Design: Retrospective Study

Sample Size: 500

Study Duration: 3 months (June-August 2020)

Sampling Method: After the approval of the Institutional Ethical Committee, data regarding

demographic details, clinical course and outcome of the patients was collected from the hospital database. Utmost care was taken to maintain the privacy and confidentiality.

Data Analysis: The data collected was coded and entered in Microsoft Excel and analyzed using SPSS version 17.0 software. Descriptive statistics was used for data analysis and the data was represented in the form of percentages, mean.

Results

A total of 500 patients were included in the present study. The study population had a male preponderance with 307(61.4%). It was observed that many patients had pre-existing comorbidity.

The study participants visited the hospital with different presenting complaints; fever, anosmia and sore throat topping the list. Other clinical features are mentioned in Table 1.

Table 1: Presenting Complaints

Clinical Features	Study Population (n=500)	Study Population (%)
Fever	500	100
Headache	402	80.4
Breathlessness	250	50
Sore throat	434	86.8
Running nose	70	14
Anosmia	477	95.4
Loss of taste	121	24.2
Body Ache	374	74.8
Diarrhoea	209	41.8
Cough	180	36

Out of 500 patients, 495 (99%) patients were tested COVID positive. 366 (73.2%) stated they had a history of exposure to COVID patients. Among the 500 patients, 400 (80%) had a positive travel history.

Various investigations were done, like, blood tests (0.2% cases), HRCT (81.8% cases), nasopharyngeal swab (11% cases) and PCR antigen test (7% cases), out of which, 495 (99%) lab tests were positive. The treatment approach differed from case to case. In most of the cases, 252 (50.4%), duration of the treatment lasted for 7-14 days. Details regarding the duration of the treatments is given in Figure 1.

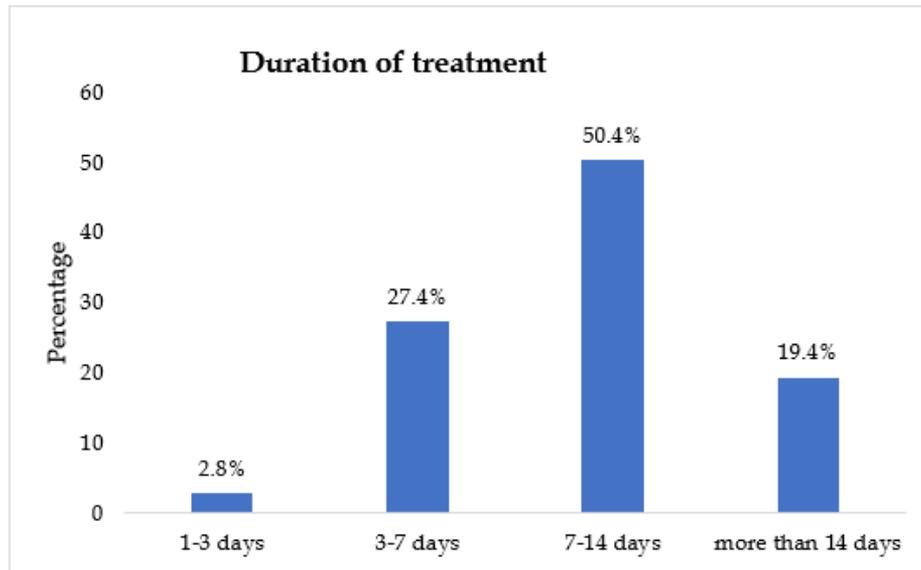


Figure 1

Majority of the patients, 338 (67.6%) were treated in the wards, whereas, the remaining 162 (32.4%) were treated in the ICU. Only, 98 (19.6%) patients required mechanical ventilator support during the course of their treatment. 255 (51%) patients required oxygen therapy. The details of oxygen therapy

administration is given in Figure 2. Majority of the patients, 401 (80.2%) did not require intubation. All the patients were on antibiotics, and majority of the patients, i.e. 499 (99.8%) were on immuno-boosters as well. Antivirals were administered in majority cases, 403 (80.6%).

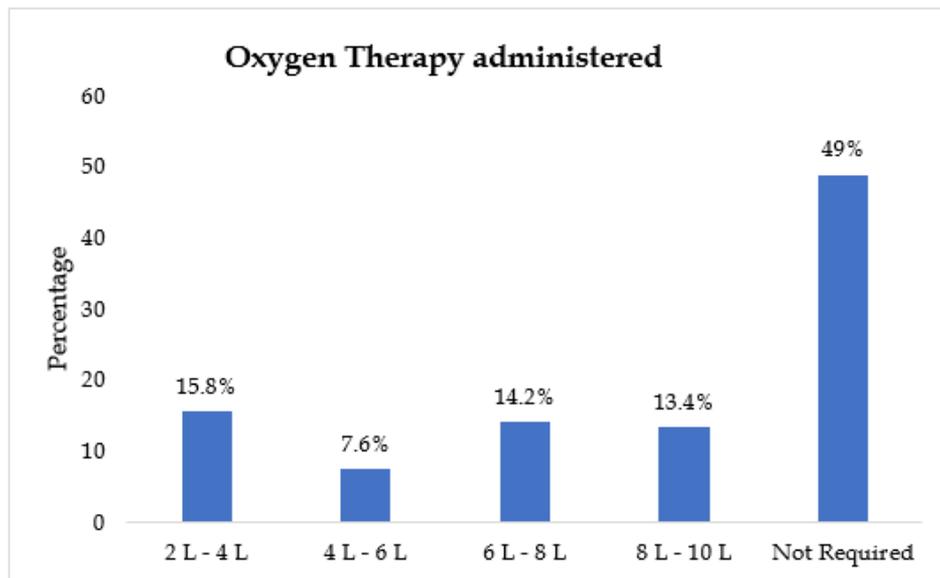


Figure 2

Out of 500 patients, 414 (82.8%) were discharged with a negative swab.

In the study population of 500 patients, out of the 86 (17.2%) patients with positive swab, mortality was

seen in 31 (6.2%) patients. 17 (3.4%) patients were referred to different centers, while the treatment of the remaining 38 (7.6%) patients was continued for a longer period of time. The details regarding the outcome is given in Figure 3.

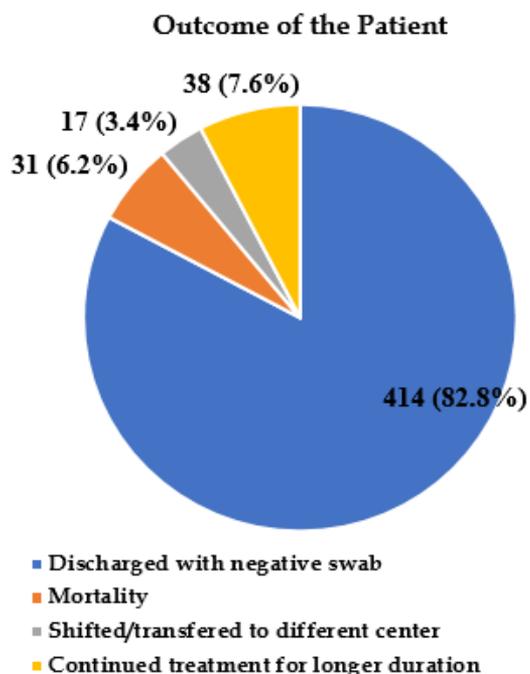


Figure 3

Discussion

A total of 500 patients were included in the present study. The study population had a male preponderance with 307(61.4%). Similar findings were observed in a meta-analysis conducted by Li LQ et al.⁹ On dividing the patients by taking 60 yrs of age as threshold, it is observed that 444 (88.8%) patients were <60yrs, which is similar to the findings of a study conducted by Lui Y et al., where majority of the study participants were aged <60 yrs.¹⁰ Majority of the individuals were found to be living in joint family. Enclosed space and overcrowding could have acted as a contributing factor.

124(24.8%) individuals had pre-existing comorbidity, in which hypertension topped the list. Similar findings were observed in a study conducted by Atkins JL et al.¹¹

Individuals related to the health care system were affected the most. In the present study, 79 (15.8%) healthcare professionals, 46 (9.2%) hospital employees and 18 (3.6%) individuals working in the COVID hospital were affected, comprising of 28.6% of the study sample. According to a recent study, it is not surprising that HCWs, especially those at the front-line in the battle against COVID-19, are at the highest risk.⁶ In global research on SARS-CoV-1, MERS-CoV and SARS-CoV-2, it can be seen that

a very large percentage of the number of infected people are health professionals struggling with them in various medical facilities. This is most often due to the fact that medical personnel is incorrectly trained in proper protection against the virus and lack of equipment that meets the relevant standards.¹² In the case of SARS-CoV-1, medical personnel accounted for 21.07% (1706/8096) of all infections, MERS-CoV 13.37% (183/1368).^{13,14} Currently, the total number of infected healthcare workers on SARS-CoV-2 is unknown due to the steadily increasing number of infections and the lack of global data on the problem.

The study participants visited the hospital with different presenting complaints, fever being the most common. Various other findings have observed the same pattern.¹⁵⁻¹⁸

Out of 500 patients, 495 (99%) patients were tested COVID positive. 366 (73.2%) stated they had a history of exposure to COVID patients. Among the 500 patients, 400 (80%) had a positive travel history. Fever screening and history taking on traveling to risk area is the general primary for screening for disease. It is found that disguising on travel history is a big problem that results ineffectiveness of using fever and travel history screening as preventive tool against COVID-19.¹⁹ A recent study used real-time mobility data from Wuhan and detailed case data including travel history to elucidate the role of case importation in transmission in cities across China and to ascertain the impact of control measures. Early on, the spatial distribution of COVID-19 cases in China was explained well by human mobility data. After the implementation of control measures, this correlation dropped and growth rates became negative in most locations, although shifts in the demographics of reported cases were still indicative of local chains of transmission outside of Wuhan. This study shows that the drastic control measures implemented in China substantially mitigated the spread of COVID-19.²⁰

Various investigations were done, like, blood tests (0.2% cases), HRCT (81.8% cases), nasopharyngeal swab (11% cases) and PCR antigen test (7% cases), out of which, 495 (99%) lab tests were positive. The treatment approach differed from case to case. In most of the cases, 252 (50.4%), duration of the treatment lasted for 7-14 days. Majority of the patients, 338 (67.6%) were treated in the wards, whereas, the remaining 162 (32.4%) were treated in the ICU. Only, 98 (19.6%) patients required mechanical ventilator

support during the course of their treatment. 255 (51%) patients required oxygen therapy. Majority of the patients, 401 (80.2%) did not require intubation. All the patients were on antibiotics, and majority of the patients, i.e. 499 (99.8%) were on immuno-boosters as well. Antivirals were administered in majority cases, 403 (80.6%).

Out of 500 patients, 414 (82.8%) were discharged with a negative swab. Age could have played a contributing factor for this result as majority of the patients, i.e. 125 (25%) lie in the age group of 21-30 yrs, followed by 111 (22.2%) in the age group of 31-40 yrs. In the study population of 500 patients, out of the 86 (17.2%) patients with positive swab, mortality was seen in 31 (6.2%) patients. 17 (3.4%) patients were referred to different centers, while the treatment of the remaining 38 (7.6%) patients was continued for a longer period of time.

Conclusion

COVID-19 has created a lot of Stigma and uncertainty regarding the viral disease progression among the patients. Patients have experienced significant psychological distress, and the levels of depressive features are correlated to the inflammatory markers in these patients. It is important to address the psychiatric symptoms for COVID-19 patients, and enhance the coping strategies and other psychological interventions. Improvement in prevention and effective management of COVID-19 will need basic public health and clinical interventions. The pathogenesis of the Novel coronavirus is still not well defined. Most patients present with a self-limited course, however, a few experience severe or even life threatening disease. The treatment approaches currently under investigation include antiviral and anti-pro-inflammatory cytokines, anti-infectious and life support therapies, monoclonal antibodies and passive immunotherapy, especially in patients with bad prognosis. Although, the therapeutic strategy against the disease is of great significance and this is the main way to prevent virus spread is the development of an effective and safe vaccine widely. It is a necessity especially in developing countries to make the vaccines available and reasonable for the entire population of the country.

We should always evaluate our own health condition before taking any long-distance public transportation. The trip should be avoided if any symptoms like fever or cough are present. Even

while going to fever clinics wear face masks and take all necessary precautions. Due to lack of data on the effectivity of face masks, further research should focus on assessing the efficacy of face masks against COVID-19. For economical benefits investigating regarding reuse of face masks and assessing compliance should also be taken into consideration. Quarantine of travelers may delay introduction or re-introduction of the virus, or may delay the peak of transmission, but it has limited evidence. There should regular disinfecting practices followed everywhere.

Presently, the summed up aggregate of number of infected healthcare workers on SARS-CoV-2 is unknown due to the continuously increasing number of infections and the lack of global data with respect to COVID 19. We often ignore the affective of COVID 19 among the HCWs, this extremely important aspect of the fight against the current pandemic, and should not be overlooked. The cost that we will be paying for this in future will force us to reconstruct the health care systems around the world and the security of medical staff needs urgent improvement.

A stepwise perspective of non-pharmaceutical interventions, screening and testing procedures, implementation and compliance to distancing, hygiene measures and use of masks at airports, railway stations, other public places with pragmatic testing and tracing are effective measures that can be implemented.

Funding: Nil

Interest of conflicts: Nil

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The Prevalence and Success Factor of Ibuprofen and Paracetamol Administration for Neonates with Patent Ductus Arteriosus at RSUD Dr. Soetomo

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How to cite this article: Rodia Amanata Rofiq, I Ketut Alit Utamayasa, Nurina Hasanatuludhhiyah et al. The Prevalence and Success Factor of Ibuprofen and Paracetamol Administration for Neonates with Patent Ductus Arteriosus at RSUD Dr. Soetomo. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):309-316.

Abstract

Background: Four thousand neonates with Persistent Ductus Arteriosus every year in Indonesia. There are two treatment options to treat Persistent Ductus Arteriosus, they are surgery and pharmacological therapy. Ibuprofen and paracetamol can be used as pharmacological therapy for Persistent Ductus Arteriosus with minimal side effects.

Objective: To analyze the prevalence and success factors in giving ibuprofen and paracetamol in neonates with persistent ductus arteriosus.

Method: This research is an observational analytic research with cross-sectional method. The determination of the research sample uses a total sampling technique by taking all members of the population in accordance with the conditions from January, 2016 to March, 2020. Bivariate analysis was performed using the Spearman rank test with 95% confidence interval ($\alpha=0.05$).

Result: From 51 samples that met the inclusion criteria, it was found that the most criteria were male (66.67%), normal birth weight (60.78%), term (76.48%), moderate defect size before being administered pharmacological therapy (47.06%), pharmacological therapy using paracetamol (88.24%), atrial septal defect in the cardiac comorbidities category (21.74%) and hyperbilirubinemia in the non-cardiac comorbidities category (13.04%). The majority of lumen defects in neonates were closed completely after being administered pharmacological therapy (72.55%). Statistical test results of Spearman rank showed that no significant relationship between birth weight and pharmacological therapy in RSUD Dr. Soetomo. There was a significant relationship between gestation and pharmacological therapy ($p = 0.000$; $r = -0.495$; $r^2 = 0.237$, 95% CI). There was a significant relationship between the size of the ductus arteriosus defect before therapy and pharmacological therapy ($p = 0.001$; $r = -0.435$; $r^2 = 0.211$, 95% CI).

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Conclusion: The success factors in administrating ibuprofen and paracetamol for neonates with persistent ductus arteriosus in RSUD dr. Soetomo from January 1, 2016 to March 3, 2020 were affected by gestation period and size of the ductus arteriosus defect prior to pharmacological therapy.

Keywords: Patent Ductus Arteriosus; neonate; ductus arteriosus.

Introduction

Four million neonates die due to asphyxia every year. Indonesia is in the fifth place of neonatal asphyxia mortality rate in ASEAN countries in 2011 of 35 per 1000.¹ The high incidence of asphyxia in newborns can cause complications such as the failure of the ductus arteriosus to be closed in neonates. Asphyxia that causes persistent ductus arteriosus can be caused by lung function that has not developed optimally and birth weight is underweight.² Besides caused by lungs that are not yet optimal, Reactive Oxygen Species (ROS) causes high levels of prostaglandins (PG), nitrogen monoxide (NO), and decreased sensitivity of calcium and oxygen to muscle blood vessels. Thus, it causes the ductus arteriosus do not close in neonates.³

The treatment of PDA (Patent Ductus Arteriosus) continues to develop until now. Initially, the management of PDA was conducted in an invasive manner through surgery.⁴ However, surgery requires a lot of money and not all hospital can provide it. It is considered ineffective to treat small to moderate PDA. Along with the development of technology and knowledge in the medical field, surgical or non-pharmacological therapy is only carried out if a large ductus arteriosus is found, it does not respond to the use of at least two therapeutic drugs, patients are on ventilators and patients with high oxygen needs. Meanwhile, pharmacological therapy can be performed by administering indomethacin (non-selective COX inhibitor) as one of the nonsteroidal anti-inflammatory drugs (NSAIDs). Indomethacin can cause side effects such as impaired kidney function, gastrointestinal bleeding, and impaired cerebral blood flow.⁴ Therefore, other NSAIDs are needed. They can be used for DAP closure but with low side effects. Ibuprofen and paracetamol can be used as closure therapy for PDA with minimal side effects.⁶

The use of ibuprofen for Persistent Ductus Arteriosus has received approval from the FDA (Food and Drug Administration) since 2009.⁷ The effectiveness of ibuprofen as a therapeutic drug in PDA is around 60% to 80%.⁸ 30% of PDA patients have closed the lumen of their ductus arteriosus in

24 hours of being administrated ibuprofen therapy.⁹ Paracetamol is used when the patient is intolerant to ibuprofen or they has conditions that contraindicate the use of ibuprofen.¹⁰ The success rate of using paracetamol in closing the ductus arteriosus is 83.3% to 100%.¹¹ Ductus Arteriosus successfully closes using paracetamol at 55/59 or about 93 % of PDA patients.⁶

The success of pharmacological therapy on Persistent Ductus Arteriosus using ibuprofen and paracetamol can be affected by several factors. Therefore, researchers are encouraged to conduct research with the main objective of obtaining information about the characteristics of neonates with Persistent Ductus Arteriosus and the results of pharmacological therapy and the factors that influence the success of pharmacological therapy in Dr. Soetomo for the period of January 1, 2016 to March 3, 2020.

Methods

This research was a type of retrospective analytic observational study with a cross-sectional study design using secondary data in the form of medical records. This research was conducted by analyzing data obtained from the medical records of persistent ductus arteriosus neonates in the pediatric inpatient room dr. Soetomo for the period of January 1, 2016 to March 3, 2020. The sample size used the total sampling technique with inclusion criteria, such as persistent ductus arteriosus neonates who needed pharmacological therapy to close the ductus arteriosus. The exclusion criteria were (1) persistent ductus arteriosus neonates who had incomplete medical records, (2) undergoing surgical therapy to close the ductus arteriosus, (3) the patient died during the pharmacological therapy of PDA. The data taken were gender, gestation period, birth weight, pharmacological therapy, defect size, results of therapy, comorbidities. The independent variables in this research were the type of pharmacological therapy selected, gestation period, birth weight, gender, and size of the ductus arteriosus defect before treatment. The dependent variable in this research was the success rate of pharmacological therapy in Persistent Ductus Arteriosus patients. Data processing was performed using SPSS version

26 software. Univariate analysis was conducted descriptively to determine the characteristics of the sample. Bivariate analysis to determine the relation between the independent variable and the dependent variable was conducted using the Spearman Rank analysis test with a 95% confidence level. Significance value of (p value) <0.05 was considered significant.

Results

From 125 neonates with persistent ductus arteriosus who were hospitalized at Dr. Soetomo from January 1, 2016 to March 3, 2020, it was obtained 51 complete medical records and met the criteria for research sample.

Tabel 1: Characteristics(n=51)

Characteristics	n	%
Gender		
Male	34	66,67
Female	17	33,33
Birth Weight		
LBW	18	35,3
NBW	31	60,78
HBW	2	3,92
Gestational Age		
Preterm	9	17,64
Aterm	39	76,48
Posterm	3	5,88
Size of Ductus Arteriosus Before Pharmacology Therapy		
Large	8	15,69
Moderate	24	47,06
Small	19	37,25
Size of Ductus Arteriosus After Pharmacology Therapy		
Large	2	3,92
Moderate	5	9,80
Small	10	19,61
Closed	34	66,67
Pharmacology Therapy		
Ibuprofen	6	11,76
Paracetamol	45	88,24

* LBW: Low Birth Weight (<2500 gram), NBW: Normal Birth Weight (2500-4000 gram), HBW : High Birth Weight (>4000 gram)

The characteristics of the sample in this research is shown in Table 1. The majority of the samples were

male (66.67%), birth weight was between 2500 - 4000 grams (60.78%), gestation period of 37-42 weeks (76.48%), the size of the defect was moderate before being given pharmacological therapy (47.06%), the choice of pharmacological therapy using paracetamol (88.24%), the comorbid Atrial Septal Defect in the comorbid cardiac disease category (21.74%) and hyperbilirubinemia in category of non-cardiac comorbidities (13.04%).

Tabel 2: Comorbidities

	Comorbidities	%
Cardiac	Atrial Septal Defect	21,74
	Tricuspid Regurgitation	10,14%
	Dextrocardia	1,45%
	Mitral Valve Regurgitation	1,45
	Hipertensi Pulmonal	1,45
Non - Cardiac	Hyperbilirubinemia	13,04
	Septic	8,70
	Asphyxia	7,25
	Pneumonia	7,25
	Hypoglycemia	4,35

Table 2 shows the top five distributions of comorbidities in each category (cardiac comorbidities and non-cardiac comorbidities) in neonates with persistent ductus arteriosus. The percentage was obtained from 51 neonates. A neonate can have more than one comorbidity. The majority of neonates with PDA suffered from atrial septal defects in the cardiac comorbidities' category and hyperbilirubinemia in the non-cardiac comorbidities category.

Tabel 3: The Spearman's Rank Correlation Test Result

Spearman's Rank		p value	r	r ²
Birth Weight	LBR	0,412	0,128	0,016
	NBR			
	HBR			
Gestation Age	Preterm	0,001	0,442	0,195
	Aterm			
	Posterm			
Size of Ductus Arteriosus	Small	0,001	0,459	0,211
	Moderate			
	Large			

* LBW: Low Birth Weight (<2500 gram), NBW: Normal Birth Weight (2500-4000 gram), HBW : High Birth Weight (>4000 gram)

The analysis result of the relation between birth weight and the success of therapy did not show a significant relation. It was evidenced by the significance value of the p value of 0.412 ($p \text{ value } (0.412) > \alpha (0.05)$). The coefficient of determination (r^2) was 0,016. Thus, it can be stated that the effect of birth weight on the success of therapy in PDA in RSUD dr. Soetomo was 1.6% and the rest was affected by other factors.

With the results of statistical tests using the Spearman SPSS correlation method, the p value = 0.000 ($p < 0.05$), it indicated that there was a significant relationship between gestation period and the success rate of pharmacological therapy in neonates with persistent ductus arteriosus. Spearman's rho correlation coefficient (r) was 0.495. It means that the level of closeness of the relation between gestation and the success of pharmacological therapy at Dr. Soetomo Hospital was in the moderate category (0.40 - 0.59). A positive sign means that the direction of the relation between the two variables was unidirectional. It means that the greater the gestation period, the greater the success of pharmacological therapy. The coefficient of determination (r^2) was 0.237. Furthermore, this value can be multiplied by 100% to convert it into a percentage. Thus, it can be stated that the effect of defect size on the success of therapy in PDA in RSUD dr. Soetomo was 23.7% and the rest was affected by other factors.

The results of the analysis of the relationship between the size of the ductus arteriosus lumen defect and the success of therapy using the SPSS correlation method were the significance value (p value) of 0.001. It means $p \text{ value } < \alpha (0.05)$. Thus, it can be stated that there was a significant relation between the size of the lumen defect and the success of pharmacological therapy in RSUD dr. Soetomo. The correlation coefficient value of Spearman's rho (r) was -0.435. It means that the degree of closeness of the relation between the size of the lumen defect and the success of pharmacological therapy in RSUD Dr. Soetomo was in the medium category (0.40 - 0.59). The negative sign means that the direction of the relation between the two variables was opposite. Thus, the larger the defect size, the smaller the success of pharmacological therapy. The coefficient of determination (r^2) was 0.211. next, this value could be multiplied by 100% to convert to a percentage. Therefore, it can be explained that the effect of defect size on the success of therapy in PDA in RSUD dr. Soetomo was 21.1% and the rest was affected by other factors.

Discussion

A. Sample Characteristic

From 51 persistent ductus arteriosus neonates in this research, it was found that a higher percentage of male babies (66.67%) than women was found. This result was not in accordance with the research conducted by James E. Dice. It showed that the incidence rate of neonatal PDA in females compared to males was 2: 1.¹² More PDA cases in males can be caused by fetuses of the female that have less prostaglandin synthase and greater prostaglandin dehydrogenase than males.¹³

The majority of PDA neonates were born with adequate birth weight of around 2500 - 4000 grams of 31 neonates (60.78%). The majority of subjects in this research were neonates with normal birth weight, because the majority of neonates who were born underweight would undergo further surgery to treat PDA. Thus, they would be included in the exclusion category in this research. The majority of neonates with sufficient birth weight with PDA would be given pharmacological therapy because the body organs were structurally and functionally mature. Thus, they were at low risk of experiencing metabolic and cardiovascular disorders.

The results of this research also showed that from a total of 51 neonates, the most samples were found in the group at term gestation (37 - 42 weeks) of 76.48%. This was not in accordance with other research stated that one third of neonates with PDA were neonates born prematurely.¹⁴ Neonates with less gestational age would worsen the ability to absorb, distribute, metabolize and excrete drugs. Thus, in preterm neonates, the safety of both short and long-term pharmacological therapy was unclear because the resulting effect of increased indirect free and total bilirubin might increase the risk of developing bilirubin encephalopathy. Organ maturity that has been already perfect in term and postterm neonates, both structurally and functionally, had a low risk of experiencing metabolic and cardiovascular disorders.¹⁵

The use of ibuprofen therapy for Persistent Ductus Arteriosus patients has been researched since 2006 and received approval from the FDA (Food and Drug Administration) in 2009. Paracetamol can't be used as a first-line drug for PDA.⁷ Yet, based on research conducted by Dang et al., Paracetamol can be used as one of the main therapies chosen to close

Ductus Arteriosus in neonates, even when Ductus Arteriosus lumen reopening occurs, paracetamol is still proven to be effective.¹⁶ Based on the results of the research, it was found that 6 neonates received ibuprofen as PDA therapy and the remaining 45 out of 51 neonates received paracetamol therapy to treat PDA. Ibuprofen used for DAP therapy can be oral or i.v.¹⁷. Ibuprofen in i.v. is not available in some countries.¹⁸ The majority of samples used paracetamol. Paracetamol is used as a therapy for Persistent Ductus Arteriosus because it has been shown to reduce prostaglandin synthesis. However, the actual mechanism of action of Paracetamol in closing AD is still a controversy. Paracetamol has only very weak peripheral PG-related effects and exerts its main effect via the central nervous system.¹⁹ Some researchers suggested that paracetamol was very beneficial over ibuprofen because paracetamol does not cause peripheral vasoconstriction. Thus, it can be used in neonates with ibuprofen contraindicated.²⁰ Paracetamol does not have the effect of gastric irritation and inflammation like other classes of NSAIDs. Therefore, it leads paracetamol to be one of the best therapeutic options for PDA cases.

Low doses of paracetamol have proven to be harmless. It does not cause side effects and it is effective in closing PDA.²¹ However, when used in large doses, paracetamol can cause severe liver necrosis to impaired liver function.²² In the research, there were no patients who had hepatotoxicity. Ibuprofen can shift the binding of bilirubin with albumin, causing hyperbilirubinemia in neonates. This occurs because newborns have low plasma protein concentrations and albumin-binding capacity. Thus, it affects their ability to bind to drugs that are extensively bound to plasma proteins. The low amount of plasma protein will cause several adverse drug effects, such as hyperbilirubinemia.¹⁶ Paracetamol has a lower risk of hyperbilirubinemia than patients using ibuprofen.¹⁰ The results of this research indicated that there were 18 neonates suffering from hyperbilirubinemia, but there were no lab results stating that hyperbilirubinemia in cases of neonatal PDA was caused by pharmacological therapy. Hyperbilirubinemia is common in newborns.

In this research, it was found that ibuprofen succeeded in closing Ductus Arteriosus by 4/6 neonates (66.67%) and paracetamol was able to close DA by 30/45 neonates DAP (66.67%). However, this result was lower than previous researches. Thus,

the further data and research are needed to study this case.

B. The Success Factor of Pharmacological Therapy Based on Test Results

The test results using the correlation method in SPSS showed that birth weight with successful therapy in Persistent Ductus Arteriosus neonates in dr. Soetomo did not show a significant relation. This could be due to the uneven distribution of the sample in each category, there were 18 neonates with DAP (35.3%) who were born in the low birth weight category (<2500 grams), 31 neonates (60.78%) were born with body weight adequate birth weight (2500 - 4000 grams), and 2 (3.92%) DAP neonates were born overweight (> 4000 grams). The sample gap occurred because neonates who were born underweight would undergo further surgery to treat PDA. Hence, they would be included in the exclusion category in this research.

The test results using the correlation method in SPSS showed that the gestation period with the success of therapy had a significant relation. The gestation period determines the quality of the newborn's health because it was related to the readiness of the structure and function of organs for extrauterine life. The lower the gestation period was the higher the risk of the neonates developing PDA because of the worsening cardiopulmonary immaturity. The gestation period would affect the formation of cells in the smooth muscle media tunica of intrauterine organs and the response to oxygen at birth. Preterm neonates tended to have a low response to oxygen but had a high sensitivity to the effects of vasodilation and prostaglandin E2 and nitric oxide.²² This was different from term or postterm neonates born, in the third trimester of pregnancy. The Ductus Arteriosus muscle would become thicker and experience sensitivity to systemic prostaglandins produced by the placenta and Ductus Arteriosus itself.²³ Neonates with a low gestation period will also have a low Ductus Arteriosus closure rate.²² Preterm neonates with PDA tend to suffer from various complications that aggravate the condition, such as RDS (respiratory distress syndrome), pulmonary bleeding, bronchopulmonary dysplasia, necrotizing enterocolitis (NEC), kidney failure, intraventricular hemorrhage (IVH), periventricular leukomalacia (PLV), cerebral palsy, until falling in a condition that requires further ventilation and even death.²⁵ The lower the gestation period, the lower the maturation

of the liver and kidneys. When the liver has not yet fully formed, this needs to be watched out when using paracetamol as a pharmacological therapy because more paracetamol is metabolized in the liver. Hence, the less gestational age will worsen the ability of drug absorption, distribution, metabolism and excretion.

The test results using the correlation method in SPSS showed that the defect size and the success of therapy had a significant relation. The larger the defect size, the smaller the success of pharmacological therapy. In normal and healthy neonates, the left side of the heart will pump clean blood (containing O₂) to the whole body and the right side of the heart will pump dirty blood (full of CO₂) to be channeled to the lungs. However, in neonates with PDA, there will be blood pumped from the aorta to the lungs or vice versa due to an open lumen. The direction of back flow (shunt) depends on the difference in resistance and pulmonary circulation with systemic circulation.²³ In large PDA, the neonate would breathe faster and louder than usual. The lungs of a neonate with large DAP would have a higher pressure because more blood is pumping in the lungs. The increase in the amount of blood in the pulmonary circulation will cause an increase in pulmonary fluid volume, decrease pulmonary compliance, difficulty breathing, and cause edema in neonates. Meanwhile, the increased blood flow in the left ventricle will cause the ventricle to overload, the ventricle will experience dilation and hypertrophy which in turn will cause a decrease in left ventricular function which can lead

to heart failure.²⁴ While a small PDA size tends to cause mild to moderate symptoms due to heart and lungs don't have to work hard. In some cases, the clinical symptoms that appear are the finding of a characteristic murmur. The size of PDA greatly affects the clinical manifestations experienced by patients²⁵. Thus, it can be concluded that the larger the size of the defect will cause more severe clinical symptoms and ultimately worsen the outcome of therapy.

Conclusion

In this research, Persistent ductus arteriosus is found more in male neonates, normal birth weight, and gestational period, moderate defect size before pharmacological therapy, pharmacological treatment options using paracetamol, comorbid diseases Atrial Septal Defect in the category of cardiac comorbidities and hyperbilirubinemia in the category of non-cardiac comorbidities. The success factors of administrating ibuprofen and paracetamol to neonates with persistent ductus arteriosus from January 1, 2016 to March 3, 2020 are affected by the gestation period and the size of the ductus arteriosus defect before being given pharmacological therapy.

Ethical Clearance: This study protocol had been approved by the Faculty of Medicine, Airlangga University, Surabaya, East Java, Indonesia.

Conflict of Interest: The authors declare that they have no conflict of interest.

Source of Funding: Self-funding.

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The incidence of Diabetic Cystopathy among Iraqi Diabetic Patients with lower Urinary Tract Symptoms

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How to cite this article: Saif AM. Abdul-Hameed, Mohammed Bassil Ismail. The incidence of Diabetic Cystopathy among Iraqi Diabetic Patients with lower Urinary Tract Symptoms. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):317-323.

Abstract

Background: Diabetic cystopathy disease is one of the most important problems facing diabetics. Determining the prevalence rate is one of the basics to know the effect of hyperglycemia on bladder patients to provide them with the best treatment services. And reduce the percentage of damage that occurs to the functioning of the bladder over a duration of disease.

Aim of study: identify the prevalence of diabetic cystopathy among diabetic patients.

Patients and methods: A cross sectional study were conducted from the first of Jun 2019 to end of December 2020. We selected convenient sample includes all Diabetic patients presenting with voiding dysfunction attending to private clinic of Dr. Saif al-haideri for urodynamic study and and who that meeting the eligibility criteria, data collection by A structured questionnaire is developed to collect information from the participation after physical examination and Urodynamic evaluation was done.

Result: The total study sample were 550 diabetic patients, out of them 112 (20.4%) were diabetic cystopathy. (56% male and 44% female) with mean age was 56.50 ± 15.50 years. The mean duration of diabetic diagnosis was (13.7 ± 8.25) years with 75% were more than 10 years, while (742.70 ± 158.29) ml the mean volume of bladder capacity and (87.35 ± 81.82) ml/cm H₂O mean of compliance and without any statically significant association ($p=0.120$ and 0.989 respectively).

Conclusion: 20.4% of Iraqi diabetic patients have diabetic cystopathy, and without any statically significant association between urodynamic finding and duration of diabetic disease.

Keyword: Diabetic cystopathy; prevalence; diabetic duration; compliance.

Introduction

Diabetes mellitus is a progressive, systemic metabolic disease that is increasingly becoming a public health

problem. Diabetes has been linked to an early onset and greater incidence of urologic disorders, which can lead to debilitating urologic complications. This urologic disorder, which include bladder

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dysfunction, have a significant impact on the quality of life of diabetic patients. Bladder disease affects more than half of all diabetic men and women.^{1,2} Bladder instability or hypersensitivity was the most common finding in a series of clinical trials involving men and women with diabetes, ranging from 39 to 61 percent of participants.^{2,3}

Diabetes cystopathy, a common urological problem, is historically described as a combination of reduced bladder sensation, rise bladder volume, and insufficient bladder emptying. Frimodt Moller identified it for the first time in 1976.⁴ Even so, people with diabetes have a major trouble from overactive bladder as well as an extensively developed lower urinary tract condition is becoming increasingly evident in the recent definition of diabetic cystopathy (LUTS).⁵ Lee et al. reported the prevalence of diabetic cystopathy with urodynamic diagnosis in 2004 to extend from 25 to 90%.⁶ A new 2011 clinical study estimated that 22.5% of diabetics had excessive bladder, 48.0% of whom had urinary incontinence.⁷

The difference in the LUTS prevalence and incidence is because the identification of diabetic cystopathy and identified relation to patients is not validated and standardized.⁸ Up to now little literature to direct the medical practice has been done about Diabetic uropathy that showed diabetic bladder dysfunction (DBD), which is more inclusive of diabetic cystopathy than the previously in vogue, DBD has become known in recent years as a series of symptoms, with a rise in frequency, urgency and sometimes even pressing incontinence described in early phases.⁹ These modifications are typical for overactive bladder-related irritative signs (OAB).⁶ As diabetes increases, recurrent DBD lead to insensate, decompensated bladder that results in high incontinence and post-residual volumes.¹⁰ Hyperglycemia overcomes the capacity of kidney glucose uptake, leading to glucosuria and osmotic polyuria in initial phases of diabetes. At first and, the bladder compensates and the urinary frequency increases. Hyperglycemia simultaneously enhances oxidative phosphorylation in the bladder like in other insulin-independent sites, causing major oxidative stress. Inverse correlation of reactive (free) radical and antioxidant protection oxidation activity resulting in the reaction of surplus radicals that destroy all cell structures, even proteins. Caused by an imbalance. [11] Many diseases, such as heart disease, cancer and especially dangerous diabetes and DBD, are caused by elevated oxidative stress. Thus, oxidative stress

in many target tissues leads to an inflammatory reaction. Over years inflammatory has been shown to play a key role in the production of various diabetic complications.¹²

Patients and Methods

A cross sectional study was conducted from the first of Jun 2019 to end of December 2020. We selected as a sample includes all Diabetic patients presenting with voiding dysfunction attending to private clinic of Dr. Saif al-haideri for urodynamic study that specialist of urology and who that meeting the eligibility criteria and accepted to participate in study were included and excluded any patients with 1. end stage renal disease, 2. Patients with medication known to interfere with function of the bladder or sphincter, 3. acute metabolic complication of DM, 4. supra-sacral lesions or any root lesion of the sacral and lumbar outflow tracts and diseases related to peripheral neuropathy other than DM; 5. prostate or bladder cancer and prostatic hyperplasia, 6. previous genitourinary surgery, pelvic organ prolapsed and 7. urolithiasis current.

We evaluated 550 diabetic patients examined in our clinic after a diabetic medical consultation, and data collection by A structured questionnaire is developed to collect information from the participation. Some information regarding clinical factors and certain other information were obtained from the medical records, (age, weight, height) while other information was obtained from the patients (duration and treatment for diabetes mellitus and the voiding symptoms). after that we do physical examination and Ultrasound of abdomen was done to assess the status of the upper tracts, any other lesions in bladder, prostate. Urine examine was done to rule out pyuria and UTI and Urodynamic evaluation consisted of multi-channel urodynamics measuring abdominal, vesical and detrusor pressures simultaneously. Data was translated into a computerized database structure. Statistical analyses were done using SPSS (Statistical Package for Social Sciences). Version 23 computer software for windows. Categorical variables were presented as frequency and percentage, Chi-square was used to test the significance of the association between categorical variables while continuous variables presented as median with interquartile range and compared using analysis of variance. With considered P. Value of ≤ 0.05 was statistically significant.

Result

The total study sample were 550 diabetic patients, 52.2% were female and 47.8% were male, with the mean patient's age of 60.78±13.45 years old. 27.8% of patients presented with over active bladder, 23.6% with bladder out let obstruction, 27.2% stress urinary incontinence and 20.4% diabetic cystopathy. all these were shown in Figure 1.

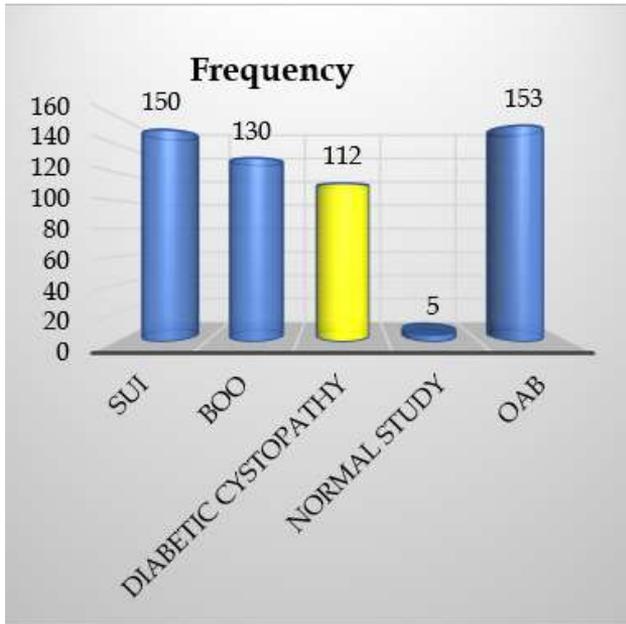


Figure 1: Distribution of study sample according to urodynamic finding. N=550

We selected all patient with diabetic cystopathy(112) for more evaluation, the result of current study showed (56% male and 44% female) with mean age of diabetic cystopathy patients was 56.50±15.50 years, and the most prevalence age was more than 60 years (45.5%). The mean duration of diabetic diagnosis was (13.7±8.25) years with 75% were more than 10 years, while (742.70±158.29 ml) the mean volume of bladder capacity with 76.8% highest part among >650ml and (87.35±81.82 ml/cm H₂O) mean of compliance.as shown in Table 1. and Figure 2. Regarding to correlation of demographic characteristic to duration of diabetic disease, the result of study found no statistical significant association between age category and duration (p=0.565), also

no any statically difference between gender and duration of diabetic (p=0.582).as shown in Table 2.



Figure 2: distribution of study sample according to gender.

Table 1: Distribution of study sample according to demographic and clinical characteristic. N=112

Age	Frequency	Percent
<40 years	17	15.2
40-60 years	44	39.3
>60 year	51	45.5
Total	112	100.0
Gender	Frequency	Percent
Female	49	43.8
Male	63	56.3
Total	112	100
Capacity volume	Frequency	Percent
350-650ml	26	23.2
>650ml	86	76.8
Total	112	100
Duration	Frequency	Percent
≤10 years	28	25.0
>10 years	84	75.0
Total	112	100.0
Compliance	Frequency	Percent
< 30ml	61	54.5
30-120 ml	21	18.8
>120ml	30	26.8
Total	112	100

Table 2: Relation duration of diabetic diagnosis to demographic characteristic.

Variables	Duration of diabetic diagnosis			P value	
	≤10 year	>10 year	Total		
Age				0.565	
18-39 years	6 5.4%	11 9.8%	17 15.2%		
40-60years	10 8.9%	34 30.4%	44 39.3%		
>60year	12 10.7%	39 34.8%	51 45.5%		
Total	28 25.0%	84 75.0%	112 100.0%		
Pearson Chi-Square= 1.141a df=2 statically significant *					
Gender	≤10 year	>10 year	Total		P value
Female	11 9.8%	38 33.9%	49 43.8%		0.582
Male	17 15.2%	46 41.1%	63 56.3%		
	28 25.0%	84 75.0%	112 100.0%		
Pearson Chi-Square=0.302a df=1 statically significant *					

Regarding the effect of the duration of diabetes on the volume of capacity, the results of the study showed that the number of patients with a capacity greater than 650 ml was the highest among patients affected for more than 10 years but without statically

differences ($p = 0.120$), as the result showed all patients who had History of diabetes more than 10 years not statistically significant association with compliance over 120, ($p = 0.989$).

Table 3: Relation duration of diabetic diagnosis to clinical characteristic

Variables	Duration of diabetic diagnosis			P value	
	≤ 10 year	>10 year	Total		
Capacity volume				0.120	
350-650ml	15 13.4%	31 27.7%	46 41.1%		
>650 ml	13 11.6%	53 47.3%	66 58.9%		
Total	28 25.0%	84 75.0%	112 100.0%		
Pearson Chi-Square =2.410 df= 1 statically significant *					
Compliance	≤ 10 year	> 10 year	Total		P value

Contd... Table 3: Relation duration of diabetic diagnosis to clinical characteristic				
< 30 ml/ cm H ₂ O	12	35	47	0.989
	10.7%	31.3%	42.0%	
30-120 ml/ cm H ₂ O	11	33	44	
	9.8%	29.5%	39.3%	
>120 ml/ cm H ₂ O	5	16	21	
	4.5%	14.3%	18.8%	
Total	28	84	112	
	25.0%	75.0%	100.0%	
Pearson Chi-Square 0.235a df= 2 statically significant *				

Discussion

Diabetes is one of the world's most prevalent chronic disease which has spread over the past years in Iraq and is relatively easily diagnosed, but neurogenic bladder condition such as diabetic cystopathy has insidious development with signs and symptoms that do not occur until the disease is advanced. So, the incidence and prevalence of diabetic cystopathy not clearly detected in Iraq. In current study we found the prevalence of diabetic cystopathy was 20.4%, while another study reported 25-95% of patients with DM have cystopathy^[13-15]. Since few studies have dealt with unselected patients, it is difficult to estimate the incidence of cystopathy in a diabetic population based on reports in the literature. One analysis¹⁶ looked at 87 randomly chosen patients, while another¹⁷ looked at 124 randomly chosen patients, and both observed cystopathy in 43 percent to 44 percent of the patients. In other documents, randomly selected patients have been selected within a restricted age (20 to 50 years). Cystopathy frequency varies between 34% and 63%.¹⁸ Some urophysiological trials have occurred in patients that either use oral hypoglycemic agents, have a diet-controlled disorder or have been given hypoglycemic and dietary treatment of these patients, 66% had supposedly primary bladder outlet disorders and only 25% had symptoms of cystopathy.¹⁷ In Iraq study conducted by Hasanain F. et al.¹⁹ recorded 42% prevalence of Diabetic cystopathy. The possible explanation of these differences might be related to variations in study setting and sample constitution and Part of this difference is that most experts agree that the specific criteria are different for diabetic cystopathy like reduced bladder sensation or increased bladder capacity.²⁰

Only diabetic men have been examined in the early research on bladder disorders. However, numerous experiments have been conducted using both sexes over the last 20 to 30 years. In present study we found the frequency of cystopathy in males and females has been found to be practically identical: 56% in males and 44% in females, mean age of diabetic cystopathy patients was 56.50±15.50 years and without any statically association with duration of diabetics and age or sex. This result similar to Takahashi O. et al.(2021) a retrospective study, that showed no. of patients was 44:(61% of males and 39% of females). Average age 67.0 ± 12.7 years; The average duration of diabetes is 16.8 ± 13.1 years and without correlation.^[21] another study by Martins U, et al.(2018) Of the 151 patients with diabetes assessed, 76 were female and 75 were male, with the average patient age being 54 years old with statically significant with age and feminine sex.²² In Hasanain F. et al.¹⁹ The study included 118 diabetic patients (71 female and 47 male) within the average age (62 ± 13) with mean duration of DM 11.3±3.7 in male, 12.2±4.1 0.2 in female with statically significant. It was found in our study, the a greater effect of prolonged diabetes duration on increased volume capacity and bladder compliance without any a statistically significant association (p=0.120 and 0.989 respectively). This result is agreed with study conducted by Somarendra K et al.²³ that found no correlation between duration of diabetes and increased maximum cystometric capacity in both males (P value-0.072) and females patients (P value-0.667). Govindarajan R et al.²⁴ reported large bladder capacity in 26% of patients and they also had no correlation with duration of diabetes mellitus.

In study conducted by Al-Shukri S. et al. showed the duration of the diabetes mellitus type II was

less than 10 years the bladder not significant. The duration of the disease was more than 10 years the predominantly bladder hypocontractility or hyposensitivity was significant while Sex and age had no significant influence on the urodynamic changes.²⁵ Several authors have shown, the longer the duration of diabetes the more likely to develop of cystopathy.^{4,7} While occurs at rate of 25% in patients with diabetes of approximately 10 years' duration, the prevalence rate increases to more than 50% in patients who have had diabetes for more than 45 year.

Early studies of cystopathy showed that the frequency of cystopathy was similar between the sexes. However, a longer duration of diabetes was associated with an increased incidence of cystopathy. And its prevalence among females with diabetes for a period of more than 10 years.²⁶ another reported by Santhanakrishnan I. et al.²⁷ found the compliance was less in individuals with duration of diabetes > 4 years compared to those with duration < 4 years, but it was not statistically significant similar to the study conducted in Poland.²⁸

Salem MA, et al. (2014) Studying the relationship between the diabetes duration and diabetic cystopathy, it was found that the duration of diabetes was not significantly related to any urodynamic finding indicating that disturbed bladder function may be independent of the duration of diabetes.²⁹ This is in concordance with the formerly mentioned study of Karavanaki et al.³⁰ which had findings suggesting that autonomic function can be impaired independent of diabetes duration.

Ethical clearance: Taken from college of medicine, university of Baghdad ethical committee

Source of funding: Self funding.

Conflict of Interest: Nil.

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The Relationship between Health Workers' Support and Frequency of Visual Inspection with Acetic Acid for Cervical Cancer Early Detection among Childbearing Age Women at Pasir Mulya Health Center

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How to cite this article: Salsalina Yuniarty G., Astry Susanti, Reny Siswanti et al. The Relationship between Health Workers' Support and Frequency of Visual Inspection with Acetic Acid for Cervical Cancer Early Detection among Childbearing Age Women at Pasir Mulya Health Center. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):324-328.

Abstract

Background: Cervical cancer could be prevented through early detection method. Visual Inspection with Acetic Acid (VIA) is one of common and simple procedures to screen early stage of cervical cancer. Health workers play a role in helping and encouraging women of childbearing age to be involved in preventing cervical cancer and thus improving the life quality of women generally. This study was aimed to determine the relationship between health workers' support and frequency of visual inspection with acetic acid (VIA) for cervical cancer early detection among childbearing age women at Pasir Mulya Health Center.

Materials and Methods: The study design used was analytical research. Participants in this study were 287 childbearing age women who regularly visited Pasir Mulya Health Center. The sampling method used was purposive sampling. The instrument in this study was questionnaire and the research data was analyzed using the Chi-square test.

Results: Out of 287 participants, 211 participants (73.5%) received support from the health workers while 76 participants (26.5%) did not receive support from the health workers. The number of childbearing age women who had done the VIA test was 146 participants, whilst 141 participants never had IVA test previously. A total of 131 childbearing age women received support and had undergone VIA test. The statistical test obtained *P-value* = 0.00.

Conclusion: There was significant relationship between health workers' support and frequency of visual inspection with acetic acid (VIA) in childbearing age women at Pasir Mulya Health Center.

Keywords: Cervical cancer; Childbearing age women; Health workers' support; Visual inspection with acetic acid.

Introduction

Cervical cancer is a major public health problem and become the second most common cancer among

women worldwide.¹ Based on the data from World Health Organization (2018), cervical cancer was the second most common cancer in Indonesia, with 9.3%

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incidence rate and 8.8% mortality rate. The Global Burden of Cancer (GLOBOCAN) estimated there were 20,928 new cases and 9,928 deaths found in Indonesia in 2012. About 70% of cervical cancer cases were already at an advanced stage upon diagnosis.² The associated risk factors of cervical cancer include young age at first intercourse less than 16 years old, multiple sexual partners, cigarette smoking, race, high parity, and lower socioeconomic status.³

The main cause of cervical cancer is the HPV virus (human papillomavirus) particularly HPV-16 and 18.⁴ These viruses are mainly transmitted through sexual contact and most people are infected with HPV shortly after the onset of sexual activity. Women who have sex with multiple partners also seem to be at higher risk to develop cervical cancer.⁵

Cervical cancer is very disturbing for sufferers both physically and psychologically. Therefore, early detection is highly recommended for all married or sexually active women.⁵

One alternative examination for detecting cervical cancer at a relatively low-cost procedure is visual inspection with acetic acid (VIA). Visual inspection with acetic acid is a direct cervical examination without using any enlargement tools. VIA is performed by washing the cervix with 3% - 5% acetic acid and waiting approximately 1 minute to check for any aceto-white areas. The result is positive when there is aceto-white area present.

The VIA screening method is relatively easy and can be done by general practitioners, midwives or nurses who have been trained to do this method. VIA also has high sensitivity and specificity to screen early stage of cervical cancer.

The high incidence of mortality rate of cervical cancer indicates that the health workers should participate actively in preventing the cervical cancer. Health workers' support toward cervical cancer prevention is essential in reducing the cervical cancer incidence rate. The aim of this study is to determine the relationship between health workers' support and frequency of visual inspection with acetic acid (VIA) for cervical cancer early detection among childbearing age women at Pasir Mulya Health Center.

Materials and Methods

The design of this study was correlational research that examined the relationship between two variables without influencing any of the variables.

This study also used a cross-sectional approach that collected and analyzed data from a sample population at a specific period.⁶

The study was conducted in December 2019 for 5 days at Pasir Mulya Health Center, Bogor City. The sample was taken using total sampling technique with a total of 287 participants. We only included the participants who gave permissions to participate in this research. The study sample involved women of childbearing age between 20-45 years old, either married women or widows.

The authors asked about the phone numbers list of the participants from Pasir Mulya Health Center's Administration Staff and sent the questionnaire via Google Form link.

The questionnaire gave information regarding the age and marital status of the participants, the number of VIA tests and support level from the health workers.

Statistical analysis was performed using SPSS for Windows. The data regarding health workers' support and visual inspection with acetic acid (VIA) were analyzed and presented as percentage of frequencies and absolute number. The Chi-square test was used to study the relationship between the health workers' support and frequency of visual inspection with acetic acid (VIA).

Result

Out of 287 participants, 211 participants (73.5%) claimed that they received support from the health workers during their visit to Pasir Mulya Health Center, while 76 participants (26.5%) did not receive support from the health workers.

Table 1: Frequency of Health Workers' Support

Support Level of Health Workers	Total (n)	Percentage (%)
Does not Support	76	26.5
Support	211	73.5
Total	287	100

Table 2: Frequency Distribution of VIA

VIA Procedure	Total (n)	Percentage (%)
No VIA	141	49.1
Took VIA	146	50.9
Total	287	100

Based on table 2, it can be seen that 141 participants (49.1%) never experienced visual inspection with

acetic acid while 146 participants (50.9%) experienced visual inspection with acetic acid.

Table 3: Relationship between health workers' support and frequency of VIA

No.	Health Personnel Support	VIA Test Examination				Total		P-value
		No		Yes		n	%	
		n	%	n	%			
1	Did Not Receive Support	61	80.3	15	19.7	76	100	0.00
2	Receive Support	80	37.9	131	62.1	211	100	
	Total	141		146		287		

Based on the table 3, out of 287 participants, as many as 131 participants (62.1%) childbearing age women received support from health workers and have conducted a VIA test. On the other hand, 15 participants (19.7%) did not receive support from the health workers but experienced visual inspection with acetic acid test. As many as 80 participants (37.9%) received support from the health workers but did not undergo the visual inspection with acetic acid. The data analysis showed *P-value* = 0.00, which means there was significant relationship between support from the health workers and frequency of visual inspection with acetic acid test among childbearing age women in Pasir Mulya Health Center.

Discussion

Cervical cancer is a preventable disease.¹⁸ An important strategy towards the reduction of its burden in a developing country is by early diagnosis and management of the premalignant lesions of the disease; this would be achieved via screening of women at risk.¹⁹

Health workers play important role in preventing cervical cancer. The role of health workers is to provide knowledge about cervical cancer and the importance of early detection, as well as to motivate childbearing age women to perform early detection of cervical cancer.¹⁷

Health workers' support is defined as physical and psychological convenience, attention, appreciation, and assistance in other forms received by individuals from health workers.⁷

Health workers are regarded as influential people and considered important by the community to promote positive attitude toward health aspect.

Based on table 1, out of 287 participants, 211 participants (73.5%) received support from the health

workers. When the health workers are encouraged to provide better health service quality to community, patients especially the childbearing age women would feel that the health workers were supportive toward healthy life preferences and thus promote the childbearing age women to participate in visual inspection with acetic acid test.

Cervical cancer as a preventable disease can be detected by performing regular screening test. There are 2 types of tests to detect cervical cancer: Pap test and HPV test. The VIA (visual inspection with acetic acid) test is one method to detect the presence of HPV in cervix area.

VIA is a visual examination of the uterine cervix after application of 3-5% acetic acid.² If the cervical epithelium contains an abnormal load of cellular proteins, the acetic acid coagulates the proteins conferring an opaque and white aspect of the concerned area.² A precancerous lesion has higher protein content when compared to normal epithelium. If the area becomes white (acetowhite), it is considered as "VIA positive".²

The VIA has gained favor in developing countries due to its low cost, simplicity, safety, and high efficacy properties. This test can be performed by any healthcare professionals, as long as they have received formal and practical VIA training.

Table 2 showed that 141 (49.1%) participants never experienced visual inspection with acetic acid while 146 (50.9%) participants experienced the VIA test. This condition indicated that less than half of the population never get themselves checked for early cervical cancer screening. Thus, the health workers need to put more effort in educating and encouraging the childbearing age women about the importance of early detection of cervical cancer. Early detection of cervical cancer could lower the mortality rate and

improve the life expectancy and health quality of childbearing age women.

Based on table 3, out of 287 participants, 131 participants (62.1%) childbearing age women received support from health workers and undergone VIA test. On the other hand, only 15 participants (19.7%) who did not receive support and had VIA. The data analysis showed $P\text{-value} = 0.00$, which means there was significant relationship between support from the health workers and frequency of visual inspection with acetic acid test among childbearing age women in Pasir Mulya Health Center. Based on this study, most of the childbearing age women would prefer the supportive health workers who were able to guide and provide answers regarding cervical cancer and the importance of early detection. The results of this study are in line with the research of Dewi (2014) which indicated that there was significant relationship between the support of health workers and the VIA examination.²¹

Health workers as the personnel who operate visual inspection with acetic acid (VIA) should receive thorough training not only in performing the correct procedure but also providing support for the childbearing age women from the beginning of check-up until completion of VIA and follow-up appointments. Knowledge factor is one of the factors that influence health behavior in society; if someone has good knowledge, that person tends to carry out healthy behavior as well. Knowledge is not the only factor that can change a person's behavior, but knowledge can also be one of the determinants of changing one's behaviour.¹² Most of a person's knowledge is obtained through the sense of hearing (ears) and the sense of sight (eyes).¹ Good health support can influence someone's behaviour toward cervical cancer screening using visual inspection with acetic acid (VIA).

Conclusion

It can be concluded that there was a significant relationship between the support of health workers and the visual inspection with acetic acid (VIA) in childbearing age women at Pasir Mulya Health Center in Bogor City.

Ethical Clearance: Ethical permission was not required.

Conflict of Interest: There was no conflict of interest in the research.

Source of Funding: Self-funded.

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Periodontal Research Wars: Navigating Through Minefield of Statistical Jargon

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How to cite this article: Shalini Mundra, Neetha J. Shetty, Srikant N et al. Periodontal Research Wars: Navigating Through Minefield of Statistical Jargon. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):329-338.

Abstract

Statistical analysis is considered to be the backbone of research and however challenging it is to a clinician, it plays a crucial part for a researcher to comprehend the various presumptions underlying the statistical methods. In the field of periodontology, statistical analysis is routinely used for management and interpretation of data. Yet, many periodontists find it difficult to understand the various statistical methods used in periodontal researches. Thus, this paper aims to cover the basic tenets of statistical analysis in a simplified manner with the help of flowcharts, specially emphasising on understanding few crucial points before choosing any statistical test. Later section of the article takes an application-based approach by incorporating the already illustrated flowcharts and crucial points.

Keywords: Statistical analysis; tests of significance; periodontal research; research methodology; data; flowcharts.

Introduction

Research is a movement from known to the unknown. (Kothari CR, 2004) It is a way to fulfil one's inquisitiveness. In the era of an ongoing pandemic, people are more inclined towards research. Curiosity about the new virus, the quest to formulate a new vaccine or mere desire to be of service to society is motivating the masses to undertake research. Over recent years, the field of Periodontology per se has observed a massive leap in varied aspects of clinical, epidemiological and molecular research – from

formulating new diagnostic criteria to solving various enigmas pertaining to the treatment aspects of periodontal disease. (Avula H et al., 2013) Recently, periodontal research is focused more on an evidence-based approach i.e., to find, evaluate and incorporate current evidence into dentists' decision making process. (Newman MG et al., 2006) Thus, research has become an integral part of periodontal studies.

The purpose of a research is to find out answers to questions through the implementation of scientific procedures. Scientific research starts with preparing

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a research question, followed by identifying research design and finally testing the null hypothesis. (Kothari CR, 2004).

The following flowchart (Fig 1) illustrates the steps necessary for the research process.(Kothari CR, 2004).

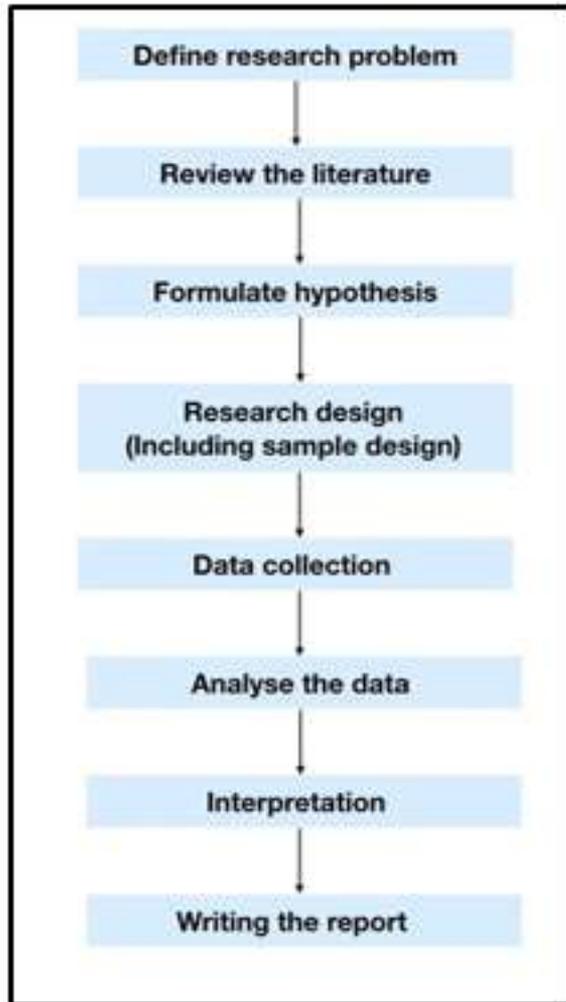


Fig 1: Flowchart of Research process

After defining research problem, formulating null hypothesis and data collection, the researchers analyse the data. (Avula H, 2013) In the process of analysis all the difference of opinions concerning

original and new hypothesis are subjected to statistical methods. A periodontal researcher mostly stumbles here in decision making process of choosing the most appropriate statistical test. Therefore, the following section discusses a few crucial points to consider before applying a statistical test.

Crucial Points

1. **Variable:** Characteristics of an observation in the population or value of something that is measured or counted in a study. The types of variables are:

- A. Dependent variable
- B. Independent variable

Dependent variable is a variable that is affected or explained by another variable and measures the outcome of interest or effects. Whereas, Independent variable causes effect on the dependent variable.⁴

For example - People with periodontitis are more susceptible to bone loss and tooth mobility. Here, periodontitis is cause therefore, an independent variable whereas bone loss and tooth mobility are the effects therefore, dependent variables.

2. **Data:** Data is the basic observation or measurement collected through primary or secondary source. Data can be classified as: (Avula H, 2013)

- A. Categorical (by ordinal and nominal scale)
- B. Continuous or discrete/counts

For example- change in pocket depth from 5mm to 3mm after Scaling and Root planing (SRP) is a continuous value. On contrary, a patient's Calculus index(CI) describes either the calculus is present/absent or it can be categorised into poor, fair & good therefore, a categorical data.

Table 1 shows various types of data in periodontology with examples.

Table 1: Types of data in periodontology

Categorical Data	Continuous Data
<p>NOMINAL: data that is described by names</p> <ul style="list-style-type: none"> • Reason for dental visit (check-up/ routine/ treatment/emergency) • Gender (male/female) • Disease(present/absent): dichotomous or binary variables. 	<p>DISCRETE: data in form of whole numbers that can be counted</p> <ul style="list-style-type: none"> • Probing depth (3mm, 4mm, 5mm) • Bone loss in mm • Width of keratinised tissue in mm • No of teeth lost due to periodontitis

<p>ORDINAL: data that can be arranged in an ordered manner</p> <ul style="list-style-type: none"> • Grades of tooth mobility (I, II, III), • Gingival recession / Furcation grades (I,II,III,IV) • Bleeding scores (mild, moderate, severe) 	<p>CONTINUOUS: data in the form of fractions or decimals</p> <ul style="list-style-type: none"> • Height (184 cm) • Weight (52.7 kg)
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The raw data collected after the study is formulated in the master table and is subjected to statistical analysis to draw statistical inferences. The

Flowchart of Data Presentation (Fig 2) describes the presentation of data for the distribution of different variables.

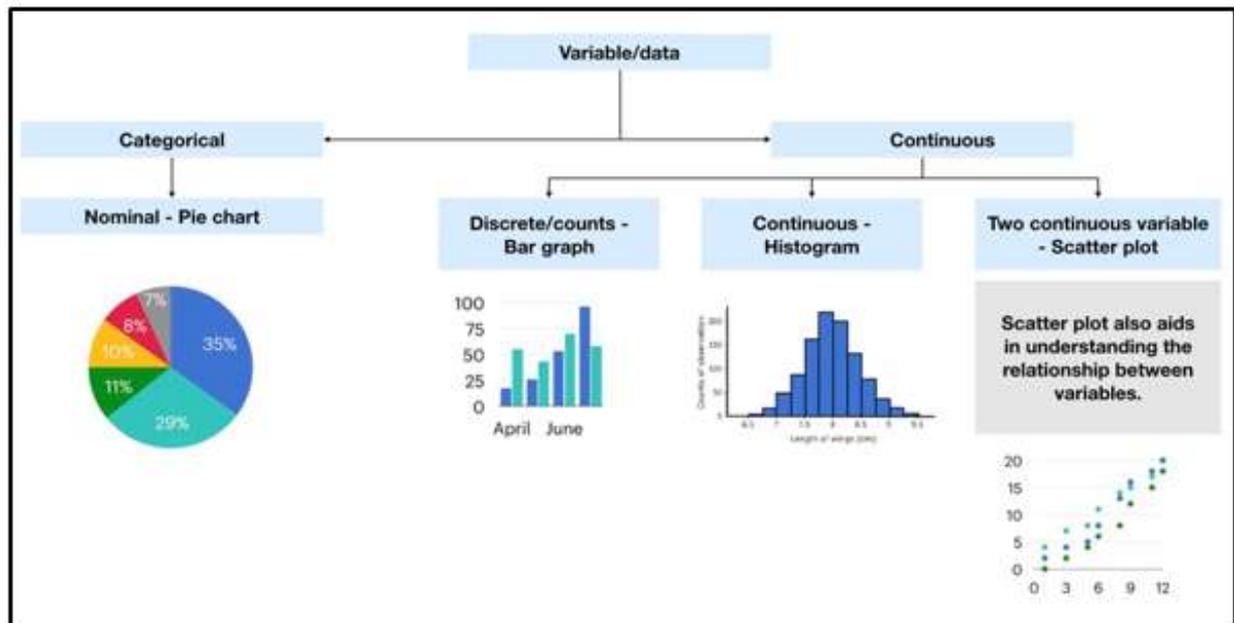


Fig. 2: Flowchart of Data Presentation

3. **Paired and Unpaired Data:** The relationship between data and population is important. (Avula H, 2013) They can be categorised into:

- Independent/unpaired data: Data obtained from two unrelated groups. For example- male or female, from two or three modalities of treatment.
- Dependent/paired data: Data obtained from same individual. For example - to determine probing depth in the same group before and after scaling and root planning(SRP), split mouth studies (data collected from left and right side is paired)

4. **Normal Distribution:** Continuous variables are defined by normal distribution. (Antonismy B, 2017) When the data is normally distributed the mean (numerical average), median

(middle value) and mode (most common value) are approximately the same. But, when the data do not follow a normal distribution, it can be skewed to either left or right side.(Fig 3)

Three important properties of distribution are: (Antonismy B, 2017)

- Location (described by averages – mean, median and mode)
- Dispersion (mean and standard deviation distribution with respect to each other)
- Skewness (asymmetry if any in a distribution)

In Figure 3

- In a normal distribution curve: mean = median = mode.

- In a negatively skewed/skewed to left curve: peak towards right and extreme values lie towards left.
 - In a positively skewed/skewed to right curve: peak towards left and extreme values lie towards right.
- o **Note:** to assess the fit of distribution for continuous variable **Kolmogorov-Smirnov test** (common test for goodness of fit) is done.

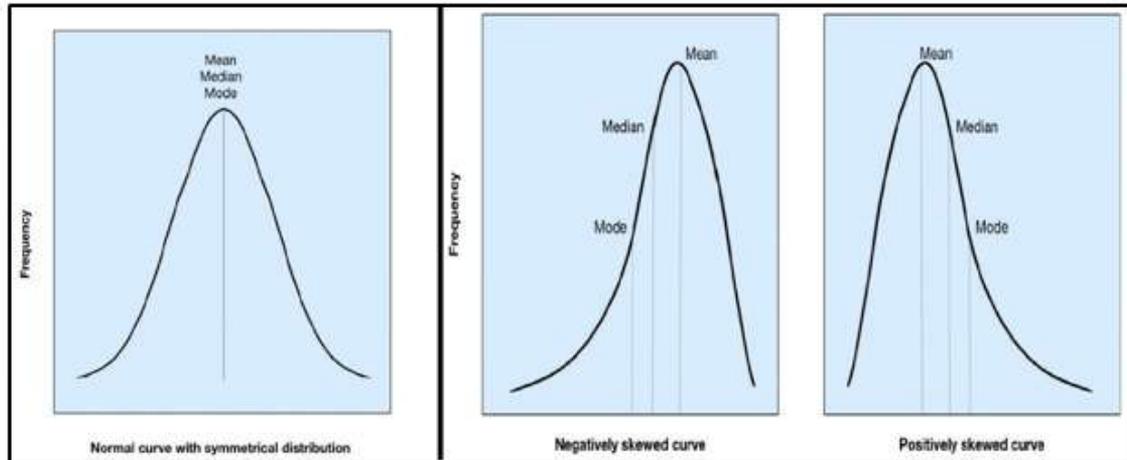


Figure 3: Data Distribution

Choosing a Statistical Test

We must be cognisant of the following questions before applying any test.

Q 1. What is your (statistical) objective?

If the objective is to describe or summarise the data, descriptive statistics is used and if it requires to make a correlation/ find associations/ draw conclusions, inferential statistics is used.

For instance, if a researcher wants to assess the oral hygiene status in a population between 18-60 years of age in India and he/she collects the following information— age, sex, occupation, number of times of brushing, dental visits per year and reason for the visits. The data obtained from this study will be compiled together, summarised and arranged in tables/ graphs/ pie charts in order to provide a description of the population. Thus, descriptive statistics are used to describe and provide statistical information about the collected data.

On contrary, if a researcher found that brushing twice daily reduces the chances of oral health problems in a sample and he/she wants to extrapolate this finding to the population and draw conclusions about the whole population using sample, then inferential statistics is applicable. (Antonisamy B, 2017)

Kindly refer to the Flowchart of Structure of Statistical Analysis (Fig 4).

- **Descriptive Statistics:** Summarizing data

Data that is collected from a large number of subjects as individual observations is described using summary measures. The Flowchart of Summary Measures (Fig 5) presents the appropriate summary measure for each type of variable.

- **Inferential Statistics:**

The inferential statistics is mainly applied to test the tentative hypothesis postulated by the researcher. In general, null hypothesis is the hypothesis that suggests an absence of difference, association or effect, the negation of which provides evidence for presence of difference, association or effect.

Tests of significance (Fig 6) are solely based on the null hypothesis. These are used to evaluate the evidence in sample as to whether or not the null hypothesis should be rejected.

To investigate the relationship between two continuous variables correlation and regression analysis are used. Correlation analysis measures the strength of the linear relationship between two continuous variables, whereas regression expresses the relationship in form of an equation, which enables one to predict the value of one variable for the given value of the other variable.

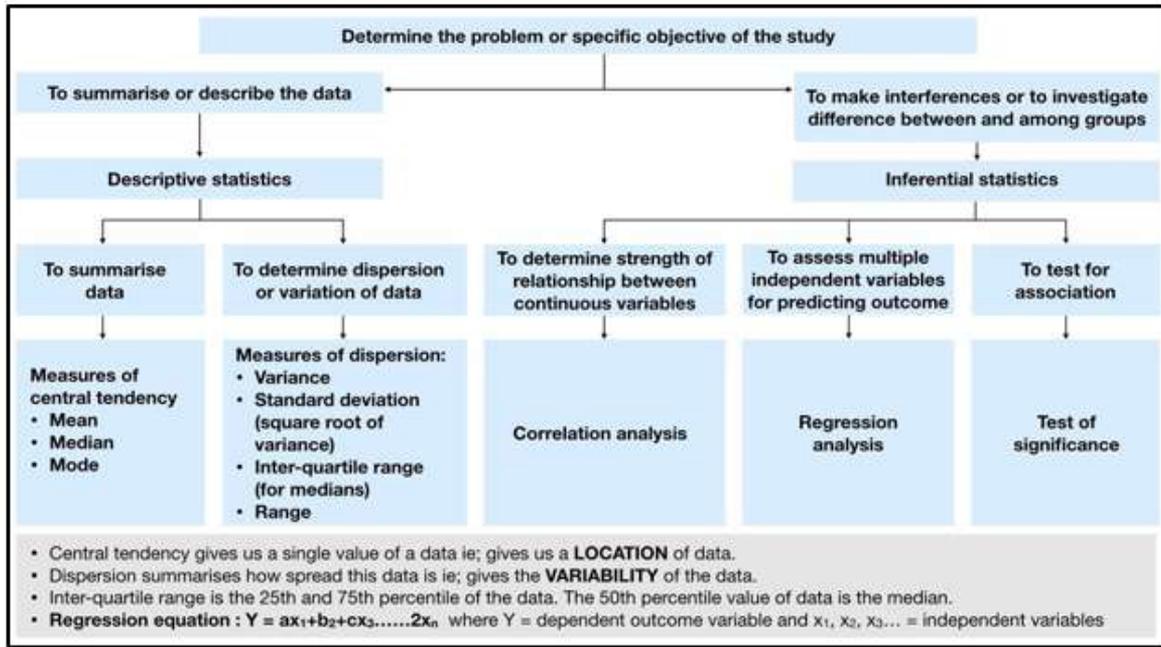


Figure 4: Flowchart of Structure of Statistical Analysis

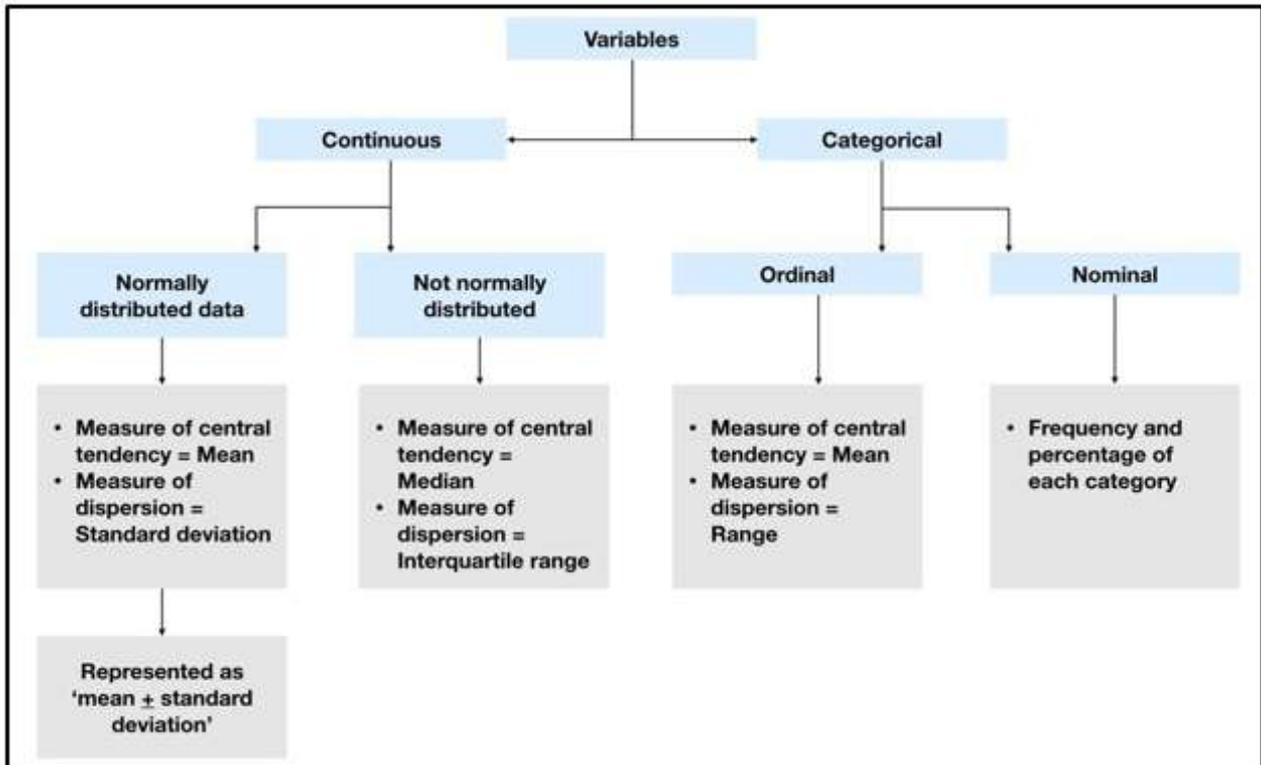


Figure 5: Flowchart of Summary Measures

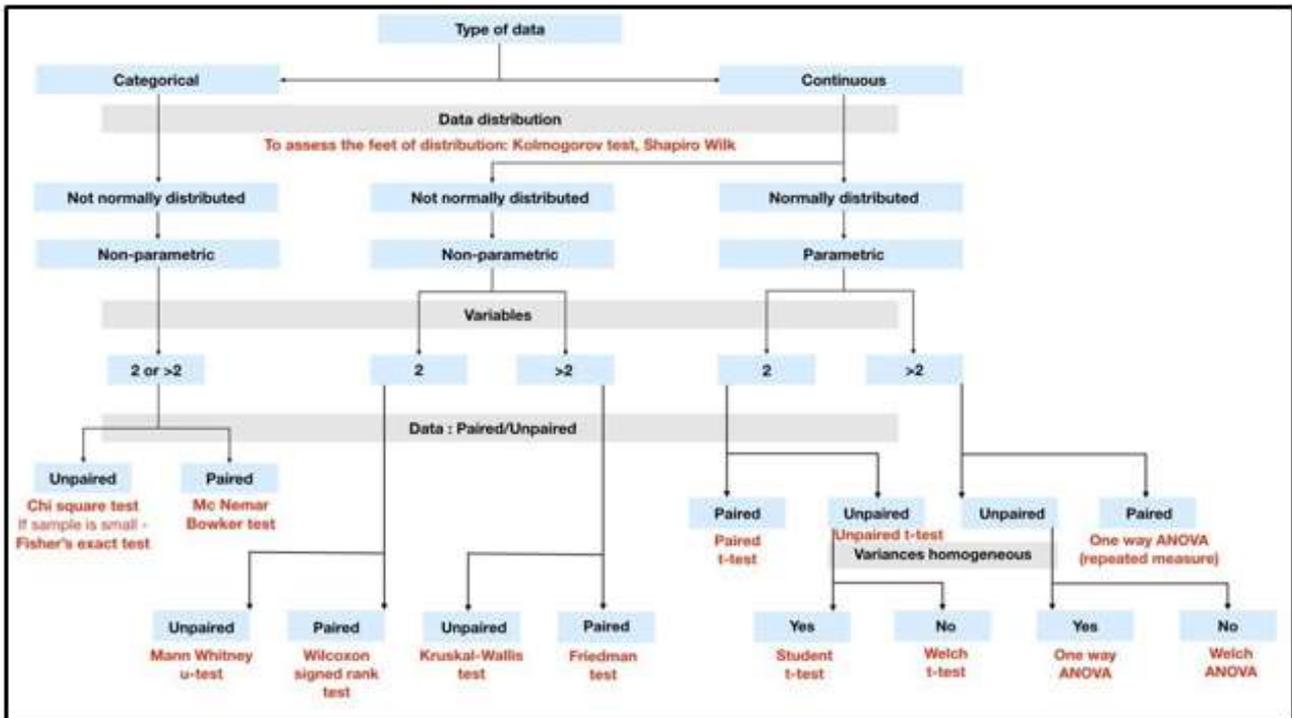


Figure 6: Flowchart of Tests of Significance

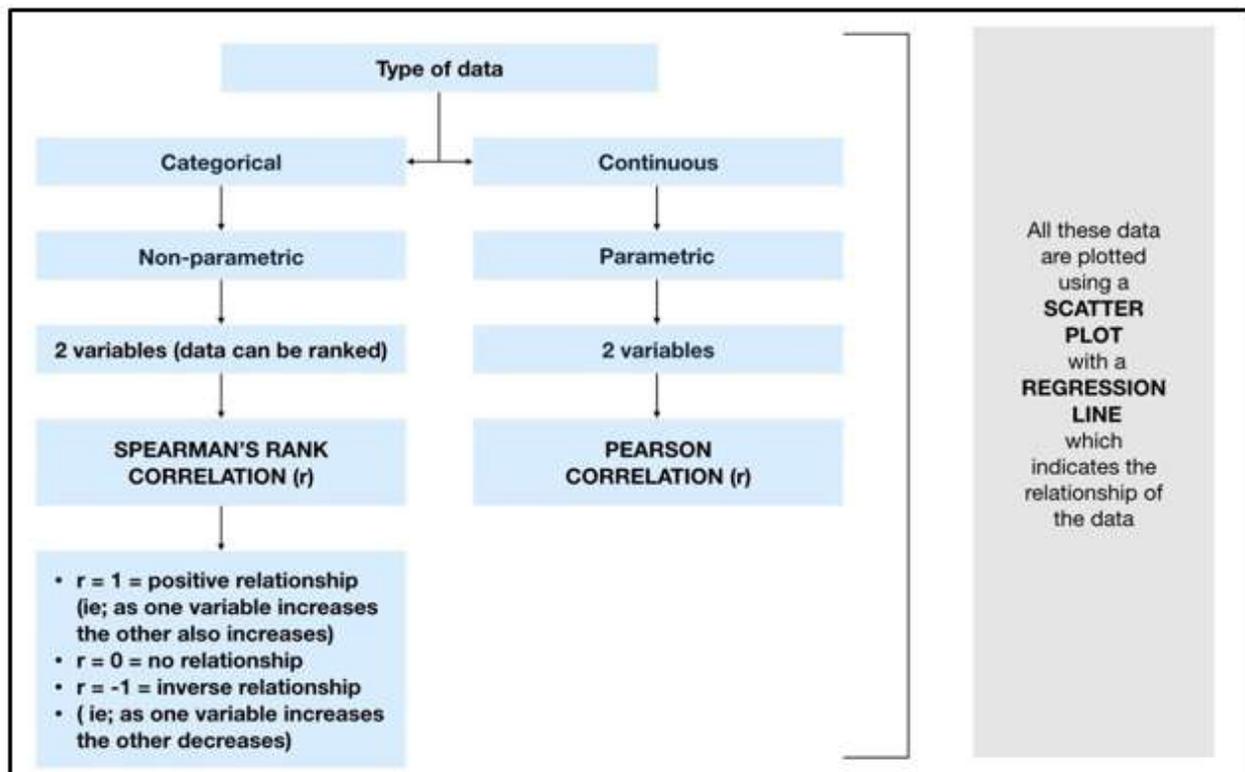


Figure 7: Flowchart of Correlation Analysis

For example, in a study on obesity and periodontitis, the relationship between the body mass index (BMI) and periodontal parameters can be examined using the two closely related techniques of correlation and regression (Fig 7). (Antonisamy B, 2017)

Q 2. What is the kind of data and how is it distributed?

The distinction between data is important because the nature of a set of data will give us the idea in choosing a particular statistical test. The categorical data is said to be non-parametric but a continuous variable has to be tested for normality.

Shapiro Wilk test or Kolmogorov Smirnov tests can be used to identify normal distribution of data. If normally distributed, parametric tests are advised but if the continuous data are highly skewed, non-parametric test are applicable.

Alternatively, when normality assumption does not hold, appropriate transformation can be tried and then parametric test can be used. If the transformation

Explanation:

1. What is the statistical objective?	<ul style="list-style-type: none"> To determine the significant difference between groups and make conclusion which is effective → Inferential statistics: Tests of Significance.
2. What is the type of data	<ul style="list-style-type: none"> Continuous data (PPD, CAL in mm) Assume normally distributed → parametric test
3. How many groups are present?	<ul style="list-style-type: none"> 2 groups: <ol style="list-style-type: none"> Deep scaling alone after 3 months Deep scaling with gel after 3 months
4. Is the data paired or unpaired?	<ul style="list-style-type: none"> Unpaired as the two groups are different patients with unmatched data.
5. Which test?	<ul style="list-style-type: none"> Independent Student -T Test

2. A study aimed to compare the efficiency of manual toothbrush v/s electric tooth brush in 100 periodontally healthy individuals. The full mouth oral hygiene status using Simplified oral

is not yielding normality, non-parametric approach is advised. (Antonisamy B, 2017)

Parametric tests are comparison for means and Standard deviation whereas non parametric tests are comparison of medians and inter-quartile range / proportion.

Q 3. How many variables or groups are present? Is the data paired or unpaired?

It is important to know the number of groups or whether the data is paired or not as there are different tests for 2 variables/ for more than two variables and for paired/unpaired data.

Examples

1. A study aims to compare the effect of deep scaling alone v/s deep scaling with metronidazole gel in chronic periodontitis patients. Periodontal parameters assessed were probing pocket dept (PPD) and clinical attachment level (CAL) after 3 months. (Abt E, 2011)

hygiene index (OHI-S) was assessed in all the individuals who regularly visited the dental clinic.

Explanation:

What is the statistical objective?	<ul style="list-style-type: none"> Compare and determine significant differences between groups → Inferential statistics: Tests of Significance.
What is the type of data	<ul style="list-style-type: none"> Categorical data (OHI-S index is assessed as excellent,good,fair and poor – ordinal data) Assume not normally distributed → non-parametric test
How many groups are present?	<ul style="list-style-type: none"> 2 groups: n = 100 a. n = 50 individual using manual tooth brushes b. n = 50 individuals using electric toothbrushes
Is the data paired or unpaired?	<ul style="list-style-type: none"> Unpaired as the two groups are different patients with unmatched data.
Which test?	<ul style="list-style-type: none"> CHI SQUARE Test

3. The study aimed to evaluate the efficacy of Vitamin D-3 supplements (2000 IU/Day - Group 1; 1000 IU/Day - Group 2; placebo group - Group 3) taken after Scaling and root planning in moderate or severe periodontitis patients.

Clinical parameters assessed were probing pocket depth (PPD); clinical attachment loss (CAL) and bleeding index (BI) after 6 months of intervention. The parameters were graded as following:

	Mild	Moderate	Severe
Probing pocket depth	4mm	4-6mm	≥6mm
Clinical attachment loss	2mm	2-3mm	≥4mm
Bleeding index	1	2	3

Explanation:

What is the statistical objective?	<ul style="list-style-type: none"> To determine significant differences between groups → Inferential statistics: Tests of Significance.
What is the type of data	<ul style="list-style-type: none"> Continuous converted to Categorical data (measured as mild, moderate and severe) Assume not normally distributed → non-parametric test
How many groups are present?	<ul style="list-style-type: none"> 3 groups a. 2000 IU/day V-D3 b. 1000 IU/day V-D3 c. Placebo
Is the data paired or unpaired?	<ul style="list-style-type: none"> Unpaired.
Which test?	<ul style="list-style-type: none"> Kruskal Wallis Test (for continuous variable) CHI SQUARE Test (for categorical variable)

4. In a split mouth study, 20 patients exhibiting multiple maxillary miller class I & II gingival recession were treated with Modified coronally advanced flap (MCAF) on and MCAF with

chorion membrane. Primary outcome variable was complete root coverage and was assessed at baseline and at 12 months post operatively.

Explanation:

What is the statistical objective?	<ul style="list-style-type: none"> To determine the significant difference between groups → Inferential statistics: Tests of Significance.
What is the type of data	<ul style="list-style-type: none"> Categorical data (gingival recession - grade I & II) Assume not normally distributed → non-parametric test
How many groups are present?	<ul style="list-style-type: none"> 2 groups: <ol style="list-style-type: none"> MCAF alone one side of split mouth MCAF with chorion on other side of split mouth
Is the data paired or unpaired?	<ul style="list-style-type: none"> Paired as the two groups are same patients (split mouth)
Which test?	<ul style="list-style-type: none"> Wilcoxon Signed Rank Test Mann Whitney U-Test if not split mouth i.e., if groups are unpaired.

5. In a study 20 patients with Grade II mandibular furcation were treated with Modified Coronally Advanced Flap (MCAF) + Guided Tissue Regeneration (GTR) membrane, MCAF + Platelet Rich Fibrin (PRF) and MCAF+Emdogain. Clinical parameters measured were probing pocket depth (PPD) at mid bifurcation, clinical attachment level (CAL) and horizontal depth of furcation at baseline and at 12 months.

Explanation:

What is the statistical objective?	<ul style="list-style-type: none"> To determine the significant difference between groups → Inferential statistics: Tests of Significance.
What is the type of data	<ul style="list-style-type: none"> Continuous data (PPD, CAL & horizontal depth of furcation in mm) Assume normally distributed → parametric test
How many groups are present?	<ul style="list-style-type: none"> 3 groups: <ol style="list-style-type: none"> MCAF + GTR MCAF + PRF MCAF + EMDOGAIN
Is the data paired or unpaired?	<ul style="list-style-type: none"> Unpaired as the two groups are different patients with unmatched data.
Which test?	<ul style="list-style-type: none"> ONE - WAY ANOVA POST HOC TUKEY TEST

Conclusion

A sound knowledge of all the intricacies pertaining to research methodology has become a pre-requisite for all the students and researcher, so as to help in understanding and critically evaluating the research studies published in their respective fields. Unfortunately, the technicalities and mathematical equations associated with statistical

methods makes it very difficult for dental researchers to implement the acquired knowledge into practice as the available material does not focus on the dental practitioners' choice of interest. A sincere attempt is made in this article by simplifying the basics of data analysis in order to facilitate dental researchers' help in implementation of various statistical tests to consequently interpret the published results in

various studies and hereby successfully negotiating a pathway through the minefield of statistical jargon.

Ethical Clearance: Taken from Institutional Ethics Committee, Manipal College of Dental Sciences, Mangalore (*A constituent institution of MAHE, Manipal*)

Source of funding: NIL

Conflict of Interest: NIL

Abbreviations

Abbreviation	Full Form
SRP	Scaling and Root planning
CI	Calculus Index
BMI	Body Mass Index
PPD	Probing Pocket Depth
CAL	Clinical Attachment Level
OHI-S	Simplified Oral Hygiene Index
BI	Bleeding Index
MCAF	Modified Coronally Advanced Flap
GTR	Guided Tissue Regeneration
PRF	Platelet Rich Fibrin

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A Prospective Study to Understand and Analyse, Correlation between Finger Prints and Lip Prints

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How to cite this article: Sharanabasavappa Karaddi, Priyanka A Ghuli, Santosh Garampalli et al. A Prospective Study to Understand and Analyse, Correlation between Finger Prints and Lip Prints. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):339-343.

Abstract

The present study was a prospective study conducted in LVD College, Raichur, Karnataka, and study period was December 2013 to November 2014 (One year). During study period, total of 210 subjects (40 male and 170 female) of Raichur origin with known blood groups were randomly selected and included in the study. The students were in the age group between 18 to 28 years. The purpose of the study was to find out the possibility of correlation between lip prints and ABO and Rh blood groups. Although a number of studies have been taken up on Dactylography and Cheiloscopy individually, studies combining features of both dactylography and cheiloscopy are very few. In the view of few literatures correlating dactylography and cheiloscopy and their importance individually in the identity fixation, the present study carries immense medico-legal importance and could prove to be a valuable step towards the identification of an individual.

Keywords: Lip prints; Finger prints; Identification.

Introduction

Identification is of paramount importance in any medico-legal investigation. Identification means the determination of the individuality of a person.¹ Personal identification is one of the most challenging processes confronted by mankind.

In the living; in civil courts - identification is required in cases such as insurance, pension and

inheritance claims, marriage, disputed sex and missing persons. In criminal courts - it is required in cases of absconding soldiers and criminals, persons accused of assault, rape, murder, etc., impersonation and interchange of babies in hospitals.² In the dead it is needed for:³ the ethical and humanitarian need to know which individual has died, especially for the information of the surviving relatives.

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The use of prints as a mean of personal identification is one of the common methods in forensic anthropology and the most popular prints are fingerprints. Even the fingerprints of twins are not similar.¹ Fingerprints of an individual are unique and remain unchanged from womb to tomb.⁴ Theory of uniqueness is the scientific principle behind finger print analysis to convince the court of law. Galton classified the types of finger prints depending upon their primary pattern as loops, whorl, arches and composite.⁵

The second prints of interest is lip prints, which are normal lines and fissures in the form of wrinkles and grooves present in the zone of transition of human lip between the inner labial mucosa and outer skin.⁶ The pattern of the furrows is unique and individualistic and can be used in identity fixation.⁷

Classification of Fingerprints

In the Henry system of classification, there are three basic fingerprint patterns: Loop, Whorl and Arch, which constitute 60–65%, 30–35% and 5% of all fingerprints respectively. There are also more complex classification systems that break down patterns even further, into plain arches or tented arches, and into loops that may be radial or ulnar, depending on the side of the hand toward which the tail points. Whorls may also have sub-group classifications including plain, accidental, double loop, peacock’s eye, composites and central pocket loop.⁸

LIP Print Classification (Suzuki and Tsuchihashi Classification - 1971).⁹

These authors considered six different types of grooves, as seen below

Classification	Groove type
Type I	Complete vertical
Type I'	Incomplete vertical
Type II	Branched
Type III	Intersected
Type IV	Reticular pattern
Type V	Irregular/other types

Materials and Methods

All the subjects were apparently healthy students of L.V.D College, in the age group between 18 to 28 years and native of Raichur district. Study period was one year i.e December 2013 to November 2014.

Sample size is 210 students. Students with permanent scar on any of the fingers, hand deformity due to injury, Birth defect or disease of the hands, Cases where there was any evidence of disease and injury of the lips that was likely to cause a change in the lip prints (dry lips, cleft lip, lip pits, lacerations and scars) are excluded from study group. Materials Required: Self-inking pad, Brown and red coloured lipstick, Lipstick applicator, Cellophane tape, White chart paper, Magnifying lens, Tissue Paper (for cleaning).

Recording of fingerprints: A self-inked pad was placed on a wooden table. The palmar aspects of the distal phalanges of a person’s right hand were inked by applying firm pressure on the ink pad starting from the little finger. The unglazed white bond paper was applied firmly over a wooden pad. Then the bond paper which was divided into two (right and left), and each further into five columns marked as thumb, index, middle, ring and little. The finger prints were taken in the respective columns on the bond paper. The same procedure was done for recording the finger prints of left hand. Thus, finger prints of both hands will be obtained and recorded and classified as per Henry classification of the various finger patterns into four main types: Loops, Whorls, Arches and Composite.

Recording of Lip prints: The subject was asked to open the mouth and lipstick was applied in a single motion, evenly the vermilion border on the upper lip, then on the lower lip. The subject was asked to rub his or her upper and lower lips together. This helped to spread the lipstick evenly on all parts of the lips. The surface of the lipstick was wiped clean with a tissue paper prior to each use for hygienic purposes.

A strip of cellophane tape was cut, the subject was asked to open mouth slightly, and to keep mouth stationary during the procedure. The glued portion of the cellophane tape was applied on the lower lip. It was held in place, applying gentle and even pressure for a few seconds, to allow the print to form on the tape. Then the tape was carefully lifted from the lip, from one end of the strip to the other, avoiding any smudging of the print.

The strip of cellophane tape was held up against light to determine its quality. If the print was not satisfactory, the above steps were repeated. If the print was satisfactory, then the strip of cellophane tape was stuck on to a piece of white bond paper and

pressed gently over the paper with a finger in order to obtain a smooth print. This procedure ensured that the lip print did not get smudged.

The above steps were repeated for the upper lip, and the strip of cellophane tape with the upper lip print was stuck just above the lower lip print, on the same piece of bond paper. The subject was provided with tissue paper to clean the lips.

The right and left sides of the print were marked on the piece of bond paper with a pencil. Then a line was drawn at the centre of the lower lip print, running vertically downwards to a point just below the cellophane tape. 5 mm on either side of this line, two parallel lines were drawn and joined together to the central line at its lower end. These lines demarcated the middle 10 mm of the lower lip, which was the area to be studied. In order to study the lip prints, the paper with the lip print was placed under the magnifying lens and the middle 10 mm of the lower lip was studied. The grooves in this area were classified according to Tsuchihashi's classification.⁹

For classification, the middle part of the lower lip (about 10 mm wide) was taken as the study area, as proposed by Sivapathasundharam et al.¹⁰ Since this fragment is almost always visible in any

trace, the determination of the pattern depends on numerical superiority of the line properties in this area. Age, sex and blood group of the students was noted down.

Statistical analysis: Descriptive statistics comprising proportion and percentage shall be used to describe the data. Chi-square of proportion shall be used to compare the statistical significance of difference between the various proportions in the study.

Results

In Table 1 when compared to other types of lip prints, distribution Type IV lip print pattern in lower lip Right quadrant shows more predominance in fingerprint pattern of Whorls (40.8%) followed by Loops (36.8%) and Arches (34.4%). In Table 2 when compared to other types of lip prints, distribution of Type IV lip print pattern in lower lip Left quadrant shows more predominance in fingerprint pattern of Whorls (38.5%) followed by Arches (37.5%) and Loops (36%). The finger print patterns show high statistical significant association with Lower Lip Right quadrant ($P < 0.0001$) in Table 1 and Lower Lip Left quadrant ($P = 0.016$) in Table 2.

Table 1: Correlation of the pattern of Lip prints in Lower Lip Right quadrant to Finger print patterns

		Lower Lip right						
		I	I'	II	III	IV	V	Total
F P P	Loop	258	212	145	102	550	229	1496
	%	17.2%	14.2%	9.7%	6.8%	36.8%	15.3%	100.0%
	Whorl	70	59	58	20	207	93	507
	%	13.8%	11.6%	11.4%	3.9%	40.8%	18.3%	100.0%
	Arch	21	19	7	8	33	8	96
	%	21.9%	19.8%	7.3%	8.3%	34.4%	8.3%	100.0%
	Composite	1	0	0	0	0	0	1
	%	100.0%	.0%	.0%	.0%	.0%	.0%	100.0%
	Total	350	290	210	130	790	330	2100
	%	16.7%	13.8%	10.0%	6.2%	37.6%	15.7%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	41.097 ^a	15	.000
Likelihood Ratio	43.336	15	.000
Linear-by-Linear Association	.003	1	.953
N of Valid Cases	2100		

6 cells (25.0%) have expected count less than 5.

The minimum expected count is .06.

Table 2: Correlation of the pattern of Lip prints in Lower Lip Left quadrant to Finger print patterns

		Lower Lip Left						
		I	I'	II	III	IV	V	Total
	Loop	284	180	147	99	539	247	1496
	%	19.0%	12.0%	9.8%	6.6%	36.0%	16.5%	100.0%
F	Whorl	63	75	66	13	195	95	507
	%	12.4%	14.8%	13.0%	2.6%	38.5%	18.7%	100.0%
P	Arch	22	15	7	8	36	8	96
	%	22.9%	15.6%	7.3%	8.3%	37.5%	8.3%	100.0%
P	Composite	1	0	0	0	0	0	1
	%	100.0%	.0%	.0%	.0%	.0%	.0%	100.0%
	Total	350	290	210	130	790	330	2100
	%	16.7%	13.8%	10.0%	6.2%	37.6%	15.7%	100.0%
Chi-Square Tests								
		Value	df	Asymp. Sig. (2sided)				
Pearson Chi-Square		29.068 ^a	15	.016				
Likelihood Ratio		28.560	15	.018				
Linear-by-Linear Association		.052	1	.820				
N of Valid Cases		2100						

6 cells (25.0%) have expected count less than 5. The minimum expected count is 0.06.

Discussion

Similar studies were done by various authors and their observations were as follows: In the study by Adamu et. al, the association between lip prints and finger print was done, the result shows that left thumb shows statistically significant association with LRM ($\chi^2 = 7.95$, $P = 0.0002$) and LLL ($\chi^2 = 5.42$, $P = 0.02$) and there was no statistically significant association with the rest of the compartments.¹¹

The study by Nandan SR et. al revealed higher prevalence of Type I lip print pattern with loop finger print pattern in females 21.31% (13/61) and Type II lip print pattern with loop finger print pattern in males 14.75% (9/61). The association of Type III lip print pattern with loop finger print pattern by Chisquare test showed statistical significance ($P = 0.05$). The Chi-square analysis of Table 4, shows Chi-square value is 27.205, Yates' Chi-square value 21.45, Yates' $P = 0.667$, degree of freedom is 25 and $P = 0.345$ suggesting the association is statistically insignificant.¹²

The study by Pranitha R et. al observed that the Inter-group comparison between two groups:

loop finger print pattern- Type 5 lip print pattern combination (25.9%) showed no statistical analysis significance.¹³

A. Nagasupriya et. al in their study they observed that the overall correlation of lip prints with finger prints in females, reticular and vertical lip print patterns show statistical significance. Vertical lip pattern associated with arch fingerprint pattern shows high statistical significance ($P = 0.009$) followed by reticular lip print pattern associated with whorl fingerprint pattern ($P = 0.05$).¹⁴

Nagasupriya A, Dhanapal R, Reena K, Saraswathi TR and Ramachandran are of the opinion that, correlative study between the lip print and finger print will be very useful in forensic science for personal identification.¹⁴

MetgudR, Kaur M, Naik S, Tak A, Patel S observed in their study in Gujrati Population concluded that correlation between lip prints and finger prints for gender identification was statistically significant. In females, branched lip pattern associated with loop (0.002) and arch (0.003) finger print pattern and

vertical lip pattern associated with whorl finger print pattern (0.041) showed significant results. In males, reticular lip pattern associated with whorl finger print pattern (0.0037), vertical lip pattern associated with arch (0.0081) and whorl (0.014) finger print, and branched lip pattern associated with arch (0.041) finger print pattern showed significant results.¹⁵

Conclusion

In our study we observed that, the finger print patterns show high statistical significant association with Lower Lip Right quadrant ($P < 0.0001$) and Lower Lip Left quadrant ($P = 0.016$). Similar observations were made by various authors, suggesting that finger print pattern and lip prints in combination, have a significant value in identity fixation.

Conflict of Interest: None

Source of Funding: Self

Ethical Clearance: Institutional Ethical Clearance Taken.

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Secondary Data Analysis of Postmortem Records in PCMC Area to Understand the Burden of Myocardial Infarction

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How to cite this article: Sharma Dharmendra, Sinnarkar Vineet V., Suryawanshi Sailee S. et al. Secondary Data Analysis of Postmortem Records in PCMC Area to Understand the Burden of Myocardial Infarction. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):344-348.

Abstract

Background: There has been a global upsurge in the cases of non-communicable diseases, especially cardiovascular diseases. Analysis of the leading causes of death in the community is very crucial in understanding and planning the health care policies. The aim of this study was to evaluate the burden of myocardial infarction in Pimpri Chinchwad city with the help of the Postmortem examination record analysis.

Methods: In this cross sectional study, the data related to 674 deaths in the period of 2019 to mid-2021 was considered. The data was obtained from the postmortem examination records of the Postmortem Centre of Talegaon Dabhade General Hospital. The obtained data was classified and analyzed with the help of descriptive statistics in order to find the burden of the leading causes of death, especially of myocardial infarction.

Conclusion: Myocardial Infarction was found to be the leading cause of death in the Pimpri Chinchwad area ranging to 33.38%. It was also seen that the deaths due to myocardial infarction was seen more in people of the age groups 25 to 64 years. These can be prevented by changing lifestyle, diet and reducing risk factors like smoking, obesity and stress. The obtained result also indicates that there is a need for increased cardiac care facilities with more cardiac ambulance and cardiac care centres in Pimpri Chinchwad city. The other leading causes of death found in the secondary analysis of the Postmortem records were death due to head injury (n= 142, 21.06%) and haemorrhagic shock (n=70, 10.38%).

Keywords: Forensic medicine, Cause of death; Myocardial Infarction; Epidemiology; Post Mortem record; Preventive medicine.

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Introduction

Thanatology is the branch of medicine which deals with the study of death. The word thanatology is derived from the Greek word *Thanatos* which means death and *logus* means science. According to medicine, death is stoppage of circulation of blood with consequent cessation of vital functions. The Law dictionary of Black states that "death is the cessation of life, the ceasing to exist and cessation of animal and vital functions consequent thereupon such as respiration, pulsation, etc."¹

The course of disease transition in India has been changeable over a substantial period of time. The disease trend has been travelling from infectious and communicable diseases, nutrition disorders to non-communicable and occupational diseases. The increase in the prevalence of non-communicable diseases has led to the rise of cardiovascular diseases in globally. Of all the non-communicable disease cases which are prevalent in India, about two-third of it belongs to the cardiovascular diseases. Both the rural and the urban parts of India equally suffer from the burden of cardiovascular diseases.² As per the Global Burden of Disease study, nearly 24.8% of all the deaths in India are a result of the cardiovascular diseases.³

The reports of World Health Organization also suggest that the patterns of death have changed in the world and the causes of death have moved from infectious to non-infectious. Heart failure, stroke, chronic obstructive pulmonary diseases and lower respiratory tract diseases are the four leading causes of death in the world.⁴

The number of cases of Coronary Artery Disease (CAD) has substantially increased from 1% to 9.6% in the urban population within 1960 to 1995. The numbers of these cases have doubled in the next ten years. A comparative study between the rural and the urban population suffering from cardiovascular diseases in India show that its incidence is more in the urban population than rural by 11%.⁵ Even in the month of August 2021, in the times of COVID-19 Pandemic, it was observed that the number of deaths in Pune was more due to Non-communicable disease than from COVID-19. This is indicative of the three fold rise in the incidence of non-communicable diseases in the city.⁶

The burden of disease in the city of Pune during the time when the survey was carried out was 4.7%

of the total population. The consumption of various unhealthy foodstuffs also contributes to the incidence of Myocardial infarction. Oily food consumption is seen in about 64.2% people suffering from cardiovascular diseases. The prevalence of tobacco chewing is seen abundantly in adults suffering from cardiovascular disease, which is further denoted in a table below.⁷ [Table 1]

Table 1: Tobacco consumption in various age groups⁷

Sr. No.	Age group	Percentage of people consuming tobacco
1	20-29	14 %
2	30-39	26 %
3	40-49	30.9 %
4	50-59	29.1 %

According to a publication in The Lancet 2000, South Asians residing in Canada are more prone to heart diseases than their European and Chinese counterparts. Some physicians claim that the South Asian population has experiences frequent famines due to which their bodies find it difficult to make a metabolic U-turn. This results in high insulin tolerance and therefore higher risks for diabetes and obesity.⁷

A postmortem examination (autopsy) is a standard, current medical procedure performed in a surgical manner, through which a thorough check of tissues and organs of a human body after death, aiming at determining the cause of death, of mechanisms that lead to that outcome. With such increasing rise in the number of communicable and non communicable diseases in the society, conducting a Post mortem examination proves vital in establishing the cause of death of the deceased. A well conducted post mortem examination also helps in improving the medical sciences and the public health. It helps one understand the mortality rate as defined by the death certificate. Mortality data aids in understanding the health of the population. Causes of death ascertained by postmortem examination are a subset of the total number of deaths registered in an area. ^[8]

Materials and Methods

Study setting

This article is a secondary data analysis of postmortem registers data which was collected from the Post mortem centre of Talegaon General Hospital in Pune from the years 2019 to 2021. Talegaon dabhade

is a town in Maval taluka at Pune district. It is situated in the outskirts of Pimpri-Chinchwad city. This area is well known for its hilly terrain and presence of several large dams such as the Pawana dam. The population of Maval taluka is approximately about 3.8 lakhs and is popular for tourist attractions. The Postmortem centre of Talegaon General Hospital is one of the main centres for conduction of postmortem examinations in the Maval taluka.

Data source

The postmortem examination registers are maintained by the Postmortem Centre of Talegaon Dabhade for each year. Data is entered by the doctor who conducted the postmortem examination. The columns in the registers include date of conduction of the postmortem examination, name, age and sex of the deceased, address (local and permanent) of the deceased, and cause of death and name of the doctor who conducted the postmortem examination. For our study, the data was collected using a predesigned format from the postmortem registers/ record whilst maintaining the confidentiality.

Ethical clearance

Since it was a secondary data analysis, ethical approval was waived off by the Institutional Ethics Committee. However, prior permission was sought for conducting the study.

Statistical analysis

The data was entered in Microsoft Excel worksheet and was cross-validated by the primary investigator. The data was analyzed with the help of descriptive statistics. Analysis of the data was based on age, sex and cause of death. The diagnoses provided in the postmortem record were categorized.

Results

A total of 674 postmortem examinations were conducted in the Talegaon General Hospital Postmortem Centre, Pune in the study period. The number of postmortem examinations may have been affected due to the ongoing COVID-19 Pandemic.

The postmortem records revealed that the most deaths that occurred in PCMC, barring the deaths due to COVID-19 Pandemic, were due to myocardial infarction. Myocardial infarction (MI) was found to be the major cause of death according to the postmortem records in the study span. It accounted for about 33%,

claiming about 225 lives. The other major causes of death found were death due to head injury (21.06%), haemorrhagic shock (10.38%), hanging (9.94%) and drowning (7.12%). The other causes of death according to the postmortem examination include congestive cardiac failure, death due to electric shock, food poisoning, hypovolemic shock and septic shock. Some decomposed bodies were also brought for postmortem examination in which elicitation of cause of death was difficult. The viscera in such cases were preserved and sent to the forensic laboratory for further evaluation. [Table 2- Original]

Table 2: Frequency distribution of causes of death according to ICD 10*

Cause of death	Frequency (N)	Percentage
Myocardial Infarction	225	33.38%
Head Injury	142	21.06%
Haemorrhagic shock	70	10.38%
Hanging	67	9.94%
Drowning	48	7.12%
Congestive Cardiac Failure	33	4.89%
Electric shock	13	1.92%
Food poisoning	10	1.48%
Hypovolemic shock	5	0.74%
Septic shock	4	0.5%
Decomposed bodies found	24	3.56%
Cases in which viscera was preserved and sent to forensic laboratory	33	4.89%

(International Classification of Diseases, 10th revision)*

Figure 1 denotes the age wise distribution of the people who died due to myocardial infarction. The grouping of age is done according to the 2001 Census Data of the Government of India.⁹ Around 16.44% (n=37) of the deceased people belonged to the age group 65 to 79 years. 2.66%, that is, 6 people above the age of 80 years succumbed to myocardial infarction during the span of study. The age groups of 25 to 44 years and 65 to 79 years show a similar graph of 38.66% (n= 87) and 39.11% (n= 88) respectively. Death due to myocardial infarction was seen the least in the age groups of 15 to 19 years and 20 to 24 years with 1.33% (n= 3) and 1.77% (n= 4) respectively. [Figure 1- Original]

Figure 2 depicts the sex wise distribution of people who died due to myocardial infarction in the study span. A huge difference has been found in the frequency of deaths due to myocardial infarction in either of the sexes. It has been seen that 193 out of

225 (85.77%) people that died due to MI were males while the frequency in females was 32 (14.22%). This is indicative of an increased burden of myocardial infarction in males than in females in PCMC area. [Figure 2- Original]

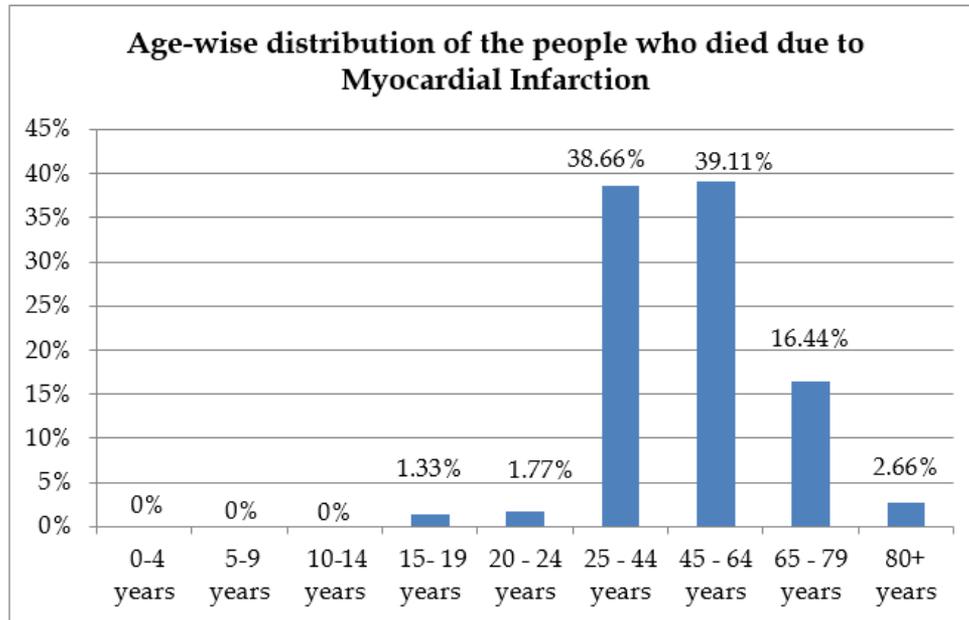


Figure 1: Age-wise distribution of the people who died due to Myocardial Infarction

Sex wise distribution of people who died due to Myocardial Infarction

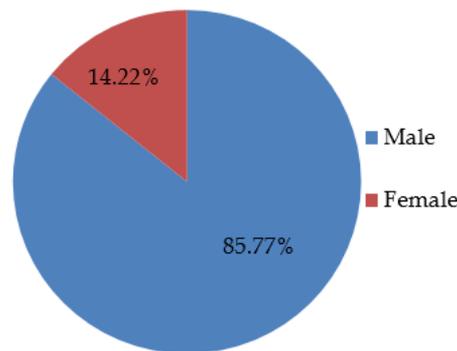


Figure 2: Sex wise distribution of people who died due to Myocardial Infarction

Conclusion

Out of the 674 postmortem examinations conducted in the Postmortem Centre of Talegaon General Hospital, Pune in the study span of 2019 to 2021, the three leading causes of death found were myocardial infarction, head injury and haemorrhagic shock. As the study span was an active COVID-19 Pandemic period, there was a relative decline in the number of postmortem examinations conducted in the said postmortem centre. Barring the deaths due to

the Corona virus affections, the maximum death rate seen in PCMC area was due to Myocardial infarction.

The postmortem examination is important to inspect public health and to assess the adequacy of the available resources in a given area. The cause of death determined by a postmortem examination is an indicator of the total number of deaths in that particular region.⁸ The present study shows a high incidence of death due to myocardial infarction in PCMC during the study span. Of the 674 postmortems

that were conducted, about 225 postmortems conducted were of myocardial infarction. This shows that the deaths due to MI in PCMC is significant and needs to be addressed.

According to the estimation of the World Health Organization, India will have about 60% of the world's cardiac patients. Due to overt globalization and changing lifestyles of people all over the world, a significant upsurge has been observed in the lifestyle disorders. Industrialization, modernization and urbanization have directly and indirectly affected the health of the people with respect to the dietary habits, the levels of physical activity and consumption of tobacco in the form of various products. The major biological risk factors for cardiovascular diseases are high blood pressure, overweight and obesity, high blood cholesterol and chronic diabetes mellitus type II. Unhealthy dietary habits such as increased consumption of saturated fats, salts and refined carbohydrates and reduced intake of fruits and vegetables also contribute to the affection of cardiovascular diseases.⁷

The increasing number of deaths due to myocardial infarction gives rise to various facets of the health care system that can be questioned. Not just is the health care system responsible but also is the changing lifestyle of people which contribute to the disease affections in people. The above study shows that the maximum deaths due to myocardial infarction were seen in the Maval region of PCMC which roughly accounted for about 39.5% (n= 89) of the total deaths due to myocardial infarction in PCMC. Also, the deaths due to myocardial infarction seen in Talegaon Dabhade region accounted for 18.22% (n=41). The high incidence in Maval region can be attributed to the poor health care facilities in the Maval region of PCMC. The availability of Cardiac ambulances and specialized Cardiac Care centres should be increased in the Maval region so as to bring down the death rate due to MI.

Ethical Clearance: Taken from the Institutional Ethical Committee

Source of Funding: Self Funded

Conflict of Interest: Nil

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Effect of Muscle Energy Technique as an Adjunct to Lumbar Stabilisation Exercise Training on Pain, Disability and Fear Avoidance belief in Patients with Chronic Low back pain with facet joint dysfunction: Randomized Controlled Trial

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How to cite this article: Shubhangi Patil, Muskan Bhojwani. Effect of Muscle Energy Technique as an Adjunct to Lumbar Stabilisation Exercise Training on Pain, Disability and Fear Avoidance belief in Patients with Chronic Low back pain with facet joint dysfunction: Randomized Controlled Trial. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):349-353.

Abstract

Background: Low back pain (LBP) is the second leading cause of disability, affecting 85 percent of people worldwide at some point in their life. LBP is viewed as a personal and societal burden due to discomfort and limited function. Facet joint dysfunction is one of the leading causes of pain and disability. While the Muscle Energy technique (MET) looks to be a promising treatment, research on MET in combination with exercise therapy is limited.

Method: Sixty patients, 18 to 40 years of age, with a history of chronic low back pain will be randomly divided into two groups. One group will receive lumbar stabilization exercise training along with muscle energy technique and the other group will receive lumbar stabilization exercise training alone respectively for 3 times a week for 4 weeks. Data will be collected pre and post the intervention on the Numeric Rating Scale, Oswestry LBP Disability Questionnaire, and Fear-avoidance belief questionnaire.

Result: Both groups will be analyzed for pain, disability, and patients' fear-avoidance belief regarding physical activity. Baseline characteristics including means and standard deviations (SDs) will be analyzed. Categorical variables will be analyzed with a chi-square test and continuous variables will be analyzed using independent t-tests.

Keywords: Facet joint dysfunction; low back pain; lumbar stabilization exercises; muscle energy technique; modified Oswestry disability index; numerical pain rating scale; fear-avoidance belief questionnaire.

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Introduction

In all developed countries, non-radicular low back pain is a serious public health concern that is routinely managed in primary care settings.¹ The majority of people experience incapacitating back pain at some point in their lives. An estimated 6.5 million people worldwide are bedridden due to this painful ailment, and physical therapists see 1.5 million new instances of back pain each month.² Low back pain is extremely common in India, with nearly 60% of Indians experiencing severe back pain at some point in their lives.³ Non specific low back pain is discomfort, muscle tension, or stiffness in the lumbosacral region that is located below the costal border and above the inferior gluteal folds but does not involve leg pain (sciatica).⁴ Pain, morning stiffness or discomfort is a common sign of this troublesome illness, which is frequently recurrent. Forward flexion, as well as returning to a standing position after commencing the movement, generates pain, which is aggravated by extension, side flexion, rotation, standing, walking, sitting, and general exercise. The pain worsens during the day and is relieved by changing positions, especially lying in the fetal position.⁵ Due to a possible pathoanatomical mechanism, the facet joint has been linked to persistent discomfort in the lower back. Facet joint pain was shown to be prevalent in up to 75% of persons with low back pain (LBP).⁶ Surgery, medication therapy, and physical and rehabilitation interventions are among the several intervention options used to treat low back pain.⁷ Exercise therapy, transcutaneous electrical nerve stimulation (TENS), low-level laser therapy (LLLT), individual patient education, massage, behavior treatment, lumbar supports, traction, therapeutic ultrasound, thermotherapy (SWD), EMG biofeedback, spinal manipulations, neural mobilizations, Mc Kenzie exercises, lumbar stabilization exercises, Muscle energy techniques, and various other manual therapy techniques are among the physical and rehabilitation medicine interventions. Physical therapists use a variety of therapies to treat LBP, but the evidence supporting their effectiveness is limited.⁸ Stabilization exercise training has been shown to help with LBP management. The purpose of lumbar stabilization training is to obtain optimal dynamic management of lumbar spine forces while avoiding repetitive harm to the spinal segments and surrounding structures.⁹ Specific stabilising workouts including

co-contraction of the deep abdominal (Transversus Abdominus and Obliquus Internus) and lumbar multifidus muscles have been documented in studies. These are useful in the short and long-term management of LBP in recent clinical trials.¹⁰ According to a recent assessment of several LBP therapies, an active strategy is successful. Another effective technique for managing chronic LBP with facet joint dysfunction is Muscle Energy Technique. However, there is relatively little information about this in the literature. The goal of the study is to see if Muscle Energy Technique along with lumbar stability exercise training is more effective than lumbar stabilization exercise training alone in lowering pain, disability, and reducing the fear-avoidance belief regarding physical activity in patients with chronic low back pain with facet joint dysfunction.

Material and Methods

Aim:

To study the effect of Muscle Energy Technique as an adjunct to Lumbar Stabilisation Exercises training on Pain, Disability and Fear Avoidance belief in Patients with Chronic Low back pain with facet joint dysfunction.

Study setting:

The study will be conducted between April 2022 to September 2022 in physiotherapy OPD of Ravi air physiotherapy college Sawangi (Meghe) Wardha after approval from Institutional Ethics Committee of Datta Meghe Institute Of Medical Sciences, Deemed to be University. The patients will be referred by the orthopedic department of AVBRH Sawangi (Meghe) Wardha. Before inclusion, all the participants will be informed regarding the aim and procedure of research. Those participants who will meet the inclusion criteria must give the written informed consent. The participants diagnosed with with chronic low back pain along with facet joint dysfunction will be enrolled for the study.

Inclusion criteria:

1. Age between 18-40 years
2. Both men and women will be included
3. The subject with chronic low back pain with facet joint dysfunction from last 3 months.
4. NPRS score more than 5.
5. Restricted ROM at lumbar spine.

Exclusion criteria:

1. Any medical red flags (Tumor, Fracture, Metabolic disease, RA, Osteoporosis, Prolonged history of steroid use)
2. Presence of any specific lumbar pathology (Lumbar canal stenosis, Lumbar spondylosis, spondylolisthesis, spondylolysis, Prolapsed intervertebral disk etc.)
3. Evidence of CNS involvement.
4. Prior surgery of the lumbar spine.

Before randomization subjects will be assessed using the questionnaire which will be self-drafted. Information regarding age, sex, occupation, the reason for visit, primary symptoms, additional symptoms, symptom description, duration of symptom, severity, and frequency of symptoms, mechanism of onset, history of recurrence, history of previous treatment, pain behavior, location of pain on body diagram, the intensity of pain, pain on scale and effect of pain on the activity of daily living (ADL) will be obtained from an interview which will contain a series of standard questions.

Group A: In group A, 15 patients will receive muscle energy technique along with lumbar stabilisation exercise therapy.

Muscle Energy Technique:

The MET group's technique is chosen based on the symptoms and diagnosis of the dysfunction's direction, as described in the textbook "Greenman's principles of manual medicine.". The paired transverse processes will be evaluated in neutral, extended, and flexed states as part of the dysfunction diagnostic technique. The transverse processes on both sides will be palpated after palpating the spinous processes to determine the lumbar vertebra level. The levels of transverse processes will be measured first in the neutral position, then in the prone position on the bed. With the patient seated on a stool and their feet on the floor, the fully flexed position will be measured. The limitation with extension, rotation, and side bend (ERS) dysfunction will be detected if the transverse process of one side is more posterior in the flexed position and symmetric in the extended position. The limitation with flexion, rotation, and side bend (FRS) will be identified if one transverse process is more evident in the extended position and symmetric in the fully flexed position. If three or more transverse processes will be evident in all

positions, a neutral malfunction will be suspected. With movement barriers, treatment will be in the same direction, but with positional diagnosis, it will be in the opposite direction. The therapist will set up the suitable position and analyse the subject's pain or resistance barrier before beginning the treatment. During treatment, the physical therapist will palpate to keep track of the problem section and muscle contractions at that precise level. Due to the persistent nature of the discomfort, light to moderate active contraction force will be used. The individual will be asked to totally relax the back after the contraction effort, and the therapist will re-engage movement limitation. The patient should be calm and his or her muscles should be stretched to a new resting length before repositioning to a new barrier resistance. These steps will be carried out three to five times.¹¹

Following MET, patients will be instructed in lumbar stabilization exercises. Exercise will be used to relearn a precise co-contraction pattern of the deep trunk muscles, including the transversus abdominis and lumbar multifidus muscles, for the LSE group. Abdominal breathing and abdominal hollowing were conducted as part of the warm-up before the activity. Abdominal hollowing, unilateral knee abduction, extension and knee raise, and bilateral knee raise will be performed in the supine position.¹² Each subject's exercise intensities and progressions will be tailored to their capacity to learn to do lumbar stabiliser co-contraction. For both the MET and LSE treatments, it will take about 25 to 30 min to finish the session.

Group B: In group B, 15 patients will receive lumbar stabilization exercise training alone.

Outcome measures:

The patients will complete outcome measures such as numerical pain rating scale, Oswestry disability questionnaire and fear-avoidance belief questionnaire

The numerical pain rating scale is an 11-point scale on which patients assess the severity of their present pain from 0 ("no pain") to 10 ("worst agony possible"); It is used to indicate the current pain intensity as well as the best and worst levels experienced over the past 24 hours. The scale has been shown to have adequate reliability, validity, and responsiveness in patients with LBP.¹³

The participants perceived disability will be assessed by using a modified Oswestry Disability Questionnaire (ODI). The test-retest reliability of the modified ODI is strong (ICC =0.90). The modified ODI consists of ten items scored from 0 to 5, with a higher score indicating more disability. The results will be then converted into a percentage out of 100 where the higher score indicates greater disability.¹⁴

The patient's fear of pain and beliefs about avoiding activity will be assessed by the Fear-Avoidance Belief Questionnaire (FABQ). The FABQ is a self-report instrument that uses a 7-point Likert scale (0=completely disagree; 6=completely agree) to examine 16 items. A higher score suggests that the fear-avoidance belief is more deeply held. With an ICC range of 0.72–0.91, the FABQ is a solid outcome measure for assessing the fear of patients with LBP.¹⁵

Data collection and statistical analysis:

The baseline characteristics, including means and standard deviations will be described. The mean differences with SD for the end measures of pain on the NPRS, disability on the ODI, and fear avoidance belief questionnaire will be calculated for the time periods of baseline to 2 weeks, and 4 weeks. An unpaired t test will be used to analyse the between-group differences for all three outcome variables (pain intensity, disability, and fear avoidance belief) at each follow-up period. A priori, a 0.05 alpha level will be used.

Discussion

The goal of this study is to evaluate the effect of Muscle Energy Technique along with core stabilisation exercises on pain, disability and fear avoidance belief in patients with chronic low back pain with facet joint dysfunction. The Muscle Energy technique along with Lumbar Stabilisation Exercise training can be a viable approach for treating chronic non-specific low back pain patient with facet joint dysfunctions but there is a lack of literature on combined effect of MET and lumbar stabilisation exercise training.

Conclusion

Based on the finding of the study, specific recommendations will be made on the use of intervention for reducing pain, disability and fear

avoidance belief among the patients with chronic low back pain with facet joint dysfunction.

Conflict of interest: Nil

Funding: None

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Allele Frequencies and Forensic Statistical Parameters for 21 Autosomal Short Tandem Repeats (STRs) loci in Northern Thai Population

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How to cite this article: Supakit Khacha-ananda, Yutti Amornlertwatana, Phatcharin Mahawong et al. Allele Frequencies and Forensic Statistical Parameters for 21 Autosomal Short Tandem Repeats (STRs) loci in Northern Thai Population. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):354-359.

Abstract

Background: Although the genetic information of STRs has been established over the entire world, the different characteristics between populations influenced the STRs information. FBI's guideline recommended to expand additional number of STRs loci to increase power of discrimination and exclusion. Unfortunately, the current data of STRs in northern Thai population have not fully covered additional loci. The aim of this study is to obtain accurate allele frequencies of STRs among northern Thai population.

Methods: The genetic profiles of unrelated individuals were characterized by Investigator 24 plex Go kit. Allele frequencies and forensic statistical parameters were calculated within GenoProof[®]3 and Arlequin ver 3.5.2.2.

Results: The SE33 and TH01 loci represented largest and lowest number of different alleles, respectively. There was no significant deviation from the Hardy-Weinberg equilibrium (HWE) after Bonferroni correction in all loci ($p=0.002$). The SE33 locus showed the greatest PD and PE, whereas the TPOX represented the lowest PD and PE. The studied population (northern Thai) appear to be most closely related to previously reported populations containing of Chinese, Japanese, and Vietnamese based on allele frequency.

Conclusion: Our results recommended that current autosomal STRs data extended the application of STRs typing in parentage analyses and human identification among the localized population in northern region of Thailand due to highly informative polymorphic data.

Keywords: short tandem repeats; northern Thai; allele frequencies; forensic genetic.

Introduction

Short tandem repeats (STRs), also known as microsatellites, is a unique region on DNA

strand.¹ This region shows the high mutation rates, so it contributes to frequent mutation and great polymorphism more than other regions in DNA.²

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Due to high variable polymorphism and small DNA fragment in STRs region, STRs typing has been applied to provide useful information for human identity testing including DNA data basing, forensic casework, missing persons/mass disaster victim identification, and parentage testing.³ In 1997, the Federal Bureau of Investigation (FBI) announced the selection of 13 loci on STR regions containing of CSF1PO, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, FGA, TH01, TPOX, and VWA. The latest recommendations regarding the NIST highlighted the need for increased number of STR loci. Therefore, STR typing were extended to seven new additional loci containing D1S1656, D2S441, D2S1338, D10S1248, D12S391, D19S433, and D22S1045 in order to decrease the number of adventitious matches, increase international compatibility, and the power of discrimination for criminal and missing person cases.⁴

Thailand is geographically located on mainland Southeast Asia. The regions of Thailand can be divided into four main regions including north, northeast, center, and south according to topography.⁵ Because of the difference in environment and geographical features, Thai population is diverse in ethnicity, race, and languages including the genetic divergence. Moreover, the people in each region of Thailand tend to have specific characteristics and appearances because of different demography, histories, and genetic structures among major Thai populations.^{6,7} Therefore, it was probable that this phenomenon was probable to influence the variation in genetic pattern of autosomal STR including the allele frequencies of STRs in individual population. It is important to understand the genetic structure in individual population for providing the database and scientifically reliable results for forensic genetic purposes.

The database of allele frequencies worldwide has been provided by many research studies. They demonstrated that the allele frequency pattern was different between populations, which some alleles were predominate or absent in each populations.⁸ Currently available official allele frequencies for northern Thai population were latest published in 2006 with three STR loci obtained from only 203 unrelated northern Thai.⁹ There was also an official report in 2006 regarding 15 STR loci obtained from only 210 unrelated Thai individuals.¹⁰ Nevertheless, the current data of autosomal STR in northern

Thai population have not fully covered the seven new additional autosomal STR according to the recommendation of FBI CODIS Core Loci Working Group's progress. Therefore, the goal of this study is to obtain more accurate allele frequencies of 21 autosomal STRs loci among localized people who lived in northern Thailand in order to increase the amount of genetic data available and update the specific northern Thai frequency databases for all autosomal STR loci.

Material and Methods

Ethics approval and consent to participate:

Written informed consent was obtained from all subjects in this study. This study was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and approved by the Ethics Committee of the Faculty of Medicine, Chiang Mai University, Thailand (Study code: No.012/2019).

Sample collection

Biological buccal swab samples were collected from 280 unrelated individuals (126 male and 154 female) living in the provinces of northern region of Thailand and have been declared ethnicity as Thai. The informed consent forms were signed by all participants.

STR typing

The genomic DNA was extracted from buccal swab using Chelex 100 method.¹¹ Genotype at 21 autosomal STRs loci (CSF1PO, D1S1656, D2S441, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D10S1248, D12S391, D13S317, D16S539, D18S51, D19S433, D21S11, D22S1045, FGA, SE33, TH01, TPOX, and vWA) and amelogenin were obtained using Investigator 24plex Go kit (QIAGEN, Germany) according to the manufacturer instructions. The DNA amplification was conducted on 9600 Thermal Cyclers (Applied Biosystems, CA, USA). The amplification products were separated on the ABI Prism 3130 Genetic Analyzer (Applied Biosystems, CA, USA). GeneMapper™ ID-X Software v1.6 (Thermo Fisher Scientific, MS, USA) was used for raw data analysis. The positive and negative control DNA included in the kit was also analyzed. The quality control was performed for day-to-day quality control, laboratory internal controls, kit controls, and genotypes for overlapping loci between kits. Moreover, the quality control for

STR typing was regularly annual participated in proficiency testing program provided by Forensic DNA Network of Thailand organization.

Statistical analysis

For all autosomal 21STRs loci of Investigator 24plex Go kit, the allele frequencies, polymorphic information content (PIC), power of discrimination (PD), power of exclusion (PE), paternity index (PI), observed heterozygosity (Ho), expected heterozygosity (He), and Hardy-Weinberg equilibrium (HWE) were calculated within GenoProof[®]3 (Qualitytype GmbH, Germany) and Arlequin ver 3.5.2.2.¹² The neighbor-joining dendrogram was constructed based on D_A genetic distance results using POPTREE software.¹³ The tree was constructed with allele frequency data based on pairwise D_A distance of 15 STR loci (CSF1P0, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D19S433, D21S11, FGA, TH01, TPOX, and vWA) for all populations which were previously reported including northeast Thai¹⁴, central Thai¹⁴ southern Thai¹⁴ Laotian¹⁵, Vietnamese¹⁶, Malaysian¹⁷, Han Chinese¹⁸, Yunnan Zhuang Chinese¹⁹, Yunnan Dai Chinese¹⁹, Yunnan Hani Chinese¹⁹, Japanese²⁰ and Hongkong Chinese²¹

Results

The largest number of different alleles was found on locus SE33, followed by FGA and D21S11, whereas the lowest number of different alleles was also observed on locus TH01. The most frequent allele among all allelic variants was the allele 8 at TPOX with allele frequency 0.5661. There were distinct microvariant alleles found in eight loci of 21 STRs markers, with one being located at TH01, three at D2S441 and D7S820, four at D1S1656, six at D19S433, seven at D21S11, seven at FGA, and nineteen at SE33.

The forensic statistical parameters for all autosomal STRs loci are provided in Table 1. The H_o ranged between 0.6179 (TPOX) and 0.9500 (SE33), the H_e ranged from 0.5945 (TPOX) to 0.9431 (SE33). All autosomal STRs markers were classified as a highly information with PIC value more than 0.6 except for locus TPOX. The potential power of a genetic marker to differentiate between any two people chosen at random was expressed as PD. The high discriminating power can be used to discriminate between members of the population. The PD ranged from 0.5613 (TPOX) to 0.9929 (SE33) with the value

of 1 for the combined PD. The PE was investigated to evaluate the loci or system efficiency for exclusion of a non-related individual in paternity testing. The SE33 locus showed the greatest PE in studied population, whereas TPOX showed the lowest PE value. Our study revealed that there was no significant deviation from HWE for autosomal STR loci (p -value greater than 0.05) except locus D13S317, D16S539, D18S51, and D19S433. However, no significant deviation from HWE was found after Bonferroni correction ($p=0.002$). As a result, it was established that the 21 autosomal STRs loci were in equilibrium.

Regarding genetic relatedness, our informative study was compared with previous data. The Neighbor-joining tree based on D_A distance represented four close cluster including northeast Thai and Laotian, Han Chinese and Japan, Yunnan Zhuang Chinese and Yunnan Hani Chinese, southern Thai and Malaysian. The D_A distance of the studied population showed that the studied population (northern Thai) and other populations consisting of Chinese, Japanese, and Vietnamese had the genetic relatedness.

Discussion

The discovery of genetic profiles in specific population is expected to expand in a future since the globalization affected the increase of population number and rapid migration between countries.⁸ To increase the power of discrimination for criminal and missing person cases as well as decrease the number of adventitious matches, the FBI launched the seven additional core loci.⁴ The allele frequency of our studied population in northern region of Thailand was slightly different from other reported population in Thailand. A possible reason was that Thailand has a diversity of ethnicity, culture, and linguistics due to the migration and settlement of different ancestor populations. The northern Thai population was recently originated from the Khon Mueang population speaking a Tai-Kadai language which had migrated from the population in southern China, however Mon group who drifted from Myanmar was a major group of Central Thai.^{23, 24} Moreover, the other reason to influence the variation in autosomal STR is to mutation process especially strand-slippage replication.²⁵

According to seven additional analyzed STR loci here, we observed microvariant alleles in D1S1656, D2S441, and D19S433 loci. A microvariant was occurred during one of the repeat units contains only one, two or three bases of the repeat motif.²⁶ The microvariants in D1S1656 was found to be 15.3, 16.3,

17.3, and 18.3 as well as D2S441 locus with 11.3 which were not observed and reported in STR database of

National Institute of Standards and Technology (NIST), U.S. department of Commerce.

Table 1: Forensic descriptive statistic of 21 autosomal STR loci among unrelated northern Thai population (n = 280)

Parameters	Polymorphic information content (PIC)	Power of exclusion (PE)	Paternity index (PI)	Power of discrimination (PD)	Combined PD	Combined PE	Observed heterozygosity (Ho)	Expected heterozygosity (He)	p-value
CSF1PO	0.6786	0.4667	1.8129	0.8174	1	1	0.71786	0.7255	0.22323
D10S1248	0.7318	0.542	2.1603	0.876	1	1	0.775	0.76992	0.43333
D12S391	0.8294	0.6898	3.2766	0.9481	1	1	0.80714	0.84892	0.29462
D13S317	0.7627	0.5872	2.4223	0.903	1	1	0.78571	0.795	0.04332
D16S539	0.7431	0.5581	2.2478	0.886	1	1	0.78929	0.77895	0.0075
D18S51	0.8428	0.712	3.5371	0.9559	1	1	0.82143	0.86018	0.00313
D19S433	0.8098	0.6564	2.9459	0.934	1	1	0.77143	0.83176	0.02393
D1S1656	0.8158	0.6631	3.0074	0.935	1	1	0.79643	0.83524	0.28387
D21S11	0.8151	0.6647	3.0226	0.9373	1	1	0.825	0.83607	0.96152
D22S1045	0.7205	0.5251	2.0736	0.864	1	1	0.73571	0.76024	0.76074
D2S1338	0.852	0.7269	3.7353	0.9604	1	1	0.85	0.86769	0.7459
D2S441	0.7685	0.5959	2.4793	0.9078	1	1	0.80357	0.79976	0.09985
D3S1358	0.6826	0.476	1.8512	0.8284	1	1	0.75	0.73122	0.4548
D5S818	0.7581	0.5804	2.3795	0.8994	1	1	0.775	0.79129	0.21171
D7S820	0.7476	0.5604	2.261	0.8845	1	1	0.77143	0.78025	0.96815
D8S1179	0.8342	0.6994	3.385	0.9527	1	1	0.85357	0.85381	0.34405
FGA	0.8783	0.7719	4.4818	0.9728	1	1	0.86786	0.89003	0.08972
SE33	0.9382	0.8805	8.5319	0.9929	1	1	0.95	0.94308	0.81684
TH01	0.6324	0.4047	1.5848	0.7535	1	1	0.65357	0.70516	0.18592
TPOX	0.5362	0.283	1.2297	0.5613	1	1	0.61786	0.59447	0.85243
vWA	0.7553	0.5753	2.3486	0.8958	1	1	0.83214	0.78851	0.42017

The forensic statistical parameters showed that all autosomal STRs markers were classified as a highly informative except for locus TPOX. Consistently with previous report, TPOX loci was also reported to have a lower PIC in Thai population.¹⁰ Iraqi Kurds population in Kurdistan Region-Iraq²⁷, Chinese Han population²⁸, and population from Kingdom of Bahrain²⁹. Moreover, we found that the greatest and lowest of PE were observed in locus SE33 and TPOX, respectively which was consistent with previous report in population of Thailiving in southern border provinces of Thailand (Pattani, Yala, Narathiwat provinces).³⁰ Obtained result is according to the previously observed from other publication in Ecuadorian and Bahraini population. It recommended that SE33 was the best STRs loci for human identification with the highest value of PIC, PD, and PE.^{29,31}

The D_A distances showed that the studied population (northern Thai) and other populations consisting of Chinese, Japanese, and Vietnamese had the genetic relatedness. Our study clearly showed that the large genetic distance of northern Thai and southern Thai population, possible due to the difference in culture, linguist, and religion since the major population in southern Thai is Thai-Malay Muslims (MUS) which was descended from population in India and distributed over Malay Peninsula according to historical evidence.³² This assumption was confirmed by the finding of close genetic relationship between southern Thai and Malaysian population in our study. Moreover, the southern Thai people showed the close genetic structure with Indian population since the migration of the Austroasiatic linguistic population into Southeast Asian region.³³ However, some studies found the close relationship between Chinese and northern Thai because the gene flow from southern China to the northern Thai people over the past one thousand years.^{18,33}

Conclusion

Our result recommended that 21 autosomal STRs loci were suitable for paternity testing and human identification among the localized population in northern region of Thailand due to high genetic polymorphism and information.

Conflict of interest: The authors declare that they have no conflicts of interest.

Source of Funding: This work was supported by Faculty of Medicine, Chiang Mai University, grant no. 102/2562.

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HbA1c and Serum Level of VEGF in Diabetic Retinopathy Patients

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How to cite this article: Surasmiati N.M.A., Wijayati, M.P, Pramita, I.A.A et al. HbA1c and Serum Level of VEGF in Diabetic Retinopathy Patients. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):360-364.

Abstract

Objective: This study aims to determine whether HbA1c level has a correlation with vascular endothelial growth factor (VEGF) serum level in diabetic retinopathy (DR) patients.

Methods: This is an analytical cross-sectional study of patients with DR due to type 2 diabetes, with a purposive sampling technique. Patients were grouped into non-proliferative DR (NPDR) and proliferative DR (PDR). HbA1c and VEGF serum levels were assessed by taking the patient's venous blood.

Results: A total of 82 samples were included, in which the mean HbA1c levels were $8.17\% \pm 1.91\%$ and the median VEGF levels were 85.78 ng/L (range 38.23-149.43 ng/L). A total of 23 out of 35 NPDR patients were female (65.7%), while 29 out of 47 PDR patients were male (61.7%). Approximately 61.7% of PDR patients had a DM duration of more than 10 years, while 62.9% of NPDR patients had DM duration of less than 10 years. There was an increase in the mean of HbA1c levels in the PDR group compared to NPDR, although it was not statistically significant ($p = 0.214$), and there was no difference in the median VEGF levels of the two groups. Spearman's correlation analysis revealed no correlation between HbA1 and VEGF levels in diabetic retinopathy, in both the NPDR and PDR groups (correlation coefficient 0.183 and -0.022 respectively).

Conclusion: No statistically significant correlation was found between HbA1c and VEGF serum levels in diabetic retinopathy patients. In this study poor glycemic control were not proven for their implications for VEGF progression. VEGF serum levels may not be used as a marker of DR severity.

Keywords: HbA1c levels; VEGF serum levels; diabetic retinopathy; HbA1c-VEGF correlation.

Introduction

Retinopathy is a microvascular complication due to chronic hyperglycemia. This complication occurs in about 42-60% of patients with type 2 diabetes, and 99% of patients with type 1 diabetes.¹⁻³ Retinopathy has been known to cause blindness in productive age (20-64 years old).⁴ The prevalence of type 2

diabetes mellitus (DM) in Indonesia is around 2.1% (5.5 million population)⁵ while the prevalence of diabetic retinopathy (DR) were 43.1%.^{2,5,6}

Vascular endothelial growth factor (VEGF) is a protein that plays a role in the angiogenesis process that occurs in DM and DR as a response to hypoxia and hyperglycemia. VEGF can stimulate endothelial

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cells to degrade the extracellular matrix in the process of forming new blood vessels. VEGF plays an important role in the early stages of retinopathy, and intraocular VEGF can be an important marker.⁷⁻⁹

HbA1c levels, which represent glycemic control in 3 months, have been associated with complications of DM. According to The Diabetes Control and Complications Trial Research Group, a reduction in HbA1c from 9% to 7% over 6.5 years can reduce the risk of retinopathy by 76%.¹⁰

This study was conducted to determine the correlation between HbA1c and VEGF serum levels in DR patients, in both non-proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR).

Methods

This research protocol has been approved by the Research Ethics Commission Faculty of Medicine, Udayana University. A total of 82 DR patients who came to the retina eye clinic of the Bali Provincial Government Eye Hospital were sampled. The study was conducted from August 2020 to January 2021. The inclusion criteria in this study were type 2 DM patients who were over 40 years old and had a retina dilated pupil examination to confirm the diagnosis of DR. Diabetic retinopathy in this study was grouped into 2, namely NPDR if microaneurysms were found without neovascularization, and PDR if there was neovascularization in the retina or optic disc. The exclusion criteria in this study were patients who had undergone avastin injection in the last 3 months, and had undergone vitrectomy surgery. Other exclusion criteria were patients on immunosuppressant drugs and patients with a history of malignancy. Subjects who met the inclusion and exclusion criteria then signed the informed consent. Data regarding the duration of DM, history of hypertension, and body mass index were recorded in the research sheet. The patient then drew 3 ml of EDTA tube venous blood for HbA1c examination by Immunoassay-Turbidimetry, and 3 ml of plain tubes for VEGF examination using the Elisa (Bioassay Technology Laboratory) method.

Statistics

All numerical data were tested for normality by Kolmogorov-Smirnov. Numerical data that were not normally distributed were subjected to a non-parametric test using the Mann-Whitney test. Chi-Square test was used for proportions of categorical

data. The relationship between 2 numerical variables was analysed by the Spearman's correlation test.

Results

A total of 82 type 2 DM patients were included in this study. It was found that there were equal numbers of male and female, as many as 41 subjects. The range of age in this study was 43-76 years old, with a median was 56 years old. The characteristics of the Body Mass Index (BMI) in this study were mostly with a normal BMI (48.8%), followed by overweight as much as 37.8%. Most of the research subjects had hypertension as many as 49 people (59.8%). Mostly it was found that the DR patients had experienced type 2 diabetes for ≥ 10 years, which was 51.2%. DR patients in this study were grouped into 2 groups, namely the PDR group and the NPDR group based on the presence or absence of retinal neovascularization after evaluation of the retina in the dilated pupil. The PDR group was found to be more numerous, which were 47 people (57.3%). The mean HbA1c levels were $8.168 \pm 1.91\%$. The median serum VEGF level was 85.779 ng/L with a range of 38,227-149,430 ng/L.

Twenty three out of 35 subjects in the NPDR group were female (65.7%), while the PDR group there were mostly male, as many as 29 (61.7%) from a total of 47 patients. There was a significant difference between these two groups with $p = 0.014$. In NPDR group was 45-75 years, with a median 60 years. While in the PDR group, it was 43-72 years old, with a median of 55 years. There was a statistically that PDR patients were younger than the NPDR group ($p=0.013$).

The Body Mass Index (BMI) in the NPDR and PDR group was mostly normal (48.8% and 51.1% respectively), followed by overweight (40% and 36.2% respectively). There was no statistically significant difference with the 2 groups ($p=0.952$).

It was found that 24 (68.6%) of NPDR patients and 25 subjects (53.2%) in PDR group had hypertension. In the NPDR group, it was found that most of the patients had type 2 diabetes for less than 10 years, as many as 22 people (62.9%). This is different from the PDR group, where it was found that the majority of subjects had type 2 diabetes mellitus for ≥ 10 years, as many as 29 patients (61.7%). This difference was statistically significant with $p = 0.028$.

The mean of HbA1c level in the NPDR group was 7.894% with a standard deviation of 1.961, while

in the PDR group it was 8.372% with a standard deviation of 1.869. There was an increase in the mean of HbA1c levels on the progression of DR, but it was not statistically significant ($p=0.214$).

The relationship between HbA1c and VEGF serum levels in DR patients, in both PDR and NPDR is shown in Table 3. The results showed no correlation between glycemic control and VEGF levels in the DR ($p = 0.634$), in the PDR group ($p = 0.884$), and in the NPDR group ($p = 0.294$).

Discussion

In the PDR group, most patients had been diagnosed with type 2 diabetes for more than 10 years. The duration of DM is associated with the onset of microvascular complications.^{11,12} The duration of DM less than 10 years generally has a low prevalence of retinopathy which is mostly non-proliferative.¹³

Most of the DR patients in this study were found to have hypertension (59.8%). This is consistent with research from Lokesh and Shivaswamy, 2018; Yin et al, 2020.^{14,15} Hypertension is associated with the incidence of DR, whether it is hypertensive conditions that are not under therapy or who have received therapy, but are not controlled.^{16,17} Based on The Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adult, 2017, suggests that a decrease in blood pressure of less than 130/80 mmHg, in DM patients, can reduce complications.¹⁸ Well-controlled hypertension will reduce the risk of progressive retinopathy by 34%.⁴

Diabetic retinopathy patients in this study mostly had normal BMI, in both the PDR and NPDR groups. This study obtained the same results as Awata et al. 2002.¹⁹ Research by Lim et al., 2010 was found BMI as a risk factor for DR, possibly because it involves platelet function, blood viscosity, aldose reductase activity and vasoproliferative parameters such as VEGF.^{20,21}

The mean HbA1c levels were higher in the PDR group. This study found an increase in the mean HbA1c level in the PDR group compared to the NPDR group, although it was not statistically significant. Decreasing HbA1c levels will reduce the complications that occur. A reduction in HbA1c levels of less than 6.5% can prevent the incidence and progression of DR. HbA1c level is one of the indicators used to assess glycemic control, and is not affected by daily blood sugar fluctuations because

it is bound to hemoglobin. Research conducted by Nakamura et al., 2008 found that HbA1c levels were associated with DR. However, several studies have found different results, that HbA1c levels not reflect the state of DR, and do not have a correlation with the severity of DR.^{19,22-24}

Serum VEGF levels in this study were not significantly different in the NPDR and PDR groups. This is similar to studies conducted by Meleth et al. 2005 and Ozturk et al. 2009.^{25,26} However, several studies have found serum VEGF levels to be associated with the progression of DR.^{27,28} The study of Sarkar et al., distinguished the DR group into mild NPDR, moderate NPDR and PDR, and the mean serum VEGF levels increased with severity. Several studies regarding the differences between vitreous and serum VEGF levels have found that there was an increase in VEGF levels, in the diabetes group compared to normal in both the vitreous and serum.^{25,29} In Murugeswari et al's study, vitreous VEGF levels were significantly higher than serum levels, indicating the occurrence of a local inflammatory process in DR.³⁰

VEGF, is a proangiogenic glycoprotein, which increases vascular permeability in DR. VEGF is expressed in vivo and in vitro and is induced by hypoxia. VEGF levels in general can be influenced by various factors, such as tissue hypoxia, hyperglycemia, oxidative stress, and the environment. The results of serum VEGF levels will be different from VEGF levels in eye tissues, such as aqueous and vitreous humor. Increased VEGF production in hyperglycaemia occurs even before any clinical features appear on funduscopy.³¹ The important role of VEGF in the pathogenesis of diabetic macular edema, makes intravitreal anti-VEGF as the main choice of therapy.³² Administration of intravitreal anti-VEGF injection therapy has also shown a decrease in serum VEGF levels.³³

This study found no relationship between HbA1c and VEGF levels. This is consistent with research from Ozturk et al., 2009.²⁶ This may occur because serum VEGF levels are influenced by many factors such as other inflammatory factors, epigenetic factors and differences in research methodology such as sampling techniques and sample processing. This study did not compare with the type 2 DM group without the complications of DR, and also with the healthy group so that it could not compare the results with the group without retinopathy This study is also

cross-sectional, so it does not describe whether the condition of retinopathy is acute or not, so the effect of serum VEGF may not be significant. Nevertheless, other studies have found a significant correlation between VEGF levels and HbA1c levels so that serum VEGF levels are considered to predict DR because they represent endothelial damage in diabetes.^{27,28}

Conclusion

This study found no significant correlation between HbA1c and VEGF serum levels in DR patients. In this study poor glycemic control were not proven for their implications for DR and VEGF progression. The difference in the results obtained with other studies could be due to inflammatory and epigenetic factors, and this may also be due to differences in the research methodology. This can be used as a basis for considering a better research methodology for future study, in terms of looking for the correlation between HbA1c and serum levels VEGF.

Acknowledgement

The author would like to thank all the staff of the eye clinic of Bali Mandara Eye Hospital Denpasar who have wholeheartedly participated in this research.

Conflict of Interest: All authors declared that they have no conflict of interest.

Funding Source: This research received a special grant from the Research and Community Service Institute of Udayana University.

Ethical Clearance: The ethical statement was taken from Faculty of Medicine, Udayana University Ethics committee on March 19, 2020 with number 671/UN14.2.2.VII.14/LT/2020

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Protein Content Test of Tilapia Fish Extract (*Oreochromis Mossambicus*) before and after Freeze Dry Using Biuret Method

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How to cite this article: Walin, Hardono Susanto, Bagoes Widjanarko et al. Protein Content Test of Tilapia Fish Extract (*Oreochromis Mossambicus*) before and after Freeze Dry Using Biuret Method. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):365-369.

Abstract

Objective: To determine the difference in protein content of tilapia extract before and after drying process.

Materials and Methods: This study was designed with a true experimental design. The research sample was tilapia fish extract with 3 replications in each sample. The measurement of protein content was carried out on samples before and after drying. The drying process of tilapia extract was carried out for 48 hours using a freeze dryer. Furthermore, the dried samples were tested for protein content using the biuret method supported by the UV-Vis Spectrophotometer instrument. The testing stages include making the standard solution, determining the maximum wavelength, determining the operating time, and making a standard curve.

Results: Showed that the protein content of tilapia extract before and after drying was 2.23% and 27.4%, respectively. The results of statistical analysis showed that there was a significant difference between the protein content of tilapia fish before and after drying.

Conclusion: There was an increase in protein content of tilapia fish extract after drying by 25.17%.

Keywords: Protein; Tilapia Fish; Extract; Freeze Dry; Biuret.

Introduction

Tilapia fish (*Oreochromis mossambicus*) is one of the freshwater fishery commodities. Tilapia fish easily live and breed in various conditions such as water with high salinity and low salinity/saltiness content. There are several nutritional contents that is very beneficial for human health, one of them is protein which help the process of growth and development of organs, membranes and cells. The protein content in tilapia fish is higher when compared to other fish, which is around 18.7%.¹ The high nutritional value

contained in tilapia fish encourages people to process it into various kinds of food products.²

Tilapia fish can also be used as a supplement with various processing techniques such as steaming, boiling, roasting and frying. Fish meat that is processed with high heating temperatures and for a long time can cause protein denaturation.³ This problem can be overcome by making the mozambique tilapia fish in extract form and dried using a freeze dryer which is safer against the risk of degradation of compounds in the extract. Freeze dryer is the best drying process

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for thermolabile products because it can reduce the nutritional degradation of a product. Freeze dryers are widely applied to the food industry, pharmaceutical industry, and health product industry.⁴

Based on the research of Asrullah et al. (2012), it is known that the decrease in content and changes in protein digestibility depend on the treatment method, so it is necessary to measure protein content before and after freeze dry using the biuret method. Determination of protein content of tilapia fish extract can be done using the biuret test supported by a UV-Vis spectrophotometer instrument.⁵ The biuret test was chosen because it is simpler and has less color deviation occurs when compared to other methods. The biuret method is based on measuring the absorption of purple light that comes from the reaction between protein and biuret reagent.⁶ This study was conducted to determine the protein content of tilapia fish extract (*Oreochromis mossambicus*) before and after the drying process freeze dryer using the biuret method.

Materials and Methods

This study was designed with a true experimental design.

Tools and materials. The tools used in this study are hydraulic press (modification), centrifuge, freeze dryer, UV spectrophotometer, measuring cup, measuring flask, test tube, beaker glass, analytical scale, water bath, napkin, gas stove, pan, volume pipette, spatula, plastic container, horn spoon, mortar, stamper, watch glass, and stirring rod. The materials used in this study are tilapia fish (*Oreochromis mossambicus*), aquadest, biuret reagent, Bovine Serum Albumin (BSA) solution.

Sample preparation. Tilapia fish with 300 grams weight is cleaned from its scales and then the contents of its stomach are removed and washed thoroughly with running water. The fins and head are cut and the body of the fish is sliced. The fish is steamed in steamer pan for 50 minutes, keeping the heat from getting too high so it doesn't boil (about 90-95°C) and the water doesn't get into the fish team. Put the fish in a high-pressure squeezer machine while it is still hot. The juice is filtered and packaged immediately to avoid contamination in a 200 ml capacity thick plastic then sealed it with electric sealer. After packing is completed, check that there are no leaks

in the packing. After all the steps are done, stored the product in the freezer with temperature below 10°C.

Freeze Dry. The water phase of tilapia fish extract was put into a tube with an equalized volume then the tube was put into the freeze dryer. Drying is processed automatically, the freeze dry state must be ensured that the power is in the "off" position, all buttons and valves are in the "off" and "close" position. After the "power" cable is connected to an electric current source, pressed the power button to "on" position (for lexol circulation) \pm 24 hours before the operation.⁷

Protein Content Test Using Biuret Method

Preparation of Solutions. A total of 0.05 grams of Bovine Serum Albumin (BSA) was dissolved with distilled water in a 10 ml volumetric flask to the mark, so that a standard solution with a concentration of 5000 ppm was obtained.

Determination of Maximum Wavelength. 0.4 ml of standard solution was added with distilled water up to 4 ml then added with 6 ml of biuret reagent, then analyzed by UV spectrophotometry at a wavelength of 400-700 nm. The results of this study indicate that the maximum wavelength is 592.8 nm at an absorbance of 0.2385.

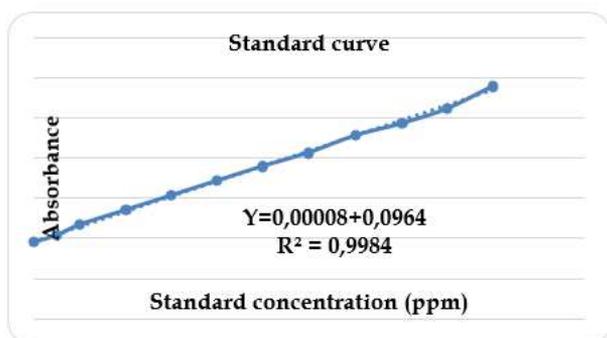
Determination of Operating Time. 0.4 ml of standard solution was added with distilled water up to 4 ml, then 6 ml of biuret reagent was added, then analyzed by UV spectrophotometry for 5-45 minutes at the maximum wavelength obtained in the previous stage. Operating time is determined by measuring the absorbance at the maximum wavelength from the data that has been generated at the determination of the maximum wavelength of 592.8 nm. The best operating time result in this study was in minutes 35 because in this time a stable product compound began to form, with an absorbance value of 0.2318.

Standard Curve Creation. Standard curve is made to determine the equation of linear regression. This is done by preparing 12 test tubes. The first tube is filled with blank solution (solvent). In the second tube and so on, it is filled with the composition according to the table below and the absorbance is measured using a spectrophotometer with a maximum wavelength then made it into a curve to obtain a linear equation.

Table 1: Composition of standard curve tube

BSA standard solution (ml)	H ₂ O (ml)	Biuret Reagent (ml)	Standard Concentration (ppm)
0.0	4	6	0
0.1	3.9	6	125
0.2	3.8	6	250
0.4	3.6	6	500
0.6	3.4	6	750
0.8	3.2	6	1000
1.0	3	6	1250
1.2	2.8	6	1500
1.4	2.6	6	1750
1.6	2.4	6	2000
1.8	2.2	6	2250
2.0	2	6	2500

Based on the concentration data of the standard solution with the absorbance of the solution, the results obtained from the linear equation $y = 0.00008x + 0.0964$ with a value of $R^2 = 0.9984$. The equation shows that b is positive, which means that the higher the concentration, the higher the absorbance value. The value of the correlation coefficient (R^2) in this study was in the range between 0.80 to 0.99 which was classified as a very strong correlation.⁸

**Figure 1: Standard curve****Table 2: Results of Protein Content Analysis of Tilapia Fish Extract**

Sample (replication)	Protein content (%)		Statistical test results paired t-test (p-value)
	Before drying	After drying	
1	2,165	27,900	0,0001
2	2,215	26,750	
3	2,310	27,550	
Average	2,230	27,400	

Results

Protein content tests were carried out on a liquid tilapia fish extract (before freeze dry) and solid tilapia fish extract (after freeze dry). The protein content test was replicated 3 times on each sample using the UV spectrophotometer. The volume of each liquid samples were 5 ml. From Table 2 it is known that the average protein content of liquid tilapia fish extract (before freeze dry) is 2.230% and the average protein content of solid fish extract (after freeze dry) is 27.400%.

The results of the statistical paired t-test showed the p-value = 0.0001 < (0.05). This means that there is a significant difference between the average level of protein of liquid tilapia fish extract (before freeze dry) and solid (after freeze dry). The difference in the average value of protein content before and after freeze dry is 25.17%. A positive difference value indicates that there is an increase in the average protein content of tilapia fish extract after freeze dry.

Discussion

The results were significantly difference between the average level of protein of liquid tilapia fish extract (before freeze dry) and solid (after freeze dry). The difference in the average value of protein content before and after freeze dry is 25.17%. A positive difference value indicates that there is an increase in the average protein content of tilapia fish extract after freeze dry. Supported by the research of Saputro et al. (2019) which stated that there was a significant difference between the protein content of snakehead fish extract before and after drying. This study is also in accordance with previous research by Andana (2018) which stated that there was an increase in protein content of processed fish products (surimi) after the drying process from 9.69% to 44.87%.^{9,10}

The drying process of fish extract can reduce the percentage of water content which can increase the percentage of protein content. This is supported by Ahmed et al. (2010) who stated that reduced water content resulted in high ash, protein and fat content. In the study of Alhanannasir et al. (2017), water absorption by freeze dry proven to be higher and shorter than the oven method. The amount of water absorbed by oven method is 4.09% for 60 minutes, while the amount of water absorbed by freeze dry can reach 45.29% in just 20 minutes. The research of Setyowati et al. (2017) also stated that the absorption of water with the freeze dry method is the best when compared to the cabinet and oven drying methods.⁽¹¹⁻¹³⁾The drier the material, the higher the protein content contained in it.⁽¹⁴⁾

In this study, the drying temperature using freeze dry reached until -100°C. The use of freeze dry method or drying at the temperature lower than the ambient temperature can prevent product damage caused

by oxidation or chemical modification.¹⁵ Supported by the research of Shen, et al., (2021) which stated that the protein content of freeze-dry was proven to be higher because it was less denatured during processing and showed better functional properties than protein which was dried by spray-drying and vacuum-drying.¹⁶

In contrast to the research, Fathonit et al. (2019) also stated that there was a decrease in protein content of cork fish extract after freeze dry up to 22.489%. The decrease in protein content can also be caused by the ineffective freeze dry process, so the frozen final product loses its nutritional composition and will most likely lose its taste.^{17,18} Smida et al. (2014) also stated that drying rate has a deleterious effect on protein content.¹⁹ Limitations of the research is : 1) Drying tilapia fish extract using the freeze dry method requires a long process and time, and 2) Not all laboratorium have freeze dry kit.

Conclusion

The research results showthat an increase in protein level of tilapia fish extract after drying from 2.23% to 27.4%. The results of statistical analysis concluded that there was a significant difference of protein content between before and after drying with freeze-dry.

What is already known on this topic?

A significant difference of protein content between before and after drying with freeze-dry. The difference in the average value of protein content before and after freeze dry is 25.17%.

What doesthis study add?

This research has confirmed that drying with freeze-dryincrease in protein level of tilapia fish extract after drying from 2.23% to 27.4%.

Potential conflicts of interest: None.

Ethical clearance

The present study was approved by the Health Research Ethics Committee of the POLTEKKES Ministry of Health in Semarang No.469/EA/KEPK/2021.

Source of funding: Self.

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Regression Models for Postmortem Interval Estimation by using Accumulated Degree Hours and Potassium in Vitreous and Blood

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How to cite this article: Warachate Khobjai, Pongpitsanu Pakdeenarong, Woratouch Vichuwanich. Regression Models for Postmortem Interval Estimation by using Accumulated Degree Hours and Potassium in Vitreous and Blood. *Indian Journal of Forensic Medicine and Toxicology* 2022;16(3):370-375.

Abstract

The biochemical changes of corpse are used for the postmortem interval (PMI) estimation, but decomposition is varied due to many factors. Ambient temperature is believed to be the most important factor which is related to biochemical change in the human corpse by using accumulated degree hours (ADH). The aim of this research was to develop exponential formula to estimate the PMI based on a potassium and ADH as the parameters. The secondary data analysis is conducted on 3 articles published in Thailand. The correlation data were analyzed by potassium, ADH, and time of death in each experiment. The temperature data was provided by the Thailand Meteorology Department (TMD). These results suggested that this developed method can use for PMI estimation. The additional step, namely to calculate the ADH from the finding date back until the predicted day of death from case reported and TMD. The regression analysis had a high reliability. The potassium level significantly estimates the PMI ($R^2 = 0.9183, 0.8296$ and 0.7142 for pig vitreous, corpse vitreous and corpse blood, respectively). When using the ADH in pig vitreous, the R^2 increased to 0.9931 . The developed potassium regression models are a practical method to measure PMI. The PMI can be estimated using this method, but caution is advised in case with a long PMI. It can be used to estimate the mortality time of the fresh-early decomposition stage, up to 48 hours. Due to the longer mortality, there may be a gradual rate of change and samples cannot be collected.

Keywords: Ambient temperature; Accumulated degree hours; Postmortem interval; Potassium.

Introduction

The time of death is extremely important to forensic medicine. There are various methods used to assessment, but often raise a question of the accuracy and precision in the PMI evaluation. The biochemistry postmortem changes after death are used to estimate

the time of death or PMI. The elements in vitreous humor (VH) are used to evaluate time since death, especially, potassium. The studies were performed on the VH^{1,2}, probably slowly autolytic changes to its being anatomically protected and isolated by its own structure.³ One method that forensic scientists have

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been explored for decades is the evaluation of the rise in potassium concentration in the VH.⁴ The several studies have found that the positive correlation between potassium concentration and PMI.⁵ Basically, most of previous equations are based on the approximation of the vitreous potassium according to both of a linear regression⁶⁻⁸ and exponential model.⁴

The various physicochemical changes of the corpse start immediately or shortly after death and continue in an orderly manner until the body decays. Each physiochemical and biochemical change has its pattern. Unfortunately, postmortem changes are strongly influenced by many unpredictable endogenous and exogenous, especially, environmental factors. Consequently, the longer the postmortem interval, the wider is the range of estimate as to when death probably occurred. In the other words, the less precise is the estimate of the time of death. The postmortem changes are a continuous process and are influenced by multivariable factors.^{9,10} The climatic differences between circumstantial regions, such as a hot or cold environment, influence the decomposition rate.¹¹ It is known from literature that temperature^{12,13}, body weight^{14,15} and clothing¹⁶ also have an effect on the decomposition process. It is said that ambient temperature is the most important variable, because it is the primary factor for all biological activity and biochemical reactions.¹⁶

In various studies, the relationship between the rise of potassium concentration in the VH and the time since death has been studied. There are many obstacles for using potassium as a tool for the time since death estimation, including some limitations and sample sizes given by different authors. Despite the numerous studies done in this field, there is still no consensus as to what formula or method is most accurate to estimate PMI from increased potassium in VH.⁴ The concentration of potassium increase in direct variable pattern with PMI.⁶ There are certain conditions that affect the methodology: correct sampling of VH, ambient temperature, body weight and temperature, metabolic status, agony preceding death, and even the cause of death are factors that can modify the results of PMI estimation.¹⁷⁻²⁰ The recent methods have been designed to determine the PMI based on the biochemistry of the VH as well as correctional factors such as ambient temperature with apparently good results.^{4,21} Nevertheless, it is essential to continue research in this field and to develop new methodologies that enable this calculation when the necessary data to apply existing

methods is not available, as well as improving the predictive power of these models. The availability of different methods of validation that integrate different parameters makes their use in daily practice more feasible and the option to choose one or another method. Based on the available data and the particular circumstances of each case helps to establish PMI in a more reliable way. In order to develop a methodology capable of estimating PMI with an easily, a quickly, and reliability obtained for tropical region. Therefore, the aim of this research was to develop exponential formula to estimate the PMI by using a potassium level and ADH.

Materials and Methods

The secondary data can be obtained from published reports, completed research papers, case studies, or published research articles about temperature changes in potassium levels and report the time of death of the corpse from papers published in journals in a manner consistent with the research objectives. The secondary data analysis is conducted from 3 articles published in Thailand. The research articles consist of time and date, ambient temperature, and potassium levels report.

Temperature collection

Ambient temperature was collected from Thai Meteorological Department (TMD) at that time in secondary data reported.

Potassium level and postmortem interval analysis

Pig vitreous carcass

The pig carcass data was obtained from Wohandee's research report.²² The objective was to study the relationship between the rate of change of various heavy metals in the VH for PMI predictable pattern. The study used 20 pig carcasses to observe the rate of body change after death and to assess the amount of electrolyte VH in 0, 1, 3, 6, 12, 24, 36, 48 hours. The researchers reported postmortem body changes as the time after death increased, environmental and body temperature, electrolytes in VH at the postmortem period increased. Therefore, these potassium data in the pig VH were used to estimate the postmortem period. The data were analyzed for new the relationship of changes in potassium levels and time after death with changes in ambient temperature. The exponential equation can be applied for estimating the time of death in corpses.

Human vitreous autopsy

The autopsy data was obtained from Soontorn’s thesis.²³ In this study, the amount of potassium, sodium and chloride ions were measured in the vitreous fluid to determine the relationship between electrolyte content and the time of death. The vitreous fluid from 50 corpses with the exact time of death was examined.

Human blood autopsy

Research data was obtained by Samroay’s thesis modification.²⁴ The research entitled “Estimation of death of dead bodies based on sodium, potassium and chloride levels in the blood”. The aim of the study was to determine the change in serum sodium, potassium and chloride levels at different times after death for use in estimating the time of death of a corpse.

Statistical analysis

The data were analyzed using GraphPad Prism 6 version 6.01 (GraphPad Software Inc. La Jolla, CA. USA). The data were analyzed for potassium, ADH and PMI. The values are displayed in the form of mean and standard deviation, linear and exponential regression.

Results and Discussion

The secondary data of Wohandee’s research report, the potassium data unit were modified from conventional unit to SI unit (mmol/L) shown in Table 1. The results shown that the potassium concentration in the VH was found to increase correlation with the time after death and ADH (Figure 1).

Table 1: potassium, PMI and ADH in pig carcass

PMI (Hours)	Potassium (Mean±SD) (mmol/L)	Accumulated degree hour (Hours)	Postmortem change
0	0.49±0.05	0.00	Fresh
1	0.67±0.11	6.60	Fresh
3	0.77±0.08	19.80	Fresh
6	1.08±0.07	39.60	Fresh
12	1.26±0.16	79.20	Early decomposition, dark skin
24	1.62±0.13	158.40	Dark brown skin,
36	1.69±0.18	237.60	Marbling
48	1.75±0.23	316.80	Late decomposition, fully bloat, bleb

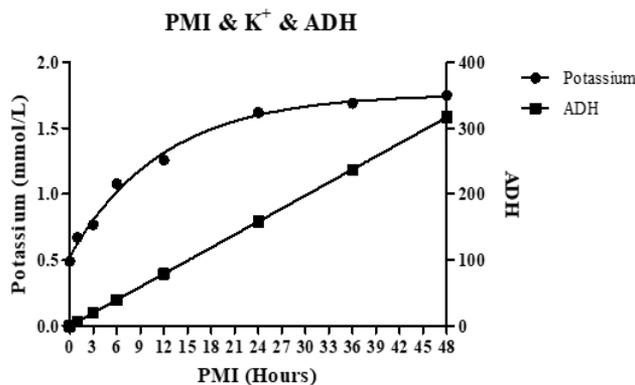


Figure 1: The relationship of potassium in pig VH, PMI and ADH

The relationships of potassium levels in pig carcass VH, PMI and ADH were shown as Figure 1. The linear regression of ADH and PMI was $Y = 6.600x - 0.0000002284$, $R^2 = 1.0000$. The exponential curve of

potassium and PMI is $Y = 0.5252 + 1.762(1 - \exp^{-0.0847X})$, R^2 is 0.9183 in Table 2. When Plateau was the Y value at infinite times, expressed in the same units as $Y = 1.762$ and K was the rate constant, expressed in reciprocal of the X axis time units = 0.0847. The relationship of exponential equation of potassium, ADH, and PMI was shown $R^2 = 0.9931$ (data not shown). The data were characterized by the correlation of potassium levels in the VH with different time periods of increased mortality consistent with the Zilg’s study.⁴ The exponential regression features have different slopes of curves at several points. The curve was initially steep and gradual, and the Y-intercept when entering fixed at Y_0 was 1.762 mmol/L during the 48 hours postmortem study period. When comparing the potassium levels of pig carcass vitreous humor at time 0, the unchanged potassium level was 7-10 times lower than that of the human

blood and vitreous humor. Comparison of initial potassium concentrations in pig VH were 1.99-2.84 times lower than human vitreous potassium levels. When applying this computational model to corpses, estimates of the duration of death may be inflated.

Table 2: Equation for postmortem interval estimation

Factors	Formula	R ²
Pig VH		
PMI-K ⁺	$Y = 0.5252 + 1.762(1 - \exp^{-0.0847X})$	0.9183
PMI-ADH	$Y = 6.6X - 0.0000002284$	1.0000
Corpse VH		
PMI-K ⁺	$Y = 2.779 + 7.491(1 - \exp^{-0.1159X})$	0.8296
Corpse blood		
PMI-K ⁺	$Y = 4.596 + 41.404(1 - \exp^{-0.06603X})$	0.7142

R² = Correlation coefficient

The secondary date of Soontorn's research report, the results shown that the potassium concentration in the VH was found to increase correlation with the time after death of corpse (Figure 2). The exponential curve of group vitreous potassium and PMI was $Y = 2.779 + 7.491(1 - \exp^{-0.1159X})$, R² = 0.8296 (Table 2). Forensic medicines estimate the time of death based on a several factors. The changes after death depend on the circumstantial environment. If the death environment was different, the change in the corpse condition would be completely different.

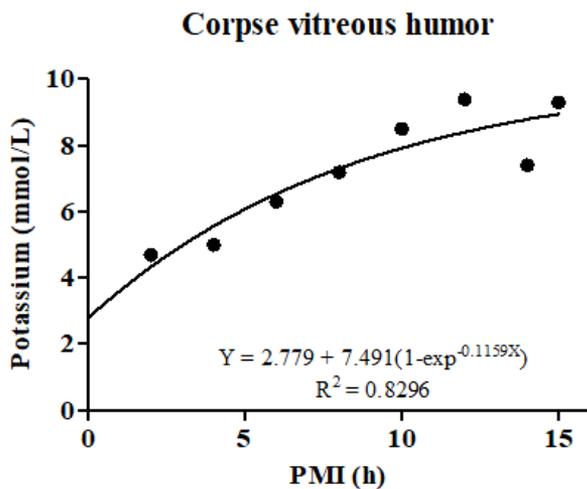


Figure 2: The exponential curve of potassium and PMI in corpses VH

The secondary data of Samroay's thesis report, the results shown that the potassium concentration in the blood was found to increase correlation with the time after death of corpse (Figure 3). The exponential curve of blood potassium and PMI was $Y = 4.596 + 41.404(1 - \exp^{-0.06603X})$, R² = 0.7142 (Table 2).

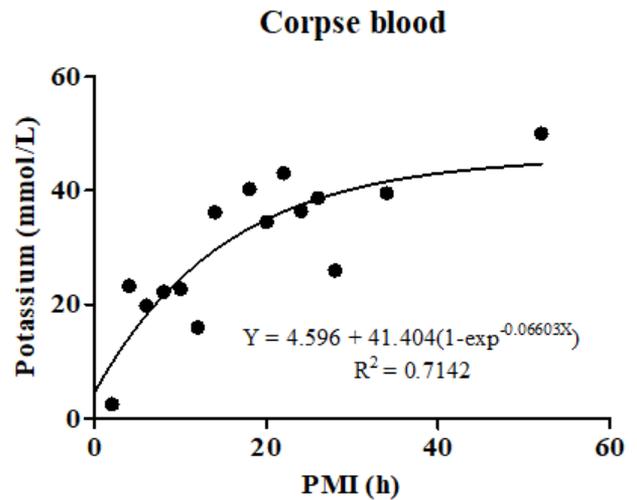


Figure 3: The exponential curve of potassium and PMI in corpse blood

The relationship of potassium levels in corpse blood and time since death was shown as Figure 3. The exponential curve of blood potassium and PMI is $Y = 3.795 + 39.575(1 - \exp^{-0.1047X})$, R² is 0.5476 in Table 2. The mean blood potassium concentration in humans was 4.14±0.46 mmol/L, which was within the reference range of 3.5-5.0 mmol/L. Comparing the equations, it was found that the exponential had better coefficients than the linear regression equations. It is suitable for early changes in potassium levels characterized by a steep curve and slows down as the mortality time increases to near constant.⁴ Potassium levels increased after death following a non-linear curve. Age factors and ambient temperature influence changes in potassium levels. The duration of injury and the high alcohol content at death were less than 1%, and the additional factors investigated did not affect the increase in potassium levels in the VH. Reported causes of death include intrathoracic hemorrhage, asphyxia from strangulation, stroke, asphyxia from choking, circulatory failure, trauma from a gunshot wound to the brain, a brain contusion, a torn lung, a torn liver and left kidney. These causes of death may be a factor in abnormal potassium levels in the blood. This is different from the concentration of potassium in the VH.

Conclusion

The present study collects data from published research on postmortem changes in biochemicals in the vitreous humor of pig carcasses and in the blood and vitreous of human corpses that can determine the timing of death. Our result can be use as data to create an equation model for estimating the time of death of dead bodies in Thailand which is characterized by a humid tropical climate. The regression equation has relationship of accumulated-degree hour to biochemical changes and decomposition processes of corpses. It can be applied to the death period after the onset of death up to 48 hours. Therefore, the PMI can be estimated using this method. However, caution is advised in the event of a prolonged death period and accumulated-degree day (ADD) is not indicative of the total heat present in the transition or decay that persists, which can be biased.

Acknowledgement

We are thankful to Graduate School, Silpakorn University for providing the facilities. We would like to thank Dr. Suebkul Kanchanasuk for recommendation on mathematical equations in this article.

Ethical clearance: Ethical committee Nation University, Thailand clearance was obtained before conducting the study (NTU.EC.1-005/2564).

Conflict of interest: None

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Histological and Histochemical Features of the Newly Lactating Udderinlocal Breed She Goat (*Caprice Hircus*)

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How to cite this article: Yousif R.Jumaah, Abdul KarimJafar Karim. Histological and Histochemical Features of the Newly Lactating Udderinlocal Breed She Goat (*Caprice Hircus*). Indian Journal of Forensic Medicine and Toxicology 2022;16(3):376-380.

Abstract

Six samples of one day post parturition mammary glands of local breed goats were used for this work. The samples of glands were dissected the tissue specimens were fixed in 10% formalin solution. The specimens were processed according to paraffin embedding technique, sectioned at 5-6 μ m and stained by hematoxylin and eosin, Masson's Trichrom stains and Verhoff stain. The newly lactating mammary gland was covered by very thick, well glandular skin that measured 210.32 \pm 9.03 μ m. The mammary parenchyma was composed of groups of huge mammary lobes which separated by well vascular thick layer of inter lobar fibrous tissue that measured 986.19 \pm 23.10 μ m (Thickness). The mean of surface area of mammary lobe was 134785.48 \pm 15.89 μ m², it was consisted of numerous small size lobules that measured 9848.79 \pm 23.75 μ m² and separated from each other by thin layer of fibro muscular inter lobular connective tissue that measured 138.16 \pm 4.11 μ m. The lobule was composed of mammary alveoli which revealed marked secretory activities. The mean surface area of alveolus was 784.79 \pm 19.09 μ m² that lined by cuboidal cells that measured 14.11 \pm 1.09 μ m. Duct system of newly lactating mammary gland was intra lobular duct, large inter lobar duct, largest lactiferous sinus, the lactiferous sinus was drainage to glandular cisterna that opened into teat cisterna and the later opened into teat canal. Gland cisterna was huge chamber had wide irregular shaped lumen. The mucosa of gland cisterna had few mucosal folds which lined by simple cuboidal epithelium and supported a lamina propria of dense fibro muscular connective tissue. The epithelium of cisterna which close to the mammary lobules was supported thick layer of well vascular fibrous connective tissue. Teat cisterna was composed of mucosal folds that supported by fibro muscular layer with venous plexus.

Keyword: Mammary gland; Goats; Post parturition mammary glands; Lactating; Histological.

Introduction

The mammary gland is an exocrine gland modified to the mammals which is adapted to the growth requirements in each species, the mammary gland develops during pregnancy and early

lactation, and regresses quickly after dry-off.¹ The mammary gland consists of the mamma and the teat, undeveloped in male and female at birth, the female mammary gland begins to develop as a secondary sex characteristic at puberty.² In the ruminants, the

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udder was divided into two distinct halves, separated by the medial or median suspensory ligament, which provided the strength to hold the udder attached to the ventral body wall.³ The Lactating mammary glands in small ruminants are classified as tubulo-alveolar glands which composed of many lobes those subdivided in to many lobules by strand of intra-lobular connective tissue. The parenchyma of mammary gland consists of alveoli, network of ducts and bundles of connective tissue. The alveolus is the basic secretory unit it is sac like or vesicle of unequal size. They are made of flattened or cuboidal epithelial cells with round nucleus. Myoepithelial cell present in between the basement membrane and epithelium lining the alveolus.⁴ The importance of small dairy ruminants has increased significantly during recent years, especially in developing countries where they are an interesting and important alternative for the supply of dairy products for human consumption, whereas in developed countries they are considered as a healthier alternative to cows' milk. Additionally, it is often considered that dairy production in developing countries is an essential tool to overcome social and economic issues such as poverty and malnutrition, particularly in infants.⁵ Several studies have investigated the factors influencing milk production, especially lactation stage are very important⁶ in cattle⁷ in camel⁸, havestudying the relationship between the external and internal udder features, teat parameters characters with machine milked of dromedary camels and in ewes have compared between immature mammary gland and lactating mammary gland.⁹ The current study was aimed to investigate the histological features of one day post parturition she goat udder.

Materials and Methods

A total six samples of healthy, lactating mammary glands of she goat were collected at one day post parturition used for this work. The sample were removed immediately from the carcasses and infused with 10% formalin throughout the teats orifices. Each sample was trimmed into small specimens involved teat canal, teat cistern, gland cistern, and parenchyma. All specimens were fixed by neutral buffer formalin 10%. The specimens were prepared with paraffin embedding technique. The tissues specimens were sectioned at 5-6 μm by rotary microtome. The prepared sections were stained with the Hematoxylin and Eosin stain, Masson's Trichrom stain and Verhoff stain¹⁰. The Histometrical measurements were the

diameters of the mammary lobes, lobules, alveoli, thicknesses of inter lobar connective tissue, thickness of inter lobular connective tissue, the heights of the alveolar epithelial cells and number of alveolar cells per alveolus. The sections were examined by light microscopy and photograph has been done by Future electronic microscopic camera, then images have analyzed and scored by using Fiji image analyzer system.¹¹

Results

During period of one day post parturient the skin of mammary gland was composed of thick hairy skin, the epidermis of skin was composed of very thick keratinized stratified squamous epithelium which measured $210.32 \pm 9.03 \mu\text{m}$, The dermis was composed of well glandular irregular denes collagenous connective tissue which enriched with groups of sebaceous, sweat glands and had no elastic fibers, the cutaneous glands were composed of groups of tubular coiled apocrine sweat glands and sebaceous glands (Fig. 1).

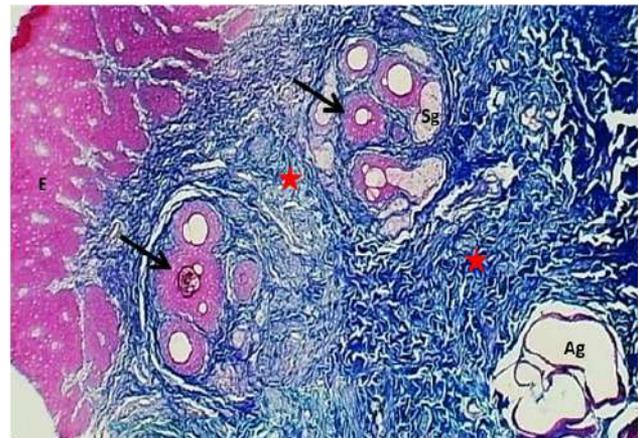


Figure 1: section of skin of goat's mammary gland (One day post parturient) shows: epidermis (E), sub dermal collagenous connective tissue (Asterisks), apocrine sweat glands (Ag), sebaceous gland (Sg), hair follicles (Black arrow). Masson's trichrom stain 40x.

The fibro muscular layer beneath skin of the newly lactating mammary gland was well developed to represent the mammary ligaments, it was thick layer of paralleled and inter crossed bundles of smooth muscle fibers that intermingled with collagen bundles close to glandular mass of the mammary quarter.

At one day post parturition the udder parenchyma was composed of groups of large size lobes which separated by very thick layer of well

vascular inter lobar fibrous connective tissue that measured $986.19 \pm 23.10 \mu\text{m}$. the inter lobar connective tissue was enriched with small size arteries, veins in addition for branches of autonomic nerves fibers (Fig. 2). The mean of surface area of mammary lobe was $134785.48 \pm 15.89 \mu\text{m}^2$ that consisted of numerous small size lobules which measured $9848.79 \pm 23.75 \mu\text{m}^2$ those separated from each other by thin layer of fibro muscular inter lobular connective tissue with mean thickness measured $138.16 \pm 4.11 \mu\text{m}$. Each mammary lobule was composed of cluster of mammary alveoli which revealed marked secretory activities.

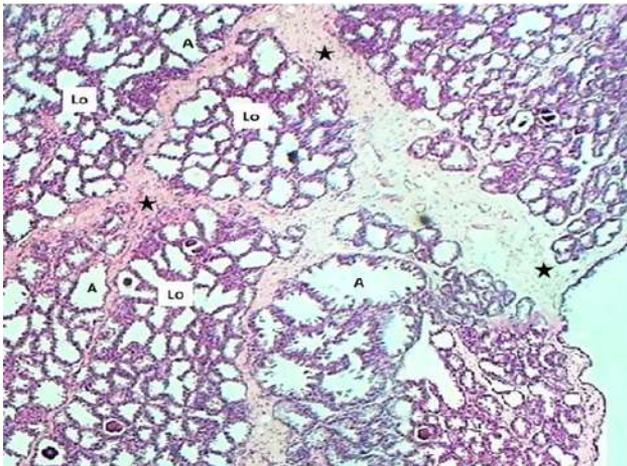


Figure 2: Section of mammary lobe (One day post parturient) shows mammary lobules (Lo), inter lobular connective tissue (Asterisks) and mammary alveoli (A). H&E stain. 40x.

The current results suggested that the secretory activities of mammary alveolar cells required enriched connective tissue that adapted for functional status of lactating gland, the fibroblasts or and precytes were the predominant cells type which was seen in the inter lobar and inter lobular connective tissue that required for angiogenesis and fibrogenesis. The mean of surface area of alveolus was $784.79 \pm 19.09 \mu\text{m}^2$ which lined by secreting cuboidal cells, containing large nucleus, dark cytoplasm and resting on basement membrane that followed by clear single layer of myoepithelial cells, the mean height of epithelial cells was $14.11 \pm 1.09 \mu\text{m}$ (Fig. 3). The number of alveolar cells per alveolus was 28.2 ± 0.37 .

Duct system of mammary gland was started by intra lobular duct which lined by simple cuboidal cells that showed secretory activities (Fig. 4). The intra lobular duct drained into large inter lobar duct that lined by stratified cuboidal cells at which the first layer of cell represents the basal cells. Both

types of ducts were supported by sub epithelial fibrous connective tissue which revealed fibroblasts and fibrocytes. The intra lobular ducts were drained to the largest lactiferous sinus which appeared as wide, had irregular folded lumen and lining by stratified cuboidal cells. The lactiferous sinus was drainage to glandular cisterna that opened into teat cisterna and the later opened into teat canal.

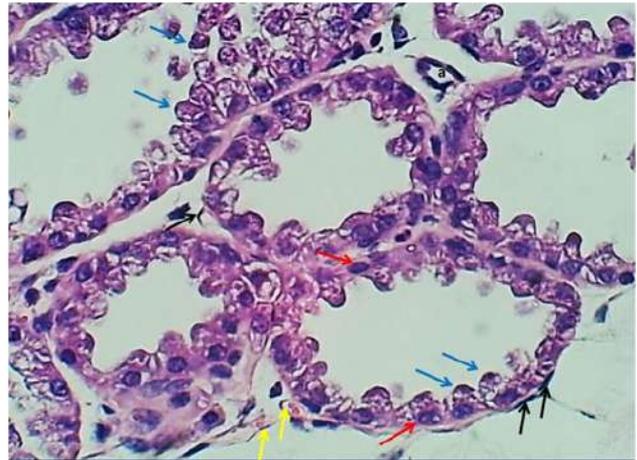


Figure 3: section of mammary alveoli (One day post parturient) shows; secretory activities (Blue arrows), myo epithelial cells (Black arrows), epithelial cells (Red arrows), blood capillaries (yellow arrows) & arteriole (a). H&E stain. 400x.

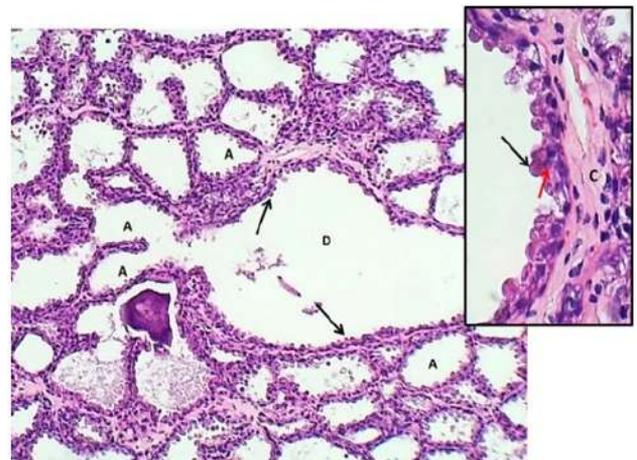


Figure 4: Section of mammary lobule (one day post parturition) shows; intra lobular duct (D) alveoli (A), epithelium (Red arrows) & secretory activities (black arrows). 100 & 400x. H&E stain.

The current result showed that the gland cisterna was huge chamber and displayed wide irregular shaped lumen. The mucosal surface of the gland cisterna was showed multiple mucosal folds. The folds were lined by simple cuboidal epithelium and

supported a lamina propria of dense fibro muscular connective tissue. The epithelium of cisterna which close to the mammary lobules was supported thick layer of well vascular fibrous connective tissue.

Teat cisterna was composed of three layers: mucosal cisterna folds (epithelium and cellular lamina propria) and fibro muscular layer with venous plexus, the folds epithelium was similar that in gland cisterna.

Discussion

The current results of epithelial type, measurements and dermal components were resemble that of immature mammary glands reported by⁹ also these results were similar to those reported by^{6,7} in she camel and cow respectively, also was reported by¹² in camel and in ewe⁴, in mare, bitch and queen.^{13,14,15} The current study fined that content of sweat and sebaceous glands were similar that reported by⁹ who mentioned that the gland parenchyma of immature and lactating ewes udder had furthermore of both apocrine sweat glands and sebaceous glands, this result disagree with that mentioned by¹⁶ who refereed for neither sebaceous nor sweat glands are found in the wall of teat of lactating cow.

The presence of the fibrous muscle layer represented by thick layer of paralleled and inter crossed bundles of smooth muscle fibers that intermingled with collagen bundles was similar to that mentioned by^{2,17} who reported that the inter lobar septa was originated from cutaneous dermis.

Thick well vascular layer of inter lobar fibrous connective tissue was recorded by^{4,7,9,12,18,19,20} and ²¹ who mentioned that the lactating udder the connective tissue became little and lobulo-alveolar tissue is mostly increased.

The type of alveolar epithelial cells at one day post parturition was similar that seen in newly lactating mammary alveoli which described by^{4,9} in lactating ewe,⁷ lactating cow, and²² in lactating she camel which was modified for milk production. The alveoli are enlarged with epithelium hypertrophic and they are often folded, they are tense with full of secretion in the mammary glands of lactating.²³ The current results revealed that the alveolar epithelium was cuboidal that disagree with¹² who referred for columnar type this difference is beyond the secretory

phase of epithelial cells. The presence of myoepithelial cells around alveoli and inter alveolar connective tissue during immature and lactating period was reported by.^{20,24,25} The size of mammary lobes and lobules during lactating period were associated with hormonal effects.^{26,27,28} The current result suggests that the stromal cells are the supporting connective tissue which was the main cellular components plays a significant part of the lactating udder, so the myo epithelial cells had supporting role and represented the contractile system which is involved in the ejection of milk from secretory units.

The results of mammary ductal system were agree with those reported by^{2,4,7,29}, while⁹ has revealed that the duct system of mammary gland of newly lactating ewe showed the epithelial cells of intra lobular duct which lined by simple cuboidal cells that showed secretory activities.

The current results suggested that the gland cisterna was huge sinus that adapted for milk storage, so the presence of sub epithelial myo epithelial cells which mentioned by^{7,9} could not beneficial, usually the myo epithelial present to support the secretory units of most exocrine glands and never seen at the end parts of duct system.

The current study showed that, the wall of the teats cisterna was similar in to report of⁹ in immature and lactating mammary glands of ewe,⁷ in cow,^{6,12} in camel and⁴ in small ruminants, the current results suggests that the composition of low part of mammary gland consider a part of storage and pumping function so, adapted for milking. The venous plexus at the wall of the teats cisterna was formed cavernous like tissue.

Conclusion

In compression to other studies the one day post parturition lactating mammary gland has small alveolar secretory size and number and the less content of elastic fibers within glandular capsule and interstitial tissue.

Ethical Clearance: All samples were taken under local anesthesia and were from multiple animals. Therefore, kindness and compassion for these animals were taken into consideration.

Source of Funding: Self.

Conflict of Interest: Nil.

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The Effectiveness of the Revitalization of “Jogo Tonggo” as Local Wisdom in Vigilance and Prevention of Transmission of COVID-19 in Central Java Province

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How to cite this article: Yulianto Prabowo, Agus Suwandono, Bagoes Widjanarko et al. The Effectiveness of the Revitalization of “Jogo Tonggo” as Local Wisdom in Vigilance and Prevention of Transmission of COVID-19 in Central Java Province. *Indian Journal of Forensic Medicine and Toxicology* 2022;16(3):381-387.

Abstract

Background: Jogo tonggo is an empowerment effort to increase community participation in preventing the transmission and spread of Covid-19 in Central Java. The study aims to assess the effectiveness of the revitalization of “jogo tonggo” as local wisdom toward increasing community knowledge, attitudes, and behavior toward COVID-19.

Methods: This study is true-experimental study involving 352 respondents, which are divided into the experimental group and the control group. The used statistical analyses were paired simple t-test, independent t-test, Mann-Whitney, and Wilcoxon.

Results: There was an increase in the mean after intervention between group 1 and group 2 including knowledge of “jogo tonggo” ($\delta=2.39$; $p=0.001$), knowledge of COVID-19 symptoms and transmission modes ($\delta=2.87$; $p=0.001$), knowledge of prevention methods of COVID-19 ($\delta=1.63$; $p=0.001$), attitudes towards “jogo tonggo” ($\delta=2.00$; $p=0.004$), attitudes towards COVID-19 ($\delta=1.58$; $p=0.011$), and COVID-19 prevention behavior ($\delta=6.04$; $p=0.001$).

Conclusion: The revitalization of “jogo tonggo” can increase knowledge, attitudes, and behavior toward COVID-19.

Keyword: Community engagement and empowerment, COVID-19, Jogo tonggo, Local wisdom

Introduction

On February 19, 2021, COVID-19 was confirmed positive in Indonesia, reaching 1,252,685, including

33,969 people died.¹ While the same date is also real time data at 09.00 WIB in Central Java, COVID-19 was confirmed positive as many as 147,609 people, with CFR 6.25%.² The majority of death cases had

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comorbidities, while the most prevalent reported comorbidities were diabetes and hypertension. Meanwhile, old age patients were more likely to associate comorbidity^[3]. The COVID-19 cases have spread to 35 cities/regencies in Central Java Province.^{4,5}

Resistance to the spread and transmission of COVID-19 must be carried out systematically, and continuously, so an effective, and strong organization is needed. Therefore, the formation of a community-based Task Force against COVID-19 is urgent to implement immediately.⁶ "Jogo tonggo" is an empowerment effort to increase community participation in preventing the transmission and spread of COVID-19 in the RW area with the principle of "gotong royong" or mutual cooperation. Exploring the potential of the community so that they are empowered and able to participate in handling COVID-19.⁶

"Gotong royong" is an activity that is carried out together and is voluntary in nature. "Gotong royong," which has been deeply rooted in people's lives, is one of the characteristics of the Indonesian nation, stated in Pancasila, namely the 3rd principle of Indonesian unity.⁷ The Javanese people, especially Central Java, really uphold Javanese customs with a culture of mutual help. The culture of "gotong royong", which is often referred to as "sambatan" is a habit for residents to help out in their daily lives, both for personal and public interests.⁸ This is of course a strong enough basic capital for community raising in dealing with COVID-19. This study aims to assess the effectiveness of the revitalization of "jogo tonggo" as a program which emphasizes community empowerment and involvement toward increasing community knowledge, attitudes, and behavior in preventing the COVID-19 occurrence.

Methods

Study design

This study is a true-experimental research design with pre-test and post-test group design.⁹

Sample and sampling method

The sampling procedure was by multi-stage sampling consisting purposive sampling for determining the district, and simple random sampling technique for selecting samples. The research was conducted in 16 sub-districts in Semarang City, Central

Java Province, Indonesia consisting 8 sub-districts as intervention subject location, and 8 sub-districts as the control subject location in 2020. Determination of the sample size using the formulation¹⁰ as follows:

$$n = \frac{N \cdot Z^2 \cdot p \cdot q}{d (N - 1) + Z^2 \cdot p \cdot q} \quad (1)$$

Note:

n = Number of samples

N = Population Size (30,613)

Z = Normal standard value for $\alpha=0.05$ (1.96)

p = Approximate proportion 50% (0.5)

q = (100%-p)

d = level of significance (0,05)

To avoid the lack of samples due to drop out, which was assumed to be 10%, the number of samples (20), as the result of the above calculation, was added by 2, so that the number of samples became 22. Finally, 22 would be multiplied by 16 (total number of RW), with the results of 352 respondents divided into two groups, experimental group and control group.

The data collection was carried out by 8 enumerators who previously given three days of training. The treatment of the intervention group was carried out by the "jogo tonggo" task force accompanied by the facilitator through providing education on the three indexes which was conducted for 4 weeks with a frequency of every day, duration of 60 minutes per intervention.

Statistical analysis

Data was analyzed using SPSS for Windows Release version 25.0 computer program consisting of descriptive analysis (mean and standard deviation) and inferential statistics (Mann-Whitney, Wilcoxon, Independent T Test, and Paired T Test).

Results and Discussion

General description of subjects

The majority of the subjects both in the experimental group and the control group were male (107 respondents, 60.8%), 25-35 years old (59 respondents, 33.5%), and as self-employed (62 people, 35.2%) (Table 1). The characteristics distributions of subject from both groups are relatively the same (Table 1).

Table 1: Characteristics of Research Subjects

Variable	Experimental Group		Control Group	
	F	%	F	%
Gender				
Male	107	60.8	107	60.8
Female	69	39.2	69	39.2
Age				
25-35 Years	59	33.5	59	33.5
36-45 Years	49	27.8	50	28.4
46-55 Years	48	27.3	49	27.8
56-60 Years	20	11.4	18	10.3
Education				
Not Completed in Primary School	2	1.1	1	0.6
Elementary School	10	5.7	9	5.1
Junior High School Graduate	18	10.2	18	10.2
Senior High School Graduate	85	48.3	86	48.9
Graduated College	61	34.7	62	35.2
Job				
Civil Servant	12	6.8	10	5.7
Private Employees	35	19.9	35	19.9
Self-Employed	62	35.2	62	35.2
Laborer	13	7.4	13	7.4
Unemployment	50	28.4	50	28.4
Pension	4	2.3	6	3.4

Based on the frequency data analysis, the result showed that before the intervention was carried out, respondents who had good knowledge of jogo tonggo were 56.8%, increasing to 90.3% after the intervention. Respondents' knowledge of the symptoms and mode of transmission of Covid-19 was originally 42.0% and after intervention it became 96.6%. Knowledge of how to prevent Covid-19 transmission before intervention was 48.3% and it became 93.2% after intervention. Attitudes towards jogo tonggo were originally 37.5% to 93.2%, and attitudes towards Covid-19 before the intervention were 25.6% to 88.6% after the intervention. Furthermore, the behavior of respondents in implementing health protocols before the intervention was 34.7% and after the intervention increased to 92.0%.

Analysis of measuring knowledges about jogo tonggo, symptoms, COVID-19 transmission, and ways to prevent COVID-19

There is a difference in the mean knowledge of "jogo tonggo" after the intervention between group 1 and group 2 which is statistically significant

with p-value=0.001 (Table 2). The mean of knowledge about "jogo tonggo" after intervention was higher at group 1 (7.99 ± 1.00), compared to group 2 (5.60 ± 1.08), with a mean difference of 2.39 (Table 2). In the group 1 or the intervention group, the mean of knowledge of "jogo tonggo" was higher (7.99 ± 1.00) after the intervention, compared to before the intervention (5.56 ± 1.25), with a mean difference of 2.43 which was statistically significant (p-value=0.001) (Table 2).

Besides, there is a difference in the mean knowledge of symptoms and modes of transmission of COVID-19 after the intervention between group 1 and group 2 which is statistically significant with p-value=0.001 (Table 2). The mean knowledge of symptoms and modes of transmission of COVID-19 after intervention was higher at group 1 (9.11 ± 0.81), compared to group 2 (6.24 ± 1.16), with the mean difference was 2.87 (Table 2). At group 1 the mean knowledge of symptoms and modes of transmission of COVID-19 was higher (9.11 ± 0.81) after intervention, compared to before intervention (6.22 ± 1.61), with a mean difference of 2.89 which was statistically significant (p-value=0.001) (Table 2).

Table 2 showed that there is a difference in the mean knowledge of how to prevent COVID-19 after the intervention between group 1 and group 2 is statistically significant with $p=0.001$. The mean knowledge of how to prevent COVID-19 after intervention was higher at group 1 (5.53 ± 0.64), compared to after intervention at G2 (3.90 ± 1.16), with a mean difference of 1.63 (Table 2). In group 1, the mean of COVID-19 prevention was higher (5.53 ± 0.64) after the intervention, compared to the average before intervention (3.81 ± 1.36), with a mean difference of 1.72 which was statistically significant ($p=0.001$) (Table 2).

The results of the quantitative analysis were strengthened by qualitative analysis result which showed that there was an increase in the knowledge from before the intervention to after the intervention. Based on the results of qualitative data analysis, the average percentage of knowledge about COVID-19 in the research subjects was 45.54%, increasing to 77.68% after the intervention, so that the level of informant knowledge increased by 70.57%. Meanwhile, the average percentage of knowledge about the “jogo tonggo” program before the intervention was 27.21% and after the intervention increased to 73.53%, so that the level of informant knowledge increased by 178.35%.

Table 2: Results of Differences Analysis in Knowledge about Jogo Tonggo, Symptoms and Modes of Transmission of COVID-19, and How to Prevent COVID-19

Measurement Phase	Jogo Tonggo			Symptoms and Modes of Transmission of COVID-19			How to Prevent COVID-19		
	G1 (Mean±SD)	G2 (Mean±SD)	P-value	G1 (Mean±SD)	G2 (Mean±SD)	P-value	G1 (Mean±SD)	G2 (Mean±SD)	P-value
Before Intervention	5.56±1.25	5.55±1.27	0.798	6.22±1.61	6.12±1.45	0.532	3.81±1.36	3.80±1.37	0.969
After Intervention	7.99±1.00	5,60±1,08	0.001**	9.11±0.81	6.24±1.16	0.001*	5.53±0.64	3.90±1.16	0.001*
Difference (Delta)	2.43±1.53	0.51±0.66	—	2.88±1.19	0.11±0.95	-	1.72±1.52	0.09±0.97	—
P-value	0.001****	0.341	—	0.001***	0.098	-	0.001***	0.107	—

SD = Standard Deviation

G1 = Experimental Group

G2 = Control Group

** = Significant based on the Mann-Whitney $p\leq\alpha$ (0.05)

**** = Significant based on the Wilcoxon $p\leq\alpha$ (0.05)

* = Significant based on the Independent T Test $p\leq\alpha$ (0.05)

*** = Significant based on the Paired T Test $p\leq\alpha$ (0.05)

The intensification program for community education through “jogo tonggo” was proven to increase the average score of respondents’ knowledges about “jogo tonggo”, symptoms and modes of transmission of COVID-19, and ways to prevent COVID-19. Interventions that involve culture in a community have the potential to increase community involvement (participation) and will have an impact on the success of the intervention. According to research conducted by Kumpfer (2020), et.al, it is shown that family strengthening programs that adapt to cultural values will increase acceptance in society by up to 40%.¹¹

Community empowerment is deemed necessary to overcome problems caused by the pandemic. The purpose of implementing community empowerment is to increase the ability to meet physical, economic and social needs. In addition, community empowerment has the ability to increase self-confidence, participate

in social activities, and the ability to be independent in carrying out life tasks.¹²

Kaim, et.al’s research in 2020 in the State of Israel which intervened in the form of short education on the characteristics of COVID-19, etiology, symptoms and signs, modes of transmission, measures to combat infection, and guidelines during home quarantine showed an increase in scores on all measured variables^[13]. Research by Sabarudin showed that there were statistically significant differences before and after the implementation of online education about the prevention of COVID-19.¹⁴

Analysis of measuring attitudes towards “jogo tonggo” and COVID-19

There is a difference in the mean attitude of “jogo tonggo” after the intervention between group 1 and group 2, which is statistically significant with a value of $p=0.004$ (Table 3). The mean of “jogo tonggo”

attitude after intervention was higher at group 1 (8.78±1.67), compared to group 2 (6.78±1.55), with a mean difference of 2.00 (Table 3). At group 1, the mean “jogo tonggo” attitude was higher (8.78±1.67) after the intervention, compared to before the intervention (6.73±1.70), with a mean difference of 2.05, which was statistically significant (p=0.001) (Table 3).

There was a difference in the mean attitude towards COVID-19 after the intervention between group 1 and group 2 which was statistically significant

with a value of p=0.011 (Table 3). The mean attitude towards COVID-19 after intervention was higher at group 1 (7.90±1.48), compared to after intervention at group 2 (6.32±1.65), with a mean difference of 1.58 (Table 3). In the group 1, the mean attitude towards COVID-19 was higher (7.90±1.48) after the intervention, compared to before the intervention (6.22±1.72), with a mean difference of 1.68, which was statistically significant (p-value=0.001) (Table 3).

Table 3: Results of the analysis of differences in attitudes towards Jogo Tonggo and COVID-19

Measurement Phase	Jogo Tonggo			COVID-19		
	G1 (Mean±SD)	G2 (Mean±SD)	P-value	G1 (Mean±SD)	G2 (Mean±SD)	P-value
Before Intervention	6.73±1.70	6.66±1.64	0.726	6.22±1.72	6.27±1.63	0.751
After Intervention	8.78±1.67	6.78±1.55	0.004*	7.90±1.48	6.32±1.65	0.011*
Difference (Delta)	2.05±1.09	0.11±0.90	–	1.78±1.20	0.04±0.86	–
P-value	0.001***	0.098	–	0.001***	0.484	–

SD = Standard deviation

G1 = Experimental group

G2 = Control group

* = Significant based on the Independent T Test $p \leq \alpha$ (0.05)

*** = Significant based on the Paired T Test $p \leq \alpha$ (0.05)

The current study is in accordance with the previous research. Study of Yudiansyah (2020) showed the “jogo tonggo” program received a positive response by the people of Central Java.¹⁵ Study from Arditama (2020) showed that “jogo tonggo” during the pandemic led to the formation of awareness and obedience of the Central Java people to the state appeal. The community’s response is clearly illustrated by the formation of social and economic networks, which are formed and managed massively in the environment. This encourages the re-strengthening of social solidarity, community togetherness at the RT/ RW level, and the existence of legal rules that contain sanctions.¹⁶ Research from Ayed, et al. (2020), who conducted COVID-19 education in school students, showed that there was a statistically significant positive relationship between scores of knowledges, attitudes, and practices after the implementation of the intervention.¹⁷

Sufficient knowledge related to the definition, mode of transmission and prevention of COVID-19

greatly affects a person’s change in attitude. Someone who knows well the benefits of the expected behavior change will have a tendency to maintain a good attitude as well.¹⁸ Attitudes are formed starting with knowledge (including awareness, feelings, etc.) and in the next stage forming actions.¹⁸

Behavior analysis to prevent COVID-19

Table 4 clearly showed that there was a difference in the mean COVID-19 prevention behavior after the intervention between group 1 and group 2 which was statistically significant with a value of p=0.001. The mean COVID-19 prevention behavior after intervention was higher at group 1 (40.69±1.58), compared to group 2 (34.65±3.04), with a mean difference of 6.04 (Table 4). At group 1 the mean prevention behavior against COVID-19 was higher (40.69±1.58) after the intervention, compared to before intervention (34.77±2.95), with a mean difference of 5.92, which is statistically significant (p-value=0.001) (Table 4).

Table 4: The Results of Analysis of Differences in COVID-19 Prevention Behavior

Measurement Phase	G1 (Mean± SD)	G2 (Mean± SD)	P-value
Before Intervention	34.77±2.95	34.55±2.93	0.492
After Intervention	40.69±1.58	34.65±3.04	0.001*
Difference (Delta)	5.92±2.58	0.09±0.86	-
P-value	0.001***	0.138	-

The present research is in line with the research previously. Research from Bavel et al. (2020) provided an explanation that knowledge has a close relationship with decision making and the achievement of positive attitudes and the embodiment of new behaviors.¹⁹ The results of research in Banyumas Regency by Kusumawardani and Triyanto (2020) found that 77% of respondents had a positive attitude and it supports behavior change. This is evidenced by the behavior of washing hands and wearing masks almost reaching 100%.²⁰

Based on the integrated theory, the behavior change model is influenced by several factors, including demographics, knowledge, attitudes, norms, habits, environment, and media. These factors complement each other which then influence each other to create the expected new behavior. Good behavior is created and can be cultured in people's daily lives through family empowerment and community empowerment.²⁰

Conclusion and Acknowledgement

In conclusion, the revitalization of "jogo tonggo" has an effect on increasing knowledge, attitudes, and behavior in preventing COVID-19 transmission. This "jogo tonggo" model has very good implications for the community to stay awake from existing health problems. Besides, the current study contributes to the literature on how jogo tonggo revitalization has affected on enhancing the awareness and behavior of the community of Central Java in preventing and controlling COVID-19, and on the involvement of the community in facing the COVID-19 outbreak. Hence, the Jogo Tonggo can be adopted by others.

The authors would like to acknowledge the heads of sub-districts, heads of community units, the "jogo tonggo" task forces, the "jogo tonggo" task force facilitators, and the community at the community unit of the research location, for their participation in the implementation of the "jogo tonggo" empowerment education intensification activities.

Conflicts of Interest: None

Funding Statement: None

Ethical considerations

The scientific procedure for ethical research permission was obtained from the Faculty of Public Health, Diponegoro University, Number: 60/EA/KEPK-FKM/2020.

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Simvastatin Toxicity Induces Alteration of Bladder Thickness in Interstitial Cystitis Rat Model

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How to cite this article: Z K Yusuf, A D I Hasanuddin, M N S Yusuf et al. Simvastatin Toxicity Induces Alteration of Bladder Thickness in Interstitial Cystitis Rat Model. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):388-392.

Abstract

Background: Interstitial cystitis (IC) is a chronic inflammatory of the bladder, while statin can increase its risk. Recently, the exact mechanism is yet known. We hypothesized simvastatin can induce alteration of bladder thickness.

Methods: Twenty-four female Wistar rats were aged 6-8 weeks old were divided into two groups and were treated with simvastatin 50 mg/kg BW or carboxymethylcellulose 0.5% by oral gavage for 30 days. Each group was then equally subdivided into three groups: control, Interstitial Cystitis (IC) day-0, and IC day-3. Either IC or control rat group was induced by intravesical instillation of protamine sulfate or buffered saline respectively. All animals in the control and IC day-0 group were sacrificed and collected for the bladder tissue in less than three hours following intravesical treatment, while animals in the IC day-3 group three days after. All collected tissue was prepared in hematoxylin-eosin staining and measured for the bladder thickness, namely the urothelial, suburothelial, and detrusor layer by image analyzer application.

Results: There was no significant difference between the groups receiving simvastatin and placebo in the thickness of the urothelium, suburothelium, and detrusor layers in all rat models, both control, IC0, and IC3 rats (all p values > 0.05). However, the thickness of the urothelium layer was consistently lower in the simvastatin group than in the placebo group in all rat models.

Conclusion: Mechanism of simvastatin toxicity on bladder tissue through urothelial denudation thus may alter the urothelial barrier function.

Keywords: Bladder Pain Syndrome; Denudation; Interstitial Cystitis; Protamin Sulfate; Statin; Toxicity; Urothelial.

Introduction

Statins are widely used to lower LDL cholesterol and prevention of cardiovascular disease.¹ Recently, statins have been found to have many health benefits

despite their hypolipidemic effects, namely anti-inflammatory, antiatherogenic, antifibrosis, and even anti-cancer. But on the other hand, this pleiotropic effect can trigger several toxicity such as increasing the incidence of new-onset diabetes

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mellitus type 2, neurological-neurocognitive effects, hepatotoxic, renal toxicity, and others.² Another statin toxicity is interstitial cystitis, while the exact mechanism is unrevealed.³

Interstitial cystitis is a complex disease and may involve a variety of unknown etiologies. One of the main processes that occur in the pathogenesis of interstitial cystitis is bladder urothelium barrier dysfunction, which can be caused by failure of urothelial cytodifferentiation, chronic inflammation in the suburothelial tissue, increased apoptotic cells and decreased proliferative cells. This dysfunction results in the leakage of water, urine, potassium, and other toxic substances in urine to the underlying tissue, triggering symptoms of urgency, frequency, and dysuria.⁴ Based on this pathogenesis, we hypothesized that statins exert a side effect of interstitial cystitis through their antiproliferative mechanism. This is reinforced by evidence that statins inhibit the proliferative activity of stem cells *in vitro*, where there is an increase in senescence and apoptosis through upregulation of p16, p53, caspase 6, caspase 8, and caspase 9.⁵ Therefore, in this study, we aim to investigate the effect of simvastatin on bladder thickness in the interstitial cystitis rat model.

Material and Method

Animal Preparation

This study used a total of 24 rat samples which were initially subjected to acclimatization for 10 days. After that, randomization was carried out using a simple random sampling method and divided equally into two groups, namely group C who only received a placebo carboxymethylcellulose (CMC) 0.5% (n = 12) and group S who received simvastatin 50 mg/kg BW (n = 12). All rats were kept in open, humid, well-ventilated cages, and life / light cycle 12 hours / 12 hours. Each cage consists of four to five rats. All rats received standard AD2 feed and free access to tap water *ad libitum*.

Simvastatin Treatment

Simvastatin was prepared from generic tablet form (Kimia Farma, Indonesia). The drug dosage was based on previous research where simvastatin 50 mg / Kg BW is a hypocholesterolemic dose in rats.^{6,7} All groups received treatment for 30 days based on previous studies which found statin can induce senescence within 20 days *in vitro*.⁸ The simvastatin tablets were turned into suspension

form with 0.5% carboxymethylcellulose (CMC) as the solvent. Simvastatin suspension or 0.5% CMC was administered by oral gavage and was adjusted according to body weight each week. The duration of administration between simvastatin doses is every 24 hours during the day or afternoon.

Induction of Interstitial Cystitis

After completing simvastatin or placebo administration, each group of rats was further divided into three subgroups, namely control rats, Interstitial Cystitis (IC) day-0 rats, and IC day-3 rats. The IC group and the control group were induced by intravesical instillation of protamine sulfate and buffered saline, respectively based on previous studies.^{9,10} Protamine sulfate (Sigma Aldrich, Japan) was dissolved in buffered saline with a concentration of 10 mg/ml, then put into an instillation tube in the form of a 1 ml spoit mounted to a sterile 22 / 24G vein catheter. Anesthetic experimental animals using ketamine injection 10% (60 mg / Kg) intraperitoneally. The rats were positioned dorsally recumbent and mildly massaged in the lower abdominal region to induce micturition. After identification of the external urethral ostium, the lubricated distal end of the instillation tube was inserted as deep as 3 mm in a cephalocaudal position parallel to the urethra, then rotate the proximal end of the instillation tube vertically about 180 degrees. After that, the distal end of the instillation tube was inserted 7 mm deep into the bladder. An amount of 0.6 ml of protamine sulfate or buffered saline was instilled with bolus for 30-45 seconds and was maintained in the bladder for 15 minutes while rotating the rats to homogenize the contact of instillation solution to the entire lumen of the bladder. Finally, the instillation tube is slowly pulled out from the urethra.

Tissue Preparation and Bladder Thickness Measurement

Tissue collection time was adjusted according to the rat model group. In the control and IC day-0 rat model, the animals were sacrificed less than 3 hours after the instillation procedure. As for the IC day-3 rat model, the animals have sacrificed three days after the instillation procedure. Initially, all rats were killed through the cervical dislocation technique then the bladder organs were taken. The tissue samples were fixed in 10% neutral formaldehyde solution overnight and then made in the form of paraffin blocks according to standard procedures. The paraffin blocks were cut using a microtome with a thickness of

5µm, followed by floating in a warm water container. After that, the specimens were placed on a slide and glued with a thin layer of albumen. The slides were then processed using the hematoxylin-eosin staining procedure.

The slides were examined using an Axio Imager. A2 microscope using a 20X magnification objective lens. Each slide was photographed to randomly obtain five different images that clearly show the full thickness of all bladder tissue layers, namely the urothelial, suburothelial, and detrusor layers. The width of each layer was calculated using the ImageJ NIH application. Five measurements on each slide were then averaged to obtain the representative data.

Statistical Analysis

All collected data were analyzed by SPSS version 17.0 with a 95% confidence interval ($\alpha = 0.05$). Data for the bladder layer thickness is expressed in terms of mean \pm standard deviation, or median \pm standard error of mean if not normally distributed. Independent Sample T-test or Mann Whitney method was used to compare the thickness of each bladder layer among treatment groups in the same mouse model. A p-value \leq of 0.05 was considered significant.

Result and Discussion

The results of tissue thickness measurements at various layers can be seen in Table 1. In the control rat model without interstitial cystitis induced, it was revealed that the thickness of the urothelium layer in the simvastatin group was lower than in the placebo group, although not significantly different (0.298 ± 0.023 mm vs. 0.321 ± 0.122 mm, $p = 0.564$). The same thing was also observed in the mouse model of interstitial cystitis on day 0 (almost significant, $p=0.061$) and day 3 ($p=0.538$). However, the thickness of the urothelium layer was consistently lower in the simvastatin group than in the placebo group in all rat models. The thickness of the suburothelial layer in the group receiving simvastatin was always higher than the group receiving placebo, except for the rat model of interstitial cystitis on day 0. Meanwhile, the thickness of the detrusor layer in the group receiving simvastatin was always lower than the group receiving placebo, except for the 3rd-day interstitial cystitis rat model. Figure 1 also shows that there are differences in tissue thickness between the treatment groups, both in control, IC0, and IC3 rats.

Table 1: Differences in bladder tissue thickness at different layers of treatment groups

Group	Urothelial Thickness (µm)		Suburothelial Thickness (µm)		Detrusor Thickness (µm)	
	Mean (SD)	P value [#]	Mean (SD)	P value [#]	Mean (SD)	P value [#]
C-C	321 (122)*	0.564 [§]	907 (233)	0.657	2,186 (464)	0.814
S-C	298 (23)		978 (195)		2,087 (664)	
C-IC0	290 (49)	0.061	983 (166)	0.386 [§]	1,741 (327)	0.467
S-IC0	205 (55)		809 (142)*		1,510 (499)	
C-IC3	344 (39)	0.538	919 (209)	0.549	1,901 (439)	0.395
S-IC3	327 (031)		1,037 (309)		2,426 (1,060)	

C-C, Carboxymethylcellulose-Control Rat; C-IC0, Carboxymethylcellulose -Interstitial Cystitis Rat Day 0; C-IC3, Carboxymethylcellulose-Interstitial Cystitis Rat Day 3; S-C, Simvastatin-Control Rat; S-IC0, Simvastatin-Interstitial Cystitis Rat Day 0; S-IC3, Simvastatin-Interstitial Cystitis Rat Day 3.

* Data were not normally distributed expressed in median \pm standard error

[#] T-independent test

[§]Mann-Whitney test

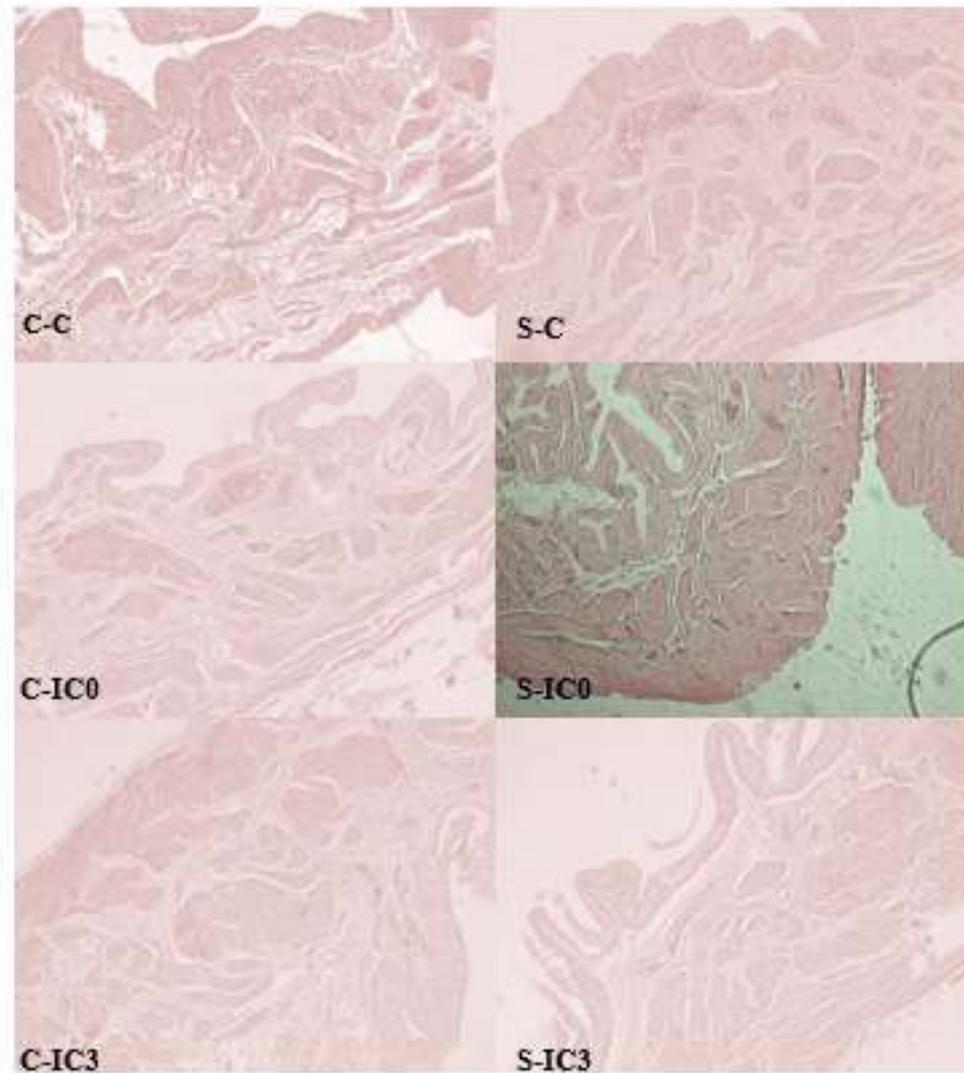


Figure 1: Representative Image of the Bladder Tissue Showing All Layers in the Treatment Group.

C-C, Carboxymethylcellulose-Control Rat; C-IC0, Carboxymethylcellulose -Interstitial Cystitis Rat Day 0; C-IC3, Carboxymethylcellulose-Interstitial Cystitis Rat Day 3; S-C, Simvastatin-Control Rat; S-IC0, Simvastatin-Interstitial Cystitis Rat Day 0; S-IC3, Simvastatin-Interstitial Cystitis Rat Day 3.

In this study, no significant difference was found between the thickness of various layers of bladder tissue between those receiving simvastatin and those receiving placebo. However, in the urothelium layer, the group receiving simvastatin always had a lower mean thickness value than the group receiving the placebo. Denudation or thinning of the urothelium is one of the typical features that indicate bladder urothelium barrier dysfunction in interstitial cystitis. The presence of this dysfunction results in the leakage of water, urine, potassium, and other toxic substances in the urine to the suburothelial tissue and the underlying bladder wall, triggering symptoms of urgency, frequency, and dysuria.⁴ On the one hand,

the difference in urothelial tissue thickness was almost significant in a mouse model of interstitial cystitis induced acutely by protamine sulfate. Instillation of protamine sulfate has been known to make umbrella cell exfoliation in the first two days of the administration, thereby exacerbating the dysfunction of the previously thinned urothelial barrier.¹¹ The impact of this dysfunction of the urothelium barrier was seen in this study, while only on day 3 after protamine sulfate instillation, the group receiving simvastatin had greater detrusor tissue thickness than the group receiving placebo. This urothelial barrier dysfunction can be caused by several factors, including failure of cytodifferentiation of the basal

layer and urothelial intermediates in response to damage to the overlying umbrella cells that may occur in this study.^{12,13}

Conclusion

Mechanism of simvastatin toxicity on bladder tissue through urothelial denudation thus may alter the urothelial barrier function.

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

Ethical Clearance: The Health Research Ethics Committee of the Faculty of Medicine, Hasanuddin University has confirmed the proposal and research protocol (No.375/UN4.6.4.5.31/PP36/2020).

Source of funding: Internal grant funds of Universitas Negeri Gorontalo

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Comparative Study on Post Mortem Analysis of Sodium and Potassium Levels of Vitreous Humour and Synovial Fluid in Determining Time Since Death

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How to cite this article: Gurpreet Singh, DK Sharma, Deepali Pathak et al. Comparative Study on Post Mortem Analysis of Sodium and Potassium Levels of Vitreous Humour and Synovial Fluid in Determining Time Since Death. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):393-398.

Abstract

Background: Time since death (TSD) is the period between the death and time of examination of a body. Numerous studies conducted on analysis of potassium in vitreous humour have shown that the vitreous potassium levels increase with rise in TSD. Studies done on postmortem biochemistry of synovial fluid have proposed a positive linear relationship between potassium and TSD. But, very few authors have compared findings of these two fluids. So, this study was undertaken to compare the accuracy of determining post-mortem interval from Sodium and Potassium levels of vitreous humour and synovial fluid.

Methods: A total of 140 cases were taken with certified time of death. These were divided into 7 groups i.e. 0-6 hours, 6-12 hours, 12-18 hours, 18-24 hours, 24-30 hours, 30-36 hours and above 36 hours. Vitreous humour and Synovial fluid from knee joint were aspirated from both sides of body and were analyzed using Medica Easylyte Na/K Analyser. Results were obtained using SPSS 24 software.

Conclusions: Potassium has a strong positive correlation with TSD in both fluids. Sodium has weak negative correlation. There is no difference between values of both sides. There is no statistically significant difference between values of Vitreous humour and Synovial Fluid. Hence, both can be used alternatively.

Keywords: Potassium; Synovial Fluid; Thanatochemistry; Time since death; Vitreous Humour.

Introduction

Time since death (TSD) is the period between the death and time of examination of a body. One of the most important objectives of post mortem examination is determination of time since death (TSD), also known as post mortem interval.¹ It is important to know when the crime was committed and helps in establishing the corpus delicti of a crime. Forensic experts being an 'expert witness'

have a great responsibility as their opinion guides the course of investigation and judicial proceedings. Thanatology is the scientific study of death in all aspects. Changes in body after death are traditionally divided into immediate, early and late changes.¹ These are helpful in estimation of TSD. The changes like cooling of body (algor mortis), eye changes, post-mortem staining (livor mortis), stiffening of body (rigor mortis), condition of stomach contents,

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bladder and bowel contents, decomposition changes and circumstantial evidence can sometimes yield reasonable results.¹ But, the estimates of post mortem interval using above changes are variable because of various internal bodily and external environmental factors and also prone to observer bias. Hence, Thanatochemistry has gained importance. It is described as the changes that occur in the chemical composition of body fluids such as blood, vitreous humour, synovial fluid and cerebrospinal fluid immediately after death.²

Blood and body fluids like cerebrospinal fluid were commonly used but since 1960's other compartmental body fluids like synovial fluid and vitreous humour are being investigated upon as they are topographically isolated and more protected.³

Intracellular concentration of potassium is 2-40 times higher than that in plasma.² After death, as the pumping mechanism of cell becomes inactive and cell wall becomes semi permeable, potassium leaks through it to reach equilibrium.⁴ Vitreous fluid is a fragile, transparent, inert, hydrogel that is present between the lens and retina and occupies 80% of eye's volume.⁵

The underpinning principle in analysing vitreous humour is that it is a closed compartment which is separated from the rest of the body. The composition of the vitreous is closely related to that of serum, aqueous humour and cerebrospinal fluid.⁶ Numerous studies have been conducted on biochemical analysis of vitreous humour. These studies have shown that the vitreous potassium level increases with increase in postmortem interval.^{2,6,7-11}

Similarly, synovial fluid is also isolated and protected from injury and decomposition. Synovial fluid can be analysed in cases of severe trauma to eye where the integrity of the eye globe is lost. It is also available in ample amounts as there are multiple sites of sample collection. Studies done on postmortem biochemistry of synovial fluid have proposed a positive linear relationship between potassium and TSD.^{2,4,12-14} The most commonly studied stable biochemical markers which can be analysed using simple techniques and minimal laboratory setup accessible for universal use have not been studied by all authors. Very few authors have pursued the comparison of these two fluids. So, this study

had been undertaken to compare the accuracy of determining post-mortem interval from Sodium and Potassium levels of vitreous humour and synovial fluid.

Material and Methods

This study was conducted at Mortuary of Department of Forensic Medicine in association with Central laboratory of the Department of Biochemistry, SMS Medical College, Jaipur. The study samples were collected between December, 2020 and August, 2021. Only cases with confirmed time of death were included in the study. The cases were recruited for the study only after obtaining consent and procedure of sample collection was done at the time of post-mortem examination. Inadequate samples and turbid or blood mixed samples were discarded and fresh case was allocated to compensate for such cases.

Inclusion criteria:

1. All the cases admitted in hospital and whose exact time of death is known and certified by treating physician.
2. All the cases without any major metabolic derangement prior to death.
3. All the cases in which guardians give consent for study.

Exclusion criteria:

1. Cases with previous history of eye or orbital injury or surgery, posterior segment diseases.
2. Cases with previous history of joint diseases, previous injury or surgery.
3. All brought dead cases at SMS Hospital emergency.

Cases were selected and organized according to known time since death in 7 groups namely between 0-6 hours, 6-12 hours, 12-18 hours, 18-24 hours, 24-30 hours, 30-36 hours and above 36 hours. 20 cases were included in each sub-group. Samples were collected from both sides of vitreous and synovial cavity.

Collection of sample of Vitreous Humour:

Vitreous humour was collected from the posterior chamber by aspirating gradually through a puncture 5-6 mm away from the limbus using a sterile 20 gauge

hypodermic needle. After sample collection, water was injected to restore cosmetic appearance.



Image 1: Correlation between TSD and K⁺ levels of Right Vitreous humour

Collection of sample of Synovial fluid:

The synovial fluid was aspirated using a sterilized 16 gauge needle by puncturing the suprapatellar pouch from lateral side, just below the patella pushing it directly backwards.



Image 2: Correlation between TSD and K⁺ levels of Right Synovial Fluid

Analysis was done within 1 hour after the synovial fluid and vitreous humour were aspirated on working days. All sample fluids were centrifuged at 3500 r.p.m. for 10 min and then the supernatant was used for analysis. On non-working days or emergency hours, the samples were centrifuged and

supernatant was stored at 4⁰C and subsequently analyzed. The samples were analyzed for sodium and potassium levels in the Department of Biochemistry section of Central Laboratory on Easylyte Plus Na/K Analyser by Ion selective method.

Prior to initiation of study, pilot samples (ten each) of vitreous humour and synovial fluid were analysed to assess efficacy of working with the assumption that being clear fluids, they could be analysed similar to serum.

Collected data was entered into Microsoft excel data sheet which was tabulated for data interpretation. This was then subjected to statistical analysis using with appropriate statistical tests (Linear regression, ANOVA and T Test) and results were obtained. Statistical analysis was performed using SPSS software 24th version.

Results

20 cases were included in each of the seven sub-groups, making a total of 140 cases in which each sub-group contributed 14.28% of cases of total cases included in the study. Cases included in the present study ranged from minimum two hours and maximum one hundred fourteen hours post mortem interval.

Amongst the study subjects, there were 36 females (25.71%) and 104 males (74.29%). There were 20, 68, 41, 9 and 2 cases in age groups of less than 20 years, 20 to 40 years, 40 to 60 years, 60 to 80 years and above 80 years respectively.

According to the cause of death, there were 63 cases (45%) of mechanical injuries, 13 cases (9.29%) of thermal injuries, 17 cases (12.14%) of asphyxia, 26 cases (18.57%) of poisoning and 21 cases (15%) of deaths due to pathology as revealed on autopsy.

Table 1: Descriptive Statistics TSD and K⁺ levels of Right Vitreous humour

Descriptive Statistics			
	Mean	Std. Deviation	N
Time Since Death (in hours)	24.26	18.658	140
Vitreous Humor (R) Potassium (mEq/L)	9.365	3.0450	140

Table 2: Correlation between TSD and K⁺ levels of Right Vitreous humour

		Time Since Death (in hours)	Vitreous Humor (R) Potassium (mEq/L)
Time Since Death (in hours)	Pearson Correlation	1	.823**
	Sig. (2-tailed)		.000
	N	140	140
Vitreous Humor (R) Potassium (mEq/L)	Pearson Correlation	.823**	1
	Sig. (2-tailed)	.000	
	N	140	140

As per table 2, Correlation is significant at the 0.01 level (2-tailed). R value of .823 indicates that there is strong positive correlation between two variables. It concludes that there is increase in levels of potassium concentration of vitreous humour of right eye as the post mortem interval increases. R² value of .678 indicated that 67.8% of total variation in Time Since Death (in hours) could be explained by potassium levels of right vitreous humour.

Similarly, Descriptive statistics, Pearson Correlation coefficient, Regression results were obtained for Sodium and Potassium levels of Vitreous Humour and Synovial Fluid of both sides. Graphical representation of correlation of K levels with TSD of right side are shown in figure 1-2. Left side graphs were also prepared.

Table 3: R values of Na⁺ and K⁺ levels of Right and Left vitreous humour and synovial fluid

	Group	R value
Vitreous Na ⁺	Right	-.343
	Left	-.448
Vitreous K ⁺	Right	.823
	Left	.827
Synovial Na ⁺	Right	-.519
	Left	-.518
Synovial K ⁺	Right	.863
	Left	.867

As per table 3, we found that potassium has strong positive correlation with TSD in both vitreous humour and synovial fluid whereas sodium had weak negative correlation with TSD.

Further, on application of T test for comparison between right and left sides, P value was >0.05. Hence, it is concluded that there is no difference among levels of sodium and potassium in left and right side of vitreous and synovial fluid.

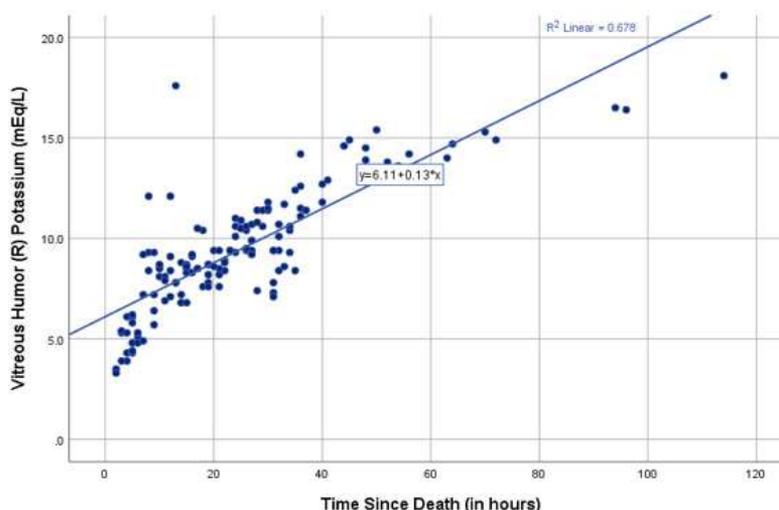


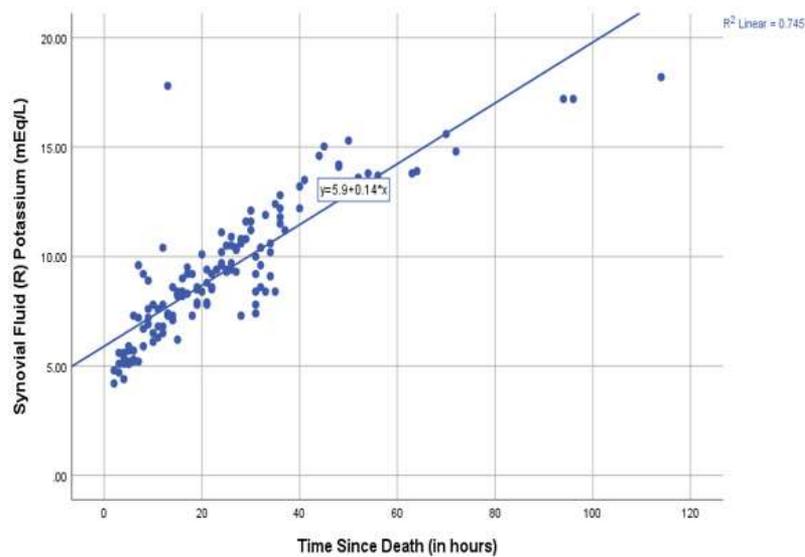
Figure 1: Correlation between TSD and K⁺ levels of Right Vitreous humour

Table 4: T- test for comparison between vitreous humour and synovial fluid

Group Statistics	Group	N	Mean	Std. Deviation	t value	p value
Right	Vitreous Na ⁺	140	140.6	12.65	-0.700	-0.484
	Synovial Na ⁺	140	141.44	6.48		
	Vitreous K ⁺	140	9.37	3.04	0.26	0.796
	Synovial K ⁺	140	9.27	3.00		
Left	Vitreous Na ⁺	140	141.5	6.66	0.05	0.935
	Synovial Na ⁺	140	141.43	6.45		
	Vitreous K ⁺	140	9.34	3.04	0.14	0.885
	Synovial K ⁺	140	9.28	2.99		

As per Table 4, P value is >0.05 when vitreous and synovial fluid are compared. The result is not significant at $p < 0.05$. Hence, it is concluded that there is no difference among levels of sodium and

potassium in vitreous and synovial fluid i.e. while determining time of death, synovial fluid reveals as accurate results as that in vitreous humour or vice versa.

Figure 2: Correlation between TSD and K⁺ levels of Right Synovial Fluid

Discussion

The magnitude of study population of the present study was quite comparable to most other studies, rather higher than most of them. Other studies were conducted on 154 cases²; 100 cases⁶ and 35 cases.¹⁵

The present study classified the subject collection in seven groups and included twenty cases in each sub-group, contributing to 14.28% cases in each group. One study² included five groups of 0-6 hours, 6-12 hours, 12-18 hours, 18-24 hours and 24-36 hours. Other study⁶ had divided into three groups of less than 12 hours, 12-24 hours and more than 24 hours.

The time since death of our study subjects ranged from 02 hours to 114 hours which is much wider than other similar studies - 1.45 hours to 35.18 hours² and 2.17 hours to 7.92 hours¹⁵; but similar to that done in Chennai. (1 to 102 hours).⁶

The cadavers were from various age groups. The youngest subject was 3 years of age and oldest 85 years. There were 104 males (74.29%) and 36 females (25.71%) in the present study. Age and gender of cadavers included in various studies has not been specified in most other studies. The mean age of study subjects in the present study was 37.17 ± 16.19 years.

The present study included 119 cases of unnatural deaths and 21 cases of natural deaths. One study done at Germany¹¹ included 42 cases of sudden natural deaths and 32 cases of sudden unnatural deaths.

In the present study, statistical analysis for electrolyte concentration in relation to time since death was studied using cumulative means of their concentrations which has also been done similarly by other study.¹¹

The present study revealed a statistically significant linear correlation of potassium levels of both vitreous humour and synovial fluid with time since death which is also reported by other authors.^{2,6,10,11,15}

There is no difference between values of right and left side. Similar inference was drawn by other authors^{6,7,15} who analyzed both right and left side samples.

From the present study, it is concluded that vitreous humour, well documented as a reliable fluid to study postmortem biochemistry can be replaced with synovial fluid and vice versa. Thus synovial fluid can be used as an alternative to vitreous humour which has also been concluded by other authors.^{6,11,15} However, the conclusion is variable to other study² which concluded that synovial fluid potassium values can be more confirmatory than vitreous humour potassium for determination of time since death.

Conclusions

There is a statistically significant linear correlation of potassium levels of both vitreous humour and synovial fluid with time since death. The samples from right and left eyes and knee joints did not reveal a significant difference in these two electrolyte biochemistry results, which implies that any side samples may be used. Synovial fluid and vitreous humour samples are equally reliable for analysis of post mortem potassium electrolyte concentrations in relation to post-mortem interval and can be used an alternate to each other.

Conflict of Interest: None

***Source of Funding:** Nil

Ethical Clearance: Taken

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