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Efficiency of Alcohol Salivary Strip Test in ‘Comparison to Breath Alcohol Analyser as Forensic Screening Tools for Blood Alcohol Analysis - A Comparative Study

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Abstract

Background: Alcohol induced intoxication is responsible for altered behavior in individuals. Blood alcohol concentration (BAC) has been the key toxicology test, which is considered as the gold standard in medico-legal evidence. BAC can be assessed by determining blood ethanol or methanol concentration. It is expensive and time-consuming procedure and so, it cannot be considered as primary choice for screening of alcohol consumption. At present breath analysis is the most common and accepted screening procedure but there are certain limitations to this procedure and hence assessment of other procedures for screening is also important.

Aim and Objective: The primary aim of the study is to assess efficiency of salivary strip test vs breath alcohol analyzer in screening for alcohol consumption among the individuals who are suspected for drunken driving by the city traffic police of Kannur district.

Setting and Design: It’s a Prospective case control study were samples are collected from Traffic aid post in Kannur district of Kerala for screening of suspected drunken driving.

Materials and Method: Individuals suspected of drunken driving by the city traffic police of Kannur district were included in the study. Subjects were screened by using alcohol breath alcohol analyzer and Alcohol salivary strip.

Statistical Analysis: It was performed using SPSS 20.0. Chi square test was used for association between variables.

Results: The result shows that both salivary alcohol strip test is as effective as breath alcohol analysis.

Conclusion: Salivary alcohol test strip is rapid, economically feasible and can easily be performed. The result given by strip is easy to interpret and can easily indicate different BAC level as it is very much sensitive even at a BAC value of 0.02%.

Keywords: Alcohol, Saliva, Breath, Blood alcohol concentration (BAC), Alcohol Salivary strips, Breathalyzer.

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Introduction

Alcohol is the key intoxicating constituent of wine, beer, spirits, and other alcoholic drinks. It has got psychoactive and in toxification effect when consumed. The term alcohol refers to a whole class of organic compounds that include a hydroxyl group – consisting of an oxygen atom and a hydrogen atom – bonded to a carbon atom and is called as ethanol.

Alcohol induced intoxication is responsible for altered behavior in individuals. Violence and Injuries – both unintentional and intentional – account for more than a third of the burden of disease attributable to alcohol consumption. Each year it is responsible for about 2.3 million premature deaths worldwide. These include injuries from road traffic crashes, burns, poisoning, falls and drowning as well as violence against oneself or others.

Blood/Breath Alcohol Concentration (BAC) is the amount of alcohol in the bloodstream or on one’s breath. BAC is expressed as the weight of ethanol, in grams, in 100 milliliters of blood, or 210 liters of breath. BAC can be measured by breath, blood, or urine test. BAC is considered as the best indicator of the level of impairment and is the gold standard test.

DUI, or driving under the influence in India is a criminal offence under the Motor Vehicle Act 1988. Section 185 of the Act states that any person who is found to have alcohol exceeding 30 mg per 100 ml of blood i.e.,0.03 % BAC in their system is breaking the law. Even with 0.03% intoxication level, a person is seven times more likely to be involved in a vehicle crash than a person who has not consumed any alcohol and the accepted legal age of consumption in Kerala is above 18 years. The impairment start at very low concentration even at BAC level of 0.02% - 0.03% and alcohol poisoning at level above 0.25%.

Alcohol from the blood passes to other biological fluids like urine and saliva. It is also present in exhaled breath. Alcohol concentration in the other biological fluids in essence reflect the blood alcohol concentration. So, these have been used as sample for assessing alcohol concentration. Breath analysis using breathalyzers is the commonly used method in different scenario but it cannot be used in patients who are in trauma, unconscious or un cooperative as it requires forceful expiration. Also, there is high chance of cross contamination as single device is used for multiple patient. Taking this into consideration, alternative methods have been tested. Saliva is a body fluid which can correctly reflect the blood alcohol concentration. It is easily available, can be easily accessed even in unconscious patients and hence can be a better sample for analyzing BAC.

Salivary strip test is a test in which we can easily assess the presence of alcohol in saliva. The strip test is based on the high specificity of alcohol oxidase for ethyl alcohol. Alcohol oxidase in the strip reacts with ethyl alcohol present in saliva in the presence of peroxidase and enzyme substrate such as tetra methyl benzidine to produce a chromatic change in the reaction pad of the strip. It is a very sensitive test and has good validity. The advantages of this method are; noninvasive, the results are not influenced by the presence of blood in the oral cavity, Rapid determination can be done, it could also be used in determining postmortem saliva ethanol levels, contact less test can a be done. Last but not least, because of the relatively low cost of the salivary strips it can be a cost-effective alternative in public health settings where mild to moderately intoxicated persons are encountered.

Aim nd Objective

Aim of the study was to assess efficiency of salivary strip test vs breath alcohol analyzer in screening of blood alcohol consumption and assess the efficiency of salivary strip test in screening for alcohol consumption in individuals who are suspected for drunken driving by the City Traffic Police Department of Kannur.

Methodology

A prospective case control study was carried out in the Kannur district of Kerala after obtaining permission from The District Police Chief, Kannur of Kerala Police Department. The study was carried out in association with the traffic police department, Kannur district.

Sixty individuals who were above 18 years of age and suspected of drunken driving by the city traffic police of Kannur district were included in the study.
Each subject was explained about the procedure and informed consent was obtained. Brief history regarding the alcohol consumption which include the time of consumption and quantity of alcohol. Subjects are screened by using breath alcohol analyzer and Alcohol salivary strip (Wondfo salivary alcohol test strip).

Subjects were asked to take a deep breath and blow steadily into the breath analyzer unit for at least 5 seconds until a deep sound to signal the completion of the testing. Once the sensor has analyzed the breath sample, it produces a green light along with the beep sound which indicate a positive result. And if no sound or no green light formation means a negative result.

Subjects are then screened using salivary alcohol test. The alcohol salivary strip with a reactive pad is checked for any chromatic change prior to test and then the pad is saturated with the saliva of the subjects either by applying directly or from a sputum cup. The results on the pad are observed after 2 min. Results after more than 2 min may not be accurate and hence not considered. The alcohol salivary strip test is based on the high specify of alcohol oxidase (ALOx) for ethyl alcohol in the presence of peroxidase and enzyme substrate such as tetramethylbenzidine (TMB) as shown in the following:

\[
\text{EtOH} + \text{TMB} \xrightarrow{\text{ALOx/Peroxidase}} \text{CH}_3\text{CHO} + \text{Colored TMB}
\]

The distinct color on reactive pad can be observed at any concentration above 0.02%.

**Interpretation of results**

**Positive**
- A distinct color developed all over the pad. The positive result indicated that the BAC was 0.02% or higher.

**Negative**
- Almost no color changes by comparing with the background. The negative result indicated that the BAC was less than 0.02%.

**Invalid**
- The test was considered as invalid if only the edge of the reactive pad turned color that might be ascribed due to insufficient sampling. The subject is re-tested.

![Figure 1: showing chromatic change and BAC level](image1)

**Statistical Analysis**

All statistical procedures were performed using Statistical Package for Social Sciences (SPSS) 20.0. All quantitative variables expressed in mean and standard Deviation. Qualitative variables will be expressed in percentages. Chi square was used for association between variables. Probability value \((p < 0.05)\) was considered statistically significant.

**Result**

The result indicate that the salivary strip test is equally efficacious as breathalyzer in identifying presence of alcohol in saliva, which indicate alcohol consumption. No significant statistical difference between the breathalyzer and alcohol salivary strip in identifying the presence of alcohol in saliva[Table 2]. On correlating the history of alcohol consumption, it was observed that the salivary strip was able to detect the presence of alcohol even after 6-7 hours after the consumption of last drink Table 1.
Table 1: Showing time duration between last drink and the test positivity

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<tr>
<td>1-2 hrs</td>
<td>1</td>
</tr>
<tr>
<td>1 hrs</td>
<td>8</td>
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<tr>
<td>2 hrs</td>
<td>16</td>
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<tr>
<td>2-3 hrs</td>
<td>4</td>
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<tr>
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<tr>
<td>3-4 hrs</td>
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<tr>
<td>45 min</td>
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<td>5-6 hrs</td>
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</tr>
<tr>
<td>5 hrs</td>
<td>2</td>
</tr>
<tr>
<td>7 hrs</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 2: Comparison between Breathalyzer and saliva alcohol strip

<table>
<thead>
<tr>
<th></th>
<th>Negative n(%)</th>
<th>Positive n(%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breathalyzer</td>
<td>3(5)</td>
<td>57(95)</td>
<td>0.12</td>
</tr>
<tr>
<td>Saliva Alcohol strip</td>
<td>0</td>
<td>60(100)</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

The adverse effect of alcohol on cognitive and psychomotor skills is well known, particularly with respect to vehicle driving performance. Increases in reaction time and performance error can be found at all doses of intoxication.

The effects of alcohol (i.e., ethanol) on various tissues depend on its concentration in the blood (blood alcohol concentration [BAC]) over time. From the blood it is distributed to other tissues and fluids and is present in other body fluids like saliva, urine as well as expelled breath. It is proven that there is a correlated change in the alcohol concentration in the saliva and in the breath with respect to the BAC (9). This fact laid the foundation for the premise that these samples can also be used to screen the presence of alcohol and that this data can be correlated to the BAC.

At present breath alcohol analysis is the most accepted screening test for alcohol consumption in many situations like law-enforcement and medico-legal cases. It has the advantages like easily obtainable, easy to perform and immediate availability of results. But this type of screening has its own limitations as it cannot be used in patients with trauma, coma patients or on any other individual who is unconscious and uncooperative. Another disadvantage is that the same equipment is used across different individuals and carries the risk of cross contamination.

It is proven that other samples can also be used for screening and among them saliva is a good sample. Salivary test strip is sensitive, reliable and easy to use tool which use saliva as a sample for screening of alcohol consumption. T M Rao et. al. determined BACs by ALCO-SCREEN 02 plastic strip in a group of people who attended an organized party. The result was statistically significant (P value 0). Phair et. al. determined BACs by ALCO-SCREEN salivary reagent strip in a series of patients who presented with suspected alcohol intoxication and the results were found to be significant (P < 0.0001). They recommended reagent strips for rapid measurement of BAC. Bates et. al. correlated BACs by alcohol sensitive saliva strips with breath estimates of BAC and found close estimates of BAC with that of saliva strips and breathalyzer; recommending alcohol saliva strips for BAC.

The Alcohol Salivary Test Strip is designed for use with human saliva only; a positive result indicates only the presence of alcohol and does not indicate or measure intoxication, and there is a possibility that technical or procedural errors, as well other substances in certain foods and medicines.

Our study was a prospective case control trial. Our results indicate that the saliva alcohol strip is as efficacious as the breath analyzer in the detection of alcohol. In our sample the time elapsed between the time of consumption of alcohol and testing ranged from 45 min to 7hrs but most of the participants were in the 2-3hrs bracket. BAC level reaches the peak about 1-2hrs after consumption and is most
easily detectable in this time range. In our study also, the test positivity was maximum between the time interval of 2-3 hrs. Both the breathalyzer and salivary strip test were equally efficacious during this time interval. Similar results were reported in previous studies done.\textsuperscript{7,13,14}

A very significant finding in our study was that the saliva alcohol strip could detect the presence of alcohol even when the time lapsed was more than 5 hrs. Positive results were seen as late as 5-7 hrs in 3 subjects while screening with salivary strip and showed negative result with breathalyzer test. This has not been reported in any previous studies. The reason could be that the breath alcohol content is expelled faster where as in saliva it stays for a longer time. This is a clear advantage of using salivary strip test in comparison to breathalyzer.

A big advantage of the salivary strip test is that it is capable of showing different colors denoting different BACs. In our study we saw light blue color in who have consumed less alcohol and dark blue color in who have consumed more alcohol. In contrast, only breathalyzers which have advanced digital readers can show the different alcoholic concentrations. These are comparatively expensive and are not commonly used as screening tools. The breathalyzer commonly used as screening tools by the police department for drunken driving do not contain digital reader and hence cannot determine the different alcohol concentration. The can only indicate the presence or absence of alcohol in breath. So salivary strip test is also advantageous in this point of view.

**Conclusion**

Our study proves that screening for alcohol consumption using salivary alcohol test strip is rapid, economically feasible and can easily be performed and it is as effective as breathalyzer. The result given by strip is easy to interpret and can easily indicate different BAC level as it is very much sensitive even at a BAC value of 0.02%.

Thus, from the results we can conclude by saying that salivary alcohol test strip is very efficacious and valid tool, and can be used as effective as breathalyzer for screening drunken driving. Moreover, it can be a useful screening tool in many medico-legal situations like trauma patients, coma patients, postmortem cases and other law and enforcement situation, where breathalyzer cannot be used.

In the present scenario of covid-19 pandemic, screening for alcohol consumption using breathalyzer has been suspended all over India and many other countries because of the fact that the same instrument is used and disinfection of the instrument is very difficult. Expelled breath can contain Corona virus and several other infective agents and disinfecting in between tests is difficult to carry out. Saliva test strips being single use strips and the fact that it can be performed without contact is a great advantage.

Based on these points it is valid to recommend that salivary strip test can be used as a screening tool instead of breathalyzer. More studies can further validate these findings. Indigenous production of saliva test strip can reduce the price of the test strips and can facilitate its extensive use.

**Conflicts of interest:** There is no conflict of interest.

**Source of funding:** Self

**Ethical clearance:** The present study has been reviewed by the institutional ethical committee and has therefore been performed in accordance with the ethical standards laid down in the 1965 Declaration of Helsinki. Permission was also obtained from The District Police Chief Kannur of Kerala Police Department.

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Cheiloscopy- An Important Technique in Forensic Science for Identification: A Review

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Abstract

Background: The concept of lip prints is clear from previous literature review that this evidence can be used for forensic purpose. While accepting that a body of data existed regarding the individuality of lip pattern, there is no credible research on the stability of the lip detail over time. The timescale in question could be measured in weeks and months or years. The former would be used in police investigations and the latter can be used by geneticist.

Keywords: Forensic science, Forensic Odontology, Lip prints, Cheiloscopy.

Introduction

Cheiloscopy, or lip print, is one of forensic methodology in case of identification. Lip prints are normal lines and fissures within the sorts of wrinkles and grooves present within the zone of transition of human lip, between the inner oral labial mucosa and outer skin or vermillion border ¹. Lip prints are exclusive for each individual and are considered as positive means of identification according to The Federal Bureau of investigation (F.B.I) and the Illinois state police². Cheiloscopy (derived from the Greek word cheilos means lips) is that the forensic investigation technique that deals with identification of humans supported lip traces³.

Review: Historical Background

The historical perspective of lip prints is that in 1902 an anthropologist named Fischer described the furrows seen on the red part of human lips. In 1932 Edmond Locard, recommended cheiloscopy in identification. In 1950 Snyder mentioned lip prints are as individualistic because the ridge characteristics on fingers in his book ‘Homicide investigation’. In 1967 Suzuki conducted investigations on the measurement of lips, use of rouge and therefore the method by which useful data might be obtained from it. In 1970 Suzuki and Tsuchihashi named the grooves on the labial mucosa (labiorum rubrorum) as ‘sulci labiorum’ and the lip prints as ‘Figura linearum labiorum rubrorum’. In 1972 Mc Donell after studying lip prints in twins, reported that identical twins, are often distinguished by lip prints, although indistinguishable by all other means. In 1981 Cottone included in his book ‘Outline of forensic dentistry’ that lip prints are one among the specialized techniques utilized in identification. In 1990 Kasprzak after doing five-year research elaborated on the practical uses of cheiloscopy⁵.

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**Collection Methods in Cheiloscopy**

The suspect’s lips can be photographed for comparison. If the prints are on a flat surface like a mirror, then it can just be photographed as well and tracings can be made of the grooves. Another method is to ask the person to press their lips to a clean piece of paper or cellophane sheet after their lips are applied with lipstick or other medium, which can help to transfer the lip prints. Even without lipstick, the latent lip prints on a suitable surface can be obtained with a method similar to that used in obtaining fingerprints, where print developing powder or a magna brush and magnetic powder may be used. A number of different powders or cyanoacrylate can be used.

For many crime scene investigators, quite half the powder they use is regular, nonmagnetic powder. Regular powders are available in colours such as black, silver/grey, bichromatic and white. To record lip prints using the magna brush method, the person should impress his or her lips against a glossy porous surface or a smooth nonporous surface. The advantage of using magnetic powder and a magna brush is that, unlike the conventional brushes which tends to smear or leave streak marks on the print, these do not. These streaks may then be interpreted as false characteristics by the comparer. But magnetic powders and magna brush are costlier compared to that of conventional ones. The development of latent successful latent lip prints experiment with reagents other than those traditionally used to develop fingerprints is required due to differences in print composition as published by before in the literature.

A number of different developments lipochromes can be used during the analysis of latent print composition such as CI solvent black 3 (Sudan Black B), CI solvent red 23 (Sudan III) or CI solvent Red 27 (Oil Red O). Lipochrome may be a generic term for compounds that have the power to dye fatty acids. Their molecular structure contains a portion that dissolves in contact with fat and another that is responsible for colour. To study the application of lipochromes the chemical composition of latent prints must be understood and this is the topic that has been widely studied. It is known that such prints contain lipids that do not depend on individual perspiration levels, being more susceptible to development using dyes that react to lipids in contrast to ninhydrin which depends on perspiration levels. Past studies showed that lip prints taken with lipochromes, the most effective was CI Solvent black 3. A review on various methods of recording and collection of lip prints at the crime scene with development of latent lip prints has emphasized on photography, lip stick method, finger printer roller and magna brush (magnetic powder) method. It was suggested that the use of red part of lips to identify a human being are wider than it is commonly thought. Collection of visible as well as latent lip prints with a suitable transferring medium is important for its consideration as positive forensic evidence.

The lips of the individual should be cleaned and a coloured lipstick is applied evenly with a single stroke on the vermillion border and asked to rub both the lips and spread the applied lipstick. After about 2 min, the glued portion of the cellophane tape should be placed over the lipstick. Lip prints should be obtained in the normal rest position by dabbing in the centre first and then pressing it comfortably towards the corner of the lips. The cellophane strip should then be stuck to a white bond for a permanent record then visualized through a hand glass or using computer aided software. The number of lines and furrows present on the middle part of the lower lip, their length, branching and combination should be noted. There are several instances where an erroneous lip print data may be obtained. Post mortem alterations tend to set in if the prints are not obtained within a day of death. The position of the lips changes depending on whether the mouth is kept open or closed. Well-formed lip prints are only available if the lips are closed. Lip print pattern is also subject to changes if affected by any pathology like mucoceles. Debris on the lips, lack of support due to loss of anterior teeth or over smudging due to application of thick layer of lipstick can all lead to alterations in lip prints. In studies regarding Post mortem changes in soft tissues reported that the physical changes of decomposition consist of alterations caused by accumulation of gases in the soft tissues as well as within the gastrointestinal and respiratory tracts. These changes are most prominent in the areas of the body which contain the most
blood, since the RBCs act as a food for the bacteria. Ordinarily, the soft tissues of the face swell first and cause eversion of lips which can make difficult for recording lip prints14,15.

When the lips are photographed, proper lighting should be focused on the lips at an angle that accentuates the contrast between the white and dark areas. The resulting Lip Print photographs should be of approximate natural size. This can be accomplished by placing a measuring device, ideally an ABFO scale No.2, or a ruler so that it will show in the photograph. Suzuki and Tsuchihashi in their study, obtained half sized negative film taken by Medical Nikkor (f 200 mm, lens 1: 5.6) and used for a contact print after enlarging to double size to get life size photographs. The photographic method involves photographing the Lip Print (either direct or latent and subsequently developed), and comparing it with photographs of the lips of the suspects or photograph of the Lip Print of the suspects. Photographing of the lips can often be very tricky and subject to errors as the central area of the lips and the angles of the lips are never in the same plane which leads to focusing errors resulting in unsharp or blurred or partial images of the lips. This invariably calls for recording the Lip Print and then photographing them and then comparing the two photographs. Thus, in spite of being a reliable method in other areas of forensics, in the recording of Lip Print images it adds to the cost but does not enhance the clarity in the same rising proportions. With the advent of digital photography, the trend is to record direct digital imaging using digital camera. Several courts particularly those in U.K. reject the evidence if the very first photograph is a direct digital image. On the grounds that there is no proof that the digital image has not been previously manipulated using any of the available digital imaging software including Adobe Photoshop. The court in U.K. insists on having the primary records based on conventional photographs and a willing to accept digital analysis of such photographs.

Analysis of lip prints:

Further studies has been done to classify lip prints, study their variations, determine the most common pattern in the study population, evaluate differences in lip prints between males and females and between different age groups, ascertain whether there's any inheritance and thereby investigate their potential role in personal identification. The lip prints were analyzed using Adobe® Photoshop® software and classified consistent with Tsuchihashi classification. Patterns of lip prints occurred in diverse combinations. The patterns were similar between males and females and varied among different age groups. Some hereditary resemblance was observed between parents and offspring. Lip prints have a good potential for use in criminal investigations. They have been used only occasionally despite their frequent occurrence at crime scenes. A place for cheiloscopy is recommended within the scope of forensic odontostomatology, along with other means of forensic identification16,17,18,19.

Concepts in cheiloscopy:

Cheiloscopy reflects sharp implication and correlation with sign and symptoms of criminology. It has been noticed that criminals are still not much aware of this specialized method of criminal identification, which often makes them exposed to the court of law. In this connection detailed study of lip print will bear definite importance. Discussing these aspects, the study of such an important human anatomical presenting feature will open new horizons in today’s rising criminal activities and medico legal disputes among the population.

Accordingly, it has been verified that lip prints do not change and recover after undergoing alterations like trauma, inflammation and disease like herpes or with environmental factors. It is interesting that the use of lip prints is not indispensable for leaving lip prints albeit the vermilion border has minor salivary glands and the edges of the lips have sebaceous glands with sweat glands in between. It is assumed that secretions of oil and moisture from these enable development of latent lip prints in most crime scenes where close contact between the victim and culprit has occurred. The main feature of dental identification is the existence of antemortem data which cannot be expected in Cheiloscopy as the only use of Cheiloscopy will be to relate lip prints to the lips that produced them20,21.

There are studies to ascertain whether lip prints behold the potential for determination of sex of an
individual. The lip prints obtained were coded, keeping in account the name and sex of the respective individuals. At the time of study, the sex of the print wasn’t disclosed\textsuperscript{22,23}.

In recent years, lipsticks used do not leave any visible trace after contact with the surfaces. They are characterized by their permanence and are referred to as persistent lip prints and can be lifted using the materials such as aluminium powder and magnetic powder. The importance of cheiloscopy in sex determination related to forensic investigation has been also discussed\textsuperscript{24,25}.

It was overviewed that lines and furrows which are present on upper and lower lips and from one corner of mouth to other, only middle portion of lip is taken into account since this portion is always visible in any traces. It was also pointed out that it is not easy to position the lip prints in the general system of traces. Also, the use of lip sticks is not indispensable for leaving lip prints\textsuperscript{26,27}.

The correlation of lip prints with blood groups may be a useful tool in forensic science for more accurate identification of an individual than with the use of lip print alone. There is no evidence to correlate lip prints with the blood groups in the literature but recently the studies has been done with the primary aim of determining the relationship between lip prints and blood group. It was found that there was no correlation between the blood groups and lip prints therefore no significant correlation exists between blood group and lip prints as lip prints play a vital role in identification because they are unique\textsuperscript{28,29,30}.

**Conclusion**

Cheiloscopy as a forensic investigation tool is gaining importance since previous couple of decades. Lip prints an important and emerging discriminatory tool in forensic criminology. If a definite description of the different parts of the upper lip and the lower lip is available for an individual by detailed study, this anti-mortem record can be used for matching the details of lip prints in post-mortem records for personal identification.

**Ethical clearance:** Taken from the ethical committee.

**Funding:** self

**Conflict of interest:** Nil

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To Describe Clinical Profile of Medico-Legal Cases at Tertiary Hospital in Ahmedabad

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Abstract

Background: Ophthalmologists play an important role in the management and fixing of legal responsibility in ocular medico legal cases (MLCs). All penetrating or non-penetrating intentional ocular trauma causing loss of an organ or part of organ and which have as consequence the facial disfigurement or post trauma complication have forensic implications.

Objective: To describe the clinical profile and meticulous documentation in ocular medico-legal cases attending the ophthalmic unit at a tertiary care hospital in Ahmedabad.

Methods: Medical records of ocular MLC from May 2018 to November 2020 were considered. A total of 112 cases of ocular injury constituted the sample size. The injuries were categorized as adnexal and globe injuries. The clinical profile was recorded in the clinical record forms and analyzed accordingly.

Results: A total of 112 patients with ocular trauma were recruited with follow up to 6 months following recruitment. The most affected age group was 26-40 years (25.89%). The most common mode of injury was assault with fist in 22 (18.74) of cases. Most patients had open globe injury (28, 25%), while 20 (16.5%) had closed globe injury, 38 (33.93%) had the adnexal injury, 7 (6.25%) had open plus adnexal, 7 (6.25%) had closed plus adnexal and 12 (10.71%) had chemical injury. Adnexal injuries were the most common n=38 (33.93%). Lid / periorbital contusion and subconjunctival hemorrhage were the most common presentations among adnexal and globe injuries respectively. Malingering was present in 3 % (3). Furthermore, among the adult population, majority 28 (25%) had ocular injury at workplace, while most pediatrics injuries 23 (20.54) occurred at home during play. The incidence of monocular blindness 2 (9%) was at 6 months follow up after the initial presentation. Factor that was associated with monocular blindness was open globe injuries.

Conclusion: Fist injury was the most common cause of trauma to the eye. A substantial number of patients who had adnexal injuries or subconjunctival hemorrhage had good vision, contrary to open globe injuries which resulted in very poor vision.

Keywords: Forensic medicine, Medico-legal case, Ophthalmic MLC, Ocular trauma

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Introduction

Ocular trauma has been a neglected disorder. Today ocular injury is one of the most common causes of unilateral blindness in the world. The national population based survey of blindness in Nepal (1981) found a blindness prevalence rate of 0.84%, with ocular trauma responsible for 7.9% of monocular blindness. Ocular injuries hold forensic significance when it occurs due to trauma to the body hampering the loss of eye ball and visual acuity. The consequences have many facets including legal, social, and economic. A medico-legal case (MLC) has been defined as an inflicted injury or disease process where the treating doctor establishes the diagnosis by a thorough history, clinical examination, and investigations and determines responsibility according to the law of the land.

Many cases of ocular trauma or illnesses are currently being registered as MLC (medico-legal cases) and present to an ophthalmologist for optimal management and to determine liability as per law. An ophthalmologist’s role is to confirm the etiology, assess the depth of injury, grade the type of injury, and establish the severity of the disability. In practice, a legal opinion of ophthalmologist is essential as a treating ophthalmologist or expert or when the legal proceeding is against the favor of ophthalmologist during medical disputes. Such precaution is necessary in civil matters where the patient claims compensation under Motor Vehicle Accident Act and Workmen’s Compensation Act. Hence, the ophthalmologist must provide evidence of detailed eye examination, correct description of wounds and appropriate investigations to support the diagnosis. If a medical practitioner completes all those processes like writing ‘wound certificate’ make a ‘written consent’ with the patient for examination and treatment, and ‘if the matter is intimated to police’, his medico-legal duties can be considered up to standard. If he fails to perform these duties, a negligence suit may be filed against him in the court of law. The eye being the bilateral organs of visual system; its involvement by injury has medico-legal implications, both of civil and criminal nature. So, every doctor must be familiar with the procedure of examination and recording of data for medico-legal purpose.

Objective

To describe the clinical profile of ocular medico-legal cases attending the ophthalmic unit at a tertiary care hospital in Ahmedabad.

Material and Method

The present study was conducted at a tertiary care ophthalmic center in Ahmedabad. This was a hospital-based retrospective study. The Institutional Ethics committee approved the study protocol. The medical records of patients registered as ophthalmic MLC from May 2018 to November 2020 were considered. The criteria for labeling a case as a MLC included a history of assault, foul play, or accident (including road traffic accidents), patient’s/patient’s legal guardian’s request to register as MLC and the medical officer’s opinion. Clinical findings were recorded in the predesigned proforma and descriptive analysis was done. Before eye examination, consent was acquired from all patients. The standard protocol for eye examination was adopted at the time of the medico-legal examination. Vision assessment on Snellen’s chart, torchlight and slit lamp assisted ocular examination along with dilated fundus examination was done in all cases. USG (B) was carried out to assess posterior segment status particularly renal detachment, vitreous hemorrhage and to rule out retained intraocular foreign body in patients with hazy media. Other relevant investigations like X-ray orbit/skull, CT scan and MRI were done whenever indicated.

The cases were grouped as adnexal (extra-ocular involving orbit and lid) and globe injuries. Globe injuries include closed and open globe injuries. A full-thickness wound of the sclera and cornea was defined as "open globe injury (OGI)." "Closed globe injury (CGI)” was defined as one in which no full-thickness wound of the sclera and cornea was present. Injuries were categorized for medico-legal purposes.

Results

Hundred and twelve patients were identified as ocular MLC. 89 (79.46%) were male. The age ranged from 6 years to 60 years with a median and mean of 28.30±19.86 years. Of this, 36 (32.14%) were less than 16 years and 3 (2.68%) patients had bilateral eye
The most common mode of injury was assault with fist in 22 (18.74%) of cases, followed by chemical 12 (%), fireworks 11(9.82%), and stone injury 10 (8.93%), RTA 9 (8.03%). Twenty five percentage of patients were injured with a sharp object like objects in (Table 1).

Table 1: Age groups and mode of ocular trauma:

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Number</th>
<th>%</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-6</td>
<td>18</td>
<td>16.07</td>
<td></td>
</tr>
<tr>
<td>7-16</td>
<td>18</td>
<td>16.07</td>
<td></td>
</tr>
<tr>
<td>17-25</td>
<td>18</td>
<td>16.07</td>
<td></td>
</tr>
<tr>
<td>26-40</td>
<td>29</td>
<td>25.89</td>
<td></td>
</tr>
<tr>
<td>41-60</td>
<td>22</td>
<td>19.64</td>
<td></td>
</tr>
<tr>
<td>&gt;60</td>
<td>7</td>
<td>6.25</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>112</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Causative agents</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blunt Fist*</td>
<td>22</td>
<td>18.74</td>
</tr>
<tr>
<td>Stone</td>
<td>10</td>
<td>8.93</td>
</tr>
<tr>
<td>Cricket ball</td>
<td>7</td>
<td>6.25</td>
</tr>
<tr>
<td>Plastic bottle</td>
<td>2</td>
<td>1.78</td>
</tr>
<tr>
<td>Wooden stick</td>
<td>7</td>
<td>6.25</td>
</tr>
<tr>
<td>Toy</td>
<td>2</td>
<td>1.78</td>
</tr>
<tr>
<td>Sharp Glass</td>
<td>3</td>
<td>2.68</td>
</tr>
<tr>
<td>Knife</td>
<td>2</td>
<td>1.78</td>
</tr>
<tr>
<td>Pencil</td>
<td>1</td>
<td>0.89</td>
</tr>
<tr>
<td>Blouse hook</td>
<td>3</td>
<td>2.68</td>
</tr>
<tr>
<td>Machine cutter</td>
<td>5</td>
<td>4.46</td>
</tr>
<tr>
<td>Finger nail</td>
<td>2</td>
<td>1.78</td>
</tr>
<tr>
<td>Kite thread</td>
<td>2</td>
<td>1.78</td>
</tr>
<tr>
<td>Edge of bed/swing/spring</td>
<td>10</td>
<td>8.93</td>
</tr>
<tr>
<td>Falls (RTA)</td>
<td>09</td>
<td>8.03</td>
</tr>
<tr>
<td>Projectiles Fire works</td>
<td>11</td>
<td>9.82</td>
</tr>
<tr>
<td>Chemical</td>
<td>12</td>
<td>10.71</td>
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<tr>
<td>Cow’s horn</td>
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<td>1.78</td>
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</table>

Workplace injuries accounted for 28 (25%) of cases where five injuries occurred due to machine cutter. About 29 (25.89%) and 23 (20.54%) of cases were recorded from household injuries and child play respectively. Adnexal injuries were most common, 38(33.93%), while open globe injuries were accounted for 71(69%) of cases and close globe injury 17 (16.5%) of cases. Adnexal and globe combined injuries accounted for about 14 (12.50%) of cases.12
Table 2: circumstances and type of ocular trauma:

<table>
<thead>
<tr>
<th>Circumstances of trauma</th>
<th>Number of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Child play*</td>
<td>23</td>
<td>20.54</td>
</tr>
<tr>
<td>Workplace*</td>
<td>28</td>
<td>25</td>
</tr>
<tr>
<td>Unintentional injury</td>
<td>1</td>
<td>0.89</td>
</tr>
<tr>
<td>Traveling</td>
<td>1</td>
<td>0.89</td>
</tr>
<tr>
<td>Assault</td>
<td>10</td>
<td>8.92</td>
</tr>
<tr>
<td>Household injuries</td>
<td>29</td>
<td>25.89</td>
</tr>
<tr>
<td>Chemical fall</td>
<td>12</td>
<td>10.71</td>
</tr>
<tr>
<td>Road traffic accident</td>
<td>09</td>
<td>8.03</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>112</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of injury</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Open globe*</td>
<td>28</td>
<td>25</td>
</tr>
</tbody>
</table>

| Open globe + adnexal                  | 7       | 6.25 |
| Close globe                           | 20      | 17.86|
| Close globe + adnexal                 | 7       | 6.25 |
| Adnexal                               | 38      | 33.93|
| Chemical                              | 12      | 10.71|
| **Total**                             | **112** | **100**|

**P value**: 0.0005

Among closed globe injuries, lid edema was present in 50 patients, subconjunctival haemorrhage present in 38, 35 patients had lid laceration and 29 patients had corneal laceration. The relative afferent pupillary defect was present in all patients with open globe injury and seven patients with closed globe injury. The common ocular presentations following trauma are shown in Table 1.

Table 3: Common traumatic presentations in medico-legal cases of ocular trauma

<table>
<thead>
<tr>
<th>Ocular Finding*</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conjunctival laceration, FB, chemosis, subconjunctival hemorrhage</td>
<td>38</td>
</tr>
<tr>
<td>Lid edema</td>
<td>50</td>
</tr>
<tr>
<td>Corneal FB, ulcer/ abrasion, epidefect, generalized edema</td>
<td>37</td>
</tr>
<tr>
<td>Lid laceration</td>
<td>35</td>
</tr>
<tr>
<td>Corneal laceration</td>
<td>29</td>
</tr>
<tr>
<td>Anterior chamber reaction, exudates, hypopyon, exudative membrane</td>
<td>11</td>
</tr>
<tr>
<td>Corneal scleral laceration</td>
<td>11</td>
</tr>
<tr>
<td>Hyphaema</td>
<td>30</td>
</tr>
<tr>
<td>Traumatic cataract</td>
<td>19</td>
</tr>
<tr>
<td>Ruptured globe with rbh</td>
<td>1</td>
</tr>
<tr>
<td>Scleral laceration</td>
<td>17</td>
</tr>
<tr>
<td>Dislocated lens</td>
<td>4</td>
</tr>
<tr>
<td>Traumatic cataract</td>
<td>7</td>
</tr>
<tr>
<td>Others</td>
<td>8</td>
</tr>
</tbody>
</table>

**Intervention Given**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lid tear repair</td>
<td>16</td>
</tr>
<tr>
<td>Medical management</td>
<td>41</td>
</tr>
<tr>
<td>Scleral wound repair</td>
<td>17</td>
</tr>
<tr>
<td>Conjunctival wound repair</td>
<td>19</td>
</tr>
<tr>
<td>Corneal wound repair</td>
<td>29</td>
</tr>
</tbody>
</table>
Marginal tear repair 19
Canalicular tear repair 19
Lateral canthal tear repair 2
Others 6

Others presentation: RD, orbital wall #, intra ocular FB, vitreous hemorrhage.

Total findings: (297) in table because of multiple finding in one eye in trauma patients.

None of the patients reported a pre-existing history of reduced vision before ocular injury and on initial assessment, (n = 50) were blind in the injured eye following trauma. Following primary treatment, the proportion of monocular blindness by the 6 months dropped to (n = 2).

Table 4: Follow up of Visual Acuity

<table>
<thead>
<tr>
<th>WHO Classification</th>
<th>Visual Impairment</th>
<th>At Presentation</th>
<th>At 1 week</th>
<th>At 2 weeks</th>
<th>At 3 months</th>
<th>At 6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (6/6&lt;6/18)</td>
<td></td>
<td>29 (26.22)</td>
<td>69 (66.1)</td>
<td>33 (56.0)</td>
<td>16 (41.0)</td>
<td>14 (63.6)</td>
</tr>
<tr>
<td>Moderate (6/18&lt;6/60)</td>
<td></td>
<td>17 (15.78)</td>
<td>12 (11.52)</td>
<td>7 (11.9)</td>
<td>5 (13.0)</td>
<td>4 (18.0)</td>
</tr>
<tr>
<td>Severe (6/60&lt;3/60)</td>
<td></td>
<td>2 (1.74)</td>
<td>4 (3.85)</td>
<td>3 (5.0)</td>
<td>3 (7.7)</td>
<td>2 (9.0)</td>
</tr>
<tr>
<td>Blind (3/60-NPL)</td>
<td></td>
<td>50 (43.48)</td>
<td>13 (12.5)</td>
<td>13 (22.1)</td>
<td>13 (33.4)</td>
<td>2 (9.0)</td>
</tr>
<tr>
<td>VA not done</td>
<td></td>
<td>14 (12.78)</td>
<td>6 (6.03)</td>
<td>3 (5.0)</td>
<td>2 (4.9)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>112 (100)</td>
<td>104 (100)</td>
<td>59 (100)</td>
<td>39 (100)</td>
<td>22 (100)</td>
</tr>
</tbody>
</table>

VA= visual acuity
NPL= no perception of light.

In the present study, within 1 week of the follow-up of the 66.1% of the patients had normal vision compared to 25.22% at presentation and those with vision 3/60 reduced from 43.48% at presentation to 12.5%.

Discussion

The present study highlighted the patient’s clinical profile and ocular presentation in medico-legal cases presenting to the ophthalmic unit at a tertiary care hospital in Ahmedabad. The most common mode of injury in the present study was assault with fist. Adnexal injuries were most common, followed by globe injury and combined injuries, respectively. Lid / periorbital contusion and sub-conjunctival haemorrhage were the most common presentation among adnexal and globe injuries, respectively. The present study highlighted the importance of meticulous eye examination to rule out malingering. It also highlighted that, at times, the final report could not be given immediately, as the patient may require additional investigations and observation to report the definitive diagnosis. Similar to previous studies on ocular trauma, the present study has also shown male dominance with a male to female ratio of 3.9:1.13

According to previous studies, a majority of injured patients were young, with an average age of around 30 years.13,14 In the present study, patient age ranged from 6-60 years with a Mean ± SD age of 28.30±19.86 years. In previous studies, the fist has been reported as the most common mode of trauma.13,14 In the present study too, the most common cause of trauma among medico-legal cases is fist injury (18.74%), followed by chemical injury (10.71%). Adnexal (33.93%) / extra ocular injuries form a major group of injuries. Among adnexal injuries, lid contusion (44.64%) and adnexal laceration (31.25%) were the most common presentations. If severe, they can lead to facial disfigurement or functional impairment, leaving a considerable impact on patients’ social, personal and psychological wellbeing. Among the injuries involving the globe, subconjunctival haemorrhage was present in (33.92%) of patients and was the most common presentation, followed by hyphaema, which was present in 26.78% of patients. Nearly 66% of patients had vision 6/18 or better, similar to previous study.13,14 Depicting adnexal and subconjunctival haemorrhage as the most common
injury. Posterior segment involvement in the form of vitreous, choroid and retinal haemorrhage, traumatic macular hole and traumatic optic neuropathy were documented. In the present study, a patient with a superficial foreign body developed a central corneal ulcer, leading to decreased vision; similarly, a macular hole was noticed once the vitreous haemorrhage was resolved in one patient. Therefore, a definitive opinion should not be given immediately; patient should be kept under observation with relevant investigations as the visual disability changes till the final outcome. In specific scenarios, though the visual acuity returns to normal following treatment, there is always a risk of severe future complications. Like in the case of traumatic cataract, visual acuity may return to normal following cataract extraction with intraocular lens implantation. However, there is loss of accommodation in young patients and the surgery is also not always free of complications. Similarly, in vitreous haemorrhage and hyphaema, visual acuity may return to normal. However, there is a future risk of complications such as secondary glaucoma, proliferative vitreoretinopathy changes and retinal detachment. Hence despite favourable vision following treatment, such types of injuries are graded as grievous considering the risk associated. In the present study, in a majority of medico legal cases, the assailant is known. These injuries may be inflicted as a result of revenge, social conflicts, or for financial reasons. Patients complain of decreased vision after recent injury for unfair advantage. This study has also witnessed malingering in 3 patients. All presented with complaints of bleeding from the eye followed by a sudden diminution of vision ranging from counting fingers close to face to no perception of light. On evaluation, these patients were found to have haemorrhage from the conjunctiva or lid. It has been noted that patients attempt to take advantage of such situations by complaining of sudden diminution of vision. The ophthalmologist should have a comprehensive understanding of the legal aspects involved in these cases and perform a meticulous examination and note all relevant findings objectively along with important negative signs. Any evidence of malingering and signs which differentiate pre-existing illness from the recent injury should be documented appropriately. In some situations the patients insistence has lead the ophthalmologists to perform electrophysiological tests to rule out blindness. Illustrating the wound diagrammatically with the measurements, determining VA (visual acuity), IOP (Intraocular pressure) and pupillary reaction are essential features of documentation. Such reports must be kept concise and comprehensible, with the doctor’s signature and saved for future references. The records should be kept for three years or until the judgement has been given by court.14

Conclusion

Ophthalmic medico legal cases are not uncommon to run into clinical practice and they are commonly caused by direct assault of fist in our cultural setting.

Recommendation

Medico-legal cases require meticulous eye examination along with clear, concise and accurate documentation. It is necessary to correlate reduced vision with signs of recent injury to rule out malingering, pre-existing ocular morbidity and foul play by patients.

Ethical clearance: Taken from IEC

Source of funding: Self

Conflict of Interest: Nil

References


Cross-Sectional Study of Sexual Dimorphism in Lip Prints among Students Doing a Professional Course in Bengaluru

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Abstract

Background: Identification of human beings forms the basis of any criminal investigation. Lip print study, also known as cheiloscopy is the study of patterns formed by the ridges and grooves on the external surface of lips. This study aids in classifying lip prints and study its correlation to sex.

Methodology: 205 student participants who volunteered were included in this study of which females were 123 and males were 82. A food safe pigment was applied to the lips. Over this pigment, the glued portion of the cellophane tape strip was placed and the subject was asked to make a lip impression in the normal rest position of the lips by dabbing it in the centre first and then pressing it uniformly toward the corners of the lips. The cellophane strip was then stuck to the white chart paper for permanent record purpose, then visualized by magnifying lens and analysed quadrant wise.

Conclusion: The most common lip print in the upper lip was found to be type 3 and in the lower lip was found to be type 4. Statistically significant difference with respect to sex was observed in first quadrant only (p = 0.01) and not in others.

Keyword: Cheiloscopy, Lip prints.

Introduction

Identification of human beings forms the basis of any criminal investigation. Lip print study, also known as cheiloscopy is the study of patterns formed by the ridges and grooves on the external surface of lips¹. It has gained importance as a prominent...
identification tool in the past few decades. It also plays a vital role in criminal investigations involving murder, rape and other sexual offences where in lip print may be the only evidence left behind by the suspect. Lip prints may also be used as a biometric modality which when combined with other techniques may further enhances the accuracy of identification.

Review of Literature

Fischer in 1902 was the first anthropologist to describe the furrows on the red part of human lips. In 1950, Synder reported in his book “Homicide Investigation” that the characteristics of the lips formed by lip grooves are as individually distinctive as the ridge characteristics of finger prints. Suzuki, in 1967, made detailed investigations of the measurement of the lips, the use and color of rouge, and the method for its extraction to obtain useful data for practical forensic application. Mc Donell in 1972 conducted a study on lip prints between two identical twins and reported that two identical twins seemed to be indistinguishable by every other means but their lip prints were different. This tool has the ability to differentiate sex in the field which could be of value when the other modalities of identification may not be applicable. Lip prints are found to be more sensitive than palatoscopy or canine odontometry. Lip prints have also been used as a potential biomarker for Type 2 diabetes mellitus.

Although this study has been around for decades, it has only been used in isolated cases in the court of law and its uniqueness and interpretation requires further research. The present study is aimed at identifying lip prints of different individuals in different parts of the lip and find out the incidence of any particular pattern in the given sex.

Aims and Objectives

1. To classify Lip Prints of the study group using classification scheme proposed by Suzuki and Tsuchihashi.
2. Correlation of lip print classification with the sex of the individual.

Materials and Methods

- **Ethical approval:** Institutional ethical clearance was obtained from the teaching institute before commencing the study.
- **Study area:** The study was conducted among students doing a professional course in a tertiary care center located in Bengaluru.
- **Study sample:**

205 student participants who volunteered were included in this study of which females were 123 and males were 82.

- **Study materials:**

1. Pigment which is food safe.
2. Cellophane tape.
3. White chart paper.

- **Inclusion criteria:** Students between 19 yrs to 24 yrs of age were included in this study. Only those students with normal lips without any pathology and a normal transition between the mucosa and the skin were included.
- **Consent:** Individual written consent was obtained before the study was conducted on the subject.
- **Technique:**

The lips of the individuals were cleaned and a pigment which is food safe was applied on the lips. Over this pigment, the glued portion of the cellophane tape strip was placed and the subject was asked to make a lip impression in the normal rest position of the lips by dabbing it in the centre first and then pressing it uniformly toward the corners of the lips. The cellophane strip was then stuck to the white chart paper for permanent record purpose and then visualized by magnifying lens. While studying the various types of lip prints, each individual’s lip was divided into four compartments, i.e., two compartments on each lip, and was allotted the digits 1-4 in a clock-wise sequence starting from the subject’s upper right quadrant thereby dividing both the lips into 4 equal quadrants named as q1, q2,
q3 and q4. Lip print in each quadrant was classified using the Suzuki and Tsuchihashi[^8]. The classification is as follows:

- **Type I** - Clear-cut grooves running vertically across the lip
- **Type I’** - The grooves are straight vertical but discontinuous, not running entirely across the lip.
- **Type II** - The grooves branch in their course in the shape of Y
- **Type III** - Intersecting grooves
- **Type IV** - Reticular pattern
- **Type V** - The grooves do not fall into any of the types I-IV, and cannot be differentiated morphologically and are irregular.

**Data analysis:** Data was tabulated, analyzed using SPSS software and interpreted with appropriate statistical parameters.

**Observation and Results**

All the lip prints analysed showed different patterns and also inter-quadrant variation. The most common lip print in q1 was type 3 (31.2%); q2 was type 3 (33.2%); q3 was type 4 (31.7%); q4 was type 4 (26.4%). Hence, the most common lip print in the upper lip was found to be type 3 and in the lower lip was found to be type 4. On performing Pearson Chi-Square test for gender correlation in each quadrant, statistical significance was observed in q1 (p = 0.010) whereas no statistical significance was found in any of the other quadrants (q2 p= 0.130; q3 p= 0.163 & q4 p= 0.428).

**Fig 1: Study Group**

Distribution of study group according to gender

**Table 1: Frequency (%) of different lip print type in different Quadrants (most common highlighted in bold)**

<table>
<thead>
<tr>
<th>Lip print type</th>
<th>Quadrant 1 (%)</th>
<th>Quadrant 2 (%)</th>
<th>Quadrant 3 (%)</th>
<th>Quadrant 4 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1’</td>
<td>20.5</td>
<td>6.8</td>
<td>24.9</td>
<td>21</td>
</tr>
<tr>
<td>1</td>
<td>13.7</td>
<td>21.5</td>
<td>15.6</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>8.3</td>
<td>10.7</td>
<td>10.2</td>
<td>8.8</td>
</tr>
<tr>
<td>3</td>
<td>31.2</td>
<td>33.2</td>
<td>12.7</td>
<td>23.4</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>14.6</td>
<td>31.7</td>
<td>26.3</td>
</tr>
<tr>
<td>5</td>
<td>8.3</td>
<td>13.2</td>
<td>4.9</td>
<td>2.4</td>
</tr>
</tbody>
</table>

**Table 2: Correlation between lip print in Quadrant 1 and gender**

<table>
<thead>
<tr>
<th>Quadrant 1: Chi-Square Tests</th>
<th>Value</th>
<th>df</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson Chi-Square</td>
<td>15.034(a)</td>
<td>5</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**Table 3: Correlation between lip print in Quadrant 2 and gender**

**Table 2: Chi-Square Tests**

<table>
<thead>
<tr>
<th>Value</th>
<th>df</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.517(a)</td>
<td>5</td>
<td>0.13</td>
</tr>
</tbody>
</table>

**Table 4: Correlation between lip print in Quadrant 3 and gender**

<table>
<thead>
<tr>
<th>Value</th>
<th>df</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.876(a)</td>
<td>5</td>
<td>0.163</td>
</tr>
</tbody>
</table>

**Table 5: Correlation between lip print in Quadrant 4 and gender**
Quadrant 4: Chi-Square Tests

<table>
<thead>
<tr>
<th>Value</th>
<th>df</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.900(a)</td>
<td>5</td>
<td>0.428</td>
</tr>
</tbody>
</table>

Discussion

As substantiated by our results and earlier studies, there exists a significant difference in lip prints from person to person and within the same person, which makes Cheiloscopy an important tool for identification. In our study we have found that the most common type of lip print in the upper lip was type 3 (31.2% and 33.2%) and in the lower lip was type 4 (31.7% and 26.4%) with overall most common type of lip print to be of type 3(64.4%) variety. This same pattern was also observed individually in the male and female sub-groups as found in study by Kiran et al.9. But this is in contrary to the studies by Gondivkar et al.10 social and legal reasons. The study of lip-prints (cheiloscopy, & Patel et. al.11 who found that type 2 pattern was most common among females and Tandon et. al.12 who found type 1 pattern to be most common in females . P Manjusha et. al.7 found that Type IV pattern of lip prints was significantly more in the diabetic patients. Basheer et. al.13 observed that most common lip print pattern in North Kerala population was type 1 and with type 2 being common in males and type 4 in females. Sexual dimorphism was observed with significant difference only in quadrant 1 in our study in which the most common type of lip print was type 3, which is in acceptance with studies by P Manjusha et. al.7, Prabhakar et. al.14 and Gondivkar et. al.10 social and legal reasons. The study of lip-prints (cheiloscopy. Whereas Kautiya et. al.15 observed that the outer four portions of the lip showed statistically significant differences in males and females . In contrast, studies by Patel et. al.11 and Basheer et. al.13 found no significant difference in lip prints of male and female.

Conclusion

This study concludes that type 3 lip print is most common among the study population with sexual dimorphism being exhibited only in the first quadrant. But this requires further validation as there is lack of consensus with studies published so far. Also, since lip prints show greater geographic and racial variation future studies in these areas may throw further light. The value of lip prints as a powerful identification tool is reiterated by this study and its use especially in medico-legal work cannot be over stressed.

Summary

205 student participants who volunteered were included in this study of which females were 123 and males were 82; all between the age groups of 19 to 24 years. Lip prints were developed using a food safe pigment and a cellophane tape which was then stuck to a paper. The lip prints were divided into 4 quadrants (q1, q2, q3 & q4) in clockwise direction starting from left upper quadrant and analysed for statistical significance. The most common lip print in q1 was type 3 (31.2%); q2 was type 3 (33.2%); q3 was type 4 (31.7%); q4 was type 4 (26.4%). Hence, the most common lip print in the upper lip was found to be type 3 and in the lower lip was found to be type 4. On performing Pearson Chi-Square test for gender correlation in each quadrant, statistical significance was observed in q1 (p = 0.010) whereas no statistical significance was found in any of the other quadrants (q2 p= 0.130; q3 p= 0.163 & q4 p= 0.428). This study concludes that type 3 lip print is most common among the study population with sexual dimorphism being exhibited only in the first quadrant.

Ethical clearance: Taken from Institutional Ethics Committee of Akash Institute of Medical Sciences and Research Centre, Bengaluru

Source of funding: Indian Council of Medical Research

Conflict of Interest: Nil

References

Profile of Autopsy Cases at District Hospital, South India in Pandemic Year 2020

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Abstract

Autopsy profile is one of the essential inputs for National Statistics. It gives an insight into the prevailing unnatural causes of death in the vicinity and subsequently helps stake holders take appropriate measures to prevent the same. The present study was conducted in district hospital, South India in the pandemic year, 2020. A total of 808 medico legal autopsies were conducted in Government District Hospital, Tumkur which is high in spite of pandemic and lockdown in place. Among them majority of victims were male constituting 78.85% and females attributing to 21.15% of total cases. Manner of death was analysed and accidental deaths formed the major chunk of cases comprising of 351 cases (43.44%). Suicidal deaths comprised of 35.87 % of cases and homicide deaths accounted for 3.71% of cases. Among males maximum number of cases was recorded in fourth decade with 147 cases. While in females maximum cases fell in third decade of life with 51 cases. Road traffic accident was the prime most cause of death with 241 cases comprising of 30% of total cases. Second most common cause was hanging with 137 cases comprising 17% of total cases. Surprisingly natural deaths constituted third most common cause with 125 cases comprising of 15% of the cases. Majority of cases were reported in middle age group 21-50 years which form the bread winner group. With adequate safety precautions and counselling these unnatural deaths could be prevented.

Key words: Profile, Pandemic, Unnatural death, autopsy

Introduction

Medico legal cases form an integral part of hospital admission. Commonly encountered medico legal cases are poisoning, road traffic accidents, asphyxia and homicide. Autopsy has to be performed in case of death occurring in above cases. Unnatural causes form majority of medico legal autopsy conducted. Study of autopsy profile throws light on demographic distribution of cases, various causes of death and help in contributing to vital statistics of the nation. Unnatural mortality form 10.3% of total deaths in India with greater incidence among population aged 10-45 years. Unnatural mortality rate in India is 0.67 per 1000 population, 0.84 among males and 0.49 among females¹. Unnatural deaths claim majority of lives in India with most of the victims in productive age group. According to accident death report, Karnataka reported 6.1% share of total deaths due to accidents in India and is categorised under high...
risk area. Autopsy profile study can enlighten us regarding most common unnatural causes plaguing the region with gender vulnerability and hence give suggestion to policy makers for implementing effective strategies to reduce mortality.

**Aim**

To study the profile of autopsy cases at District hospital, South India in the year 2020.

To suggest effective strategies to prevent unnatural deaths in the region.

**Material and Methods:**

The present retrospective study was conducted in Government District hospital, Tumkur, Karnataka. Period of study was from 1st January 2020 to 31st December 2020. Data was collected from Inquest report form 146 (i)&(ii), autopsy register, post-mortem reports and information given by relatives of the deceased. Autopsy profile was tabulated according to manner of death, gender distribution and cause of death.

**Results**

A total of 808 medico legal autopsies were conducted in Government District Hospital, Tumkur in the year 2020. Among them majority of victims were male with 637 autopsies which constitute 78.85% and 171 autopsies were conducted on females attributing to 21.15% of total cases. Manner of death was analysed and accidental deaths formed the major chunk of cases comprising of 351 cases (43.44%). Suicidal deaths constituted 35.87 % of cases i.e. 290 cases and homicide deaths accounted for 3.71% of cases i.e. 30 cases.

Most of the victims were in fourth decade of life which constitutes 183 cases. Next age group which forms majority of cases is third decade comprising of 179 cases. Nearly half of cases were in these two age groups. Among males maximum number of cases was recorded in fourth decade with 147 cases. While in females maximum cases fell in third decade of life with 51 cases. Least cases were recorded in extremes of age group, 1.35% in first decade and 0.37% in ninth decade.

Road traffic accident was the prime most cause of death with 241 cases comprising of 30% of total cases. Second most common cause was hanging with 137 cases comprising 17% of total cases. Surprisingly natural deaths constituted third most common cause with 125 cases comprising of 15% of the cases. Burns represent least number among cause of death.

In gender distribution, road traffic accident was most common cause of death among both genders followed by hanging. In males, burns and in females electrocution was least common.

Distribution of cause of death among different age group was studied and majority of cases were observed in middle age group between 21 to 50 years.

**Table 1: Distribution of cases among gender**

<table>
<thead>
<tr>
<th>Gender</th>
<th>Cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>637</td>
<td>78.83%</td>
</tr>
<tr>
<td>Female</td>
<td>171</td>
<td>21.16%</td>
</tr>
<tr>
<td>Total</td>
<td>808</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2: Distribution of cases based on manner of death**

<table>
<thead>
<tr>
<th>Manner of Death</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accident</td>
<td>351</td>
</tr>
<tr>
<td>Suicide</td>
<td>290</td>
</tr>
<tr>
<td>Homicide</td>
<td>30</td>
</tr>
</tbody>
</table>

**Table 3: Distribution of cases based on age**

<table>
<thead>
<tr>
<th>Age</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>9</td>
<td>6</td>
<td>15</td>
<td>1.85%</td>
</tr>
<tr>
<td>11-20</td>
<td>42</td>
<td>15</td>
<td>57</td>
<td>7%</td>
</tr>
<tr>
<td>21-30</td>
<td>128</td>
<td>51</td>
<td>179</td>
<td>22.15%</td>
</tr>
<tr>
<td>31-40</td>
<td>147</td>
<td>36</td>
<td>183</td>
<td>22.64%</td>
</tr>
<tr>
<td>41-50</td>
<td>134</td>
<td>18</td>
<td>152</td>
<td>18.81%</td>
</tr>
<tr>
<td>51-60</td>
<td>93</td>
<td>19</td>
<td>112</td>
<td>13.86%</td>
</tr>
<tr>
<td>61-70</td>
<td>66</td>
<td>20</td>
<td>86</td>
<td>10.64%</td>
</tr>
<tr>
<td>71-80</td>
<td>16</td>
<td>05</td>
<td>21</td>
<td>2.59%</td>
</tr>
<tr>
<td>81-90</td>
<td>02</td>
<td>01</td>
<td>03</td>
<td>0.37%</td>
</tr>
</tbody>
</table>
Table 4: Distribution of cases based on cause of death

<table>
<thead>
<tr>
<th>Cause of Death</th>
<th>Cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burns</td>
<td>5</td>
<td>0.61%</td>
</tr>
<tr>
<td>Assault</td>
<td>30</td>
<td>3.71%</td>
</tr>
<tr>
<td>RTA</td>
<td>241</td>
<td>29.82%</td>
</tr>
<tr>
<td>Poisoning</td>
<td>120</td>
<td>14.85%</td>
</tr>
<tr>
<td>Hanging</td>
<td>137</td>
<td>16.95%</td>
</tr>
<tr>
<td>Natural Death</td>
<td>125</td>
<td>15.47%</td>
</tr>
<tr>
<td>Railway Accident</td>
<td>28</td>
<td>3.46%</td>
</tr>
<tr>
<td>Fall From Height</td>
<td>40</td>
<td>4.95%</td>
</tr>
<tr>
<td>Drowning</td>
<td>57</td>
<td>7.05%</td>
</tr>
<tr>
<td>Animal/Snake Bite</td>
<td>12</td>
<td>1.48%</td>
</tr>
<tr>
<td>Electrocution</td>
<td>13</td>
<td>1.6%</td>
</tr>
</tbody>
</table>

Table 5: Distribution of age based on cause of death

<table>
<thead>
<tr>
<th>Cause of Death</th>
<th>1-10Y</th>
<th>11-20</th>
<th>21-30</th>
<th>31-40</th>
<th>41-50</th>
<th>51-60</th>
<th>61-70</th>
<th>71-80</th>
<th>81-90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burns</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Assault</td>
<td>1</td>
<td>3</td>
<td>12</td>
<td>8</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>RTA</td>
<td>13</td>
<td>55</td>
<td>50</td>
<td>45</td>
<td>33</td>
<td>32</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Poisoning</td>
<td>1</td>
<td>7</td>
<td>31</td>
<td>33</td>
<td>17</td>
<td>15</td>
<td>13</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Hanging</td>
<td>2</td>
<td>14</td>
<td>46</td>
<td>63</td>
<td>23</td>
<td>8</td>
<td>7</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Natural Death</td>
<td>1</td>
<td>2</td>
<td>10</td>
<td>18</td>
<td>40</td>
<td>32</td>
<td>23</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Railway Accident</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>2</td>
<td>5</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Fall From Height</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>6</td>
<td>10</td>
<td>9</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Drowning</td>
<td>3</td>
<td>11</td>
<td>8</td>
<td>17</td>
<td>5</td>
<td>7</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Animal/Snake Bite</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>17</td>
<td>5</td>
<td>7</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Electrocution</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>7</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6: Distribution of cause of death among gender

<table>
<thead>
<tr>
<th>Cause of Death</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burns</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Assault</td>
<td>19</td>
<td>11</td>
</tr>
<tr>
<td>RTA</td>
<td>200</td>
<td>41</td>
</tr>
<tr>
<td>Poisoning</td>
<td>93</td>
<td>27</td>
</tr>
<tr>
<td>Hanging</td>
<td>98</td>
<td>39</td>
</tr>
<tr>
<td>Natural Death</td>
<td>105</td>
<td>20</td>
</tr>
<tr>
<td>Railway Accident</td>
<td>22</td>
<td>6</td>
</tr>
<tr>
<td>Fall From Height</td>
<td>37</td>
<td>3</td>
</tr>
<tr>
<td>Drowning</td>
<td>39</td>
<td>18</td>
</tr>
<tr>
<td>Animal/Snake Bite</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Electrocution</td>
<td>12</td>
<td>1</td>
</tr>
</tbody>
</table>

Discussion

A total number of 808 autopsies were conducted at Government District Hospital, Tumkur from January to December 2020. Magnitude of cases was still high considering implementation of lockdown owing to pandemic. Reason may be increase in suicides due to pandemic and relaxation of lockdown in parts. Most of the victims were males, which is consistent with earlier studies conducted at other district hospitals, Belagavi³, Bikaner⁴, Bagalkot⁵ and Lucknow⁶. Most of the autopsies conducted on males belonged to age group of 31 to 40 years which is contrast to earlier studies done at Belagavi³, Bikaner⁴, and Bagalkot⁵ where third decade was mostly affected. Male predominance in cases is known fact that they are the bread winners who venture out for a living. In females third decade had more number of cases similar to earlier studies.
In our study death due to road traffic accident formed majority of cases which is in accordance with study findings at Belagavi\textsuperscript{3}, Bikaner\textsuperscript{4}, Lucknow\textsuperscript{6}, but in contrast to study at Bagalkot where burns was most common cause of death. Road traffic accidents constituted majority of cases as Tumkur being District Headquarters and surrounded by highways on either side, accidents are common feature and victims are shifted to tertiary care centre for management. After road traffic accident, death due to hanging was second commonest cause of death which is in contrast to earlier studies where hanging is distant third cause. Surprisingly natural death was third most common cause in autopsies conducted. Probable reason may be due to stress of Pandemic and also hesitancy to seek medical attention in spite of being sick due to fear of Covid.

Accidental deaths formed major chunk of cases followed by suicidal deaths and homicidal deaths which is in consistent with finding of previous study at Belagavi\textsuperscript{3}. Poisoning cases was placed at distant fourth in terms of number of cases in contrast to other study\textsuperscript{3} where it was second most common cause. Hanging was most common method adopted for suicide among both sexes.

**Conclusion**

Unnatural deaths share an important portion of death statistics in any part of country. The present study conducted in District hospital, South India, being a tertiary care centre; throw light upon profile of autopsy cases done in Pandemic year 2020. Most of the victims were males with third and fourth decade being more vulnerable to unnatural deaths. Road traffic accidents claimed dubious distinction of being leading cause of death. From this what we can infer is that precious young lives are being lost who are bread winners for the family due to unnatural deaths. Even though lockdown was in place for several months due to pandemic, the amount of unnatural deaths is painfully more. This can be prevented by effective safety precautions like wearing helmets; following traffic rules. Suicidal deaths claimed 35\% of total unnatural deaths. It may be attributed to pandemic anxiety, loss of near ones and loss of employment in unpredictable situation. Another interesting feature was natural deaths emerging as third commonest cause of death in autopsy which may be due to stress of Pandemic and also hesitancy to seek medical attention in spite of being sick due to fear of Covid. I would like to stress upon adequate safety measures must be employed to avoid accidents. There is also scope for prevention of suicidal deaths through identifying vulnerable population and effective counselling especially in this testing period of Pandemic.

**Conflict of interest:** NIL

**Source of funding:** self

**Ethical clearance:** obtained from institutional ethical committee

**References**

2. Accidental deaths and suicides in India, 2019; ncrb.gov.in
A Cross sectional Study on Estimation of Stature from Forearm length in the age group of 18 to 25 years in Telangana population

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Abstract

A cross sectional study on correlation of the stature from forearm length was conducted in the population of Telangana region of India. A total of 150 members participated in the study among 75 males and 75 females in the age group of 18 to 25 years. The aim of the study is to estimate stature from a fragmentary body part forearm in the population of Telangana and to derive a separate regression equation for both right and left hand and for both male and female individuals. Measurement of the body part forearm length has taken as distance from the tip of the olecranon to proximal wrist crease in semi flexed posture measured by using vernier caliper.

Statistical analysis was done by descriptive statistics like mean standard deviation, correlation coefficient and linear regression. P value <0.05 was considered as statistical significant. The data was analyzed by using SPSS version 25 software. The formula for right forearm is \( y = 50.76 + 4.18 \times X \), left forearm is \( y = 50.89 + 4.19 \times X \) for total study group. For male population the formula for right forearm is \( y = 67.80 + 3.62 \times X \), left forearm is \( y = 72.84 + 11.19 \times X \). For female population the formula for right forearm is \( Y = 68.45 + 8.13 \times X \) and for left forearm is \( Y = 64.88 + 3.60 \times X \).

We found highly significant correlation between the forearm length and height of the individual. We observed a separate regression equation for right and left hand in both male and female population yield better result. We also observed left forearm equation is more accurate than right forearm. The height of the individual is about 6.1 times of forearm length. Linear regression formula developed in our study group is more appropriate for Telangana population.

Key word: Stature, Forearm length, linear regression.

Introduction

Stature means a height of a person from crown to heel length. Identity of person is basic necessity in modern world, every person identified by different biometric criteria’s like face recognition, fingerprints, Iris pattern, etc. but identification in dead body can be
relied on different identification features, like age, sex, stature, and other identification marks, among them stature is the most characteristic of identification.

Identification of the individual by height is easy when the entire body is found at crime scene, but in certain cases like aero plane crash, bomb blast, Railway injuries, earth quakes, war crimes and other mass disasters where mutilated body parts or amputated limbs of the body are found, at such situations forensic anthropology study methods like regression equations are more useful to estimate stature and identification.

In living body diurnal variation of stature is common, a difference of one and half to two cm is observed at different times of a day, it is less in the afternoon and evening due to reduced elasticity of the intervertebral discs and longitudinal vertebral muscles. After the age of 30, due to the natural process of senile degeneration can gradually decreases stature by about 0.6mm per year on an average. The stature also varies in different postures of the body it is greater by 1-3 cm on lying down position than erect posture. After death the height of the body increases by about 2cm due to complete relaxation of muscles of the body including vertebral and para vertebral muscles.

Ancient Italian artist Leonardo da Vinci’s painting of Vitruvius man describes the estimation of stature by means of different body measurements. The length of the outspread hands is equal to the height of a man. The length from chin to the top of the head is equal to one eighth of the height of a person. The normal width of the shoulders is a quarter of the height of an individual. The distance from the elbow to the tip of the hand is a quarter of the height of a man.

Several studies reveal that the regression equation yield better results for estimation of stature. Regression formula derived from one population does not always give accurate result for other population. The variations occur because of genetical, environmental and nutritional factors (Krogmen and Iscan 1986, Duyar and Pelin 2010).3

The aim and objective of the study is to estimate stature from a fragmentary body part forearm in the population of Telangana and to derive a separate regression equation for both right and left hand and for both male and female individuals. This study will be useful to forensic scientist’s anthropologists and law enforcement authorities to estimate stature in this region which in turn helpful in crime investigation.

Materials and Methods

A cross sectional study on correlation of forearm length in relation to the height of an individual was conducted in medical students of Telangana region in the age group of 18-25yrs. The maximum height usually attained in this age group, hence these age group individuals were selected for this study. Body part forearm length of both right and left hand measured separately in male and female individuals. A total of 150 individuals among 75 males and 75 females were taken as participants in this study, an informed consent was obtained from all the participants before commencement of study.

A healthy individual of normal skeletal growth without any skeletal abnormalities were selected from the study. Individuals with skeletal disorders, genetic disorders, hormonal and nutritional disorders are not considered.

Instruments used in the study are vernier caliper, measuring scale, stadio meter (height stand) and weighing machines. Height of an individual was measured by using stadio meter in standing posture. Measurement of body part forearm length taken as distance from the tip of the olecranon to proximal wrist crease in semi flexed posture was measured by using vernier caliper. The participants were asked to place their forearm of hand on the table in semi flexed with palm facing upward position and the measurements were taken.

Statistical Analysis

Statistical analysis was done by descriptive statistics like mean standard deviation, correlation coefficient and linear regression. P value <0.05 was considered as statistical significant. The data was analysed by using SPSS version 25 software. The formula of linear regression for estimation of height is y=a + bx. Y=dependant variable (height), a=constant, b=independent variable coefficient and x=independent variable i.e. length of the forearm.
A cross sectional study on estimation of stature from forearm length was conducted in the region of Telangana, among 150 students 75 were males and 75 were females, the following results were observed.

**Table 1: Mean standard deviation of age and weight for total study group.**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD)</td>
<td>19.80 ± 1.21</td>
<td>19.51 ± 1.26</td>
<td>19.66 ± 1.24</td>
<td>0.14</td>
</tr>
<tr>
<td>Weight (mean ± SD)</td>
<td>57.05 ± 10.49</td>
<td>50.42 ± 11.45</td>
<td>53.80 ± 11.43</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Table 2: Mean standard deviation of right and left forearm for total study group.**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right forearm (mean ± SD)</td>
<td>28.14 ± 1.41</td>
<td>25.59 ± 1.55</td>
<td>26.89 ± 1.95</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Left forearm (mean ± SD)</td>
<td>28.05 ± 1.46</td>
<td>25.51 ± 1.48</td>
<td>26.80 ± 1.94</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The mean length of right forearm in males was 28.14 and in female population were 25.59 and for total population 26.89 whereas the length of left forearm in males was 28.05, female population 25.51 and for total population 26.80. P value less than 0.001 considered as good.

**Table 3: Linear regression formula for total study group.**

<table>
<thead>
<tr>
<th>Independent Variables: (Length)</th>
<th>Derived Formula: ( y = a + bx )</th>
<th>R square</th>
<th>P value</th>
<th>Standard Error</th>
<th>Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right Forearm (cm)</td>
<td>( y = 50.76 + 4.18 x )</td>
<td>0.74</td>
<td>&lt;0.001</td>
<td>0.20</td>
<td>3.78 – 4.58</td>
</tr>
<tr>
<td>Left Forearm (cm)</td>
<td>( y = 50.89 + 4.19 x )</td>
<td>0.73</td>
<td>&lt;0.001</td>
<td>0.20</td>
<td>3.79 – 4.60</td>
</tr>
</tbody>
</table>

**Table 4: Linear regression formula of male individual.**

<table>
<thead>
<tr>
<th>Independent Variables: (Length)</th>
<th>Formula: ( y = a + bx )</th>
<th>R square</th>
<th>P value</th>
<th>Standard Error</th>
<th>Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right Forearm (cm)</td>
<td>( y = 67.80 + 3.62 x )</td>
<td>0.71</td>
<td>&lt;0.001</td>
<td>0.40</td>
<td>2.80 – 4.43</td>
</tr>
<tr>
<td>Left Forearm (cm)</td>
<td>( y = 72.84 + 11.19 x )</td>
<td>0.50</td>
<td>&lt;0.001</td>
<td>0.39</td>
<td>2.66 – 4.24</td>
</tr>
</tbody>
</table>
Table 5: Linear regression formula of female individual.

<table>
<thead>
<tr>
<th>Independent Variables: (Length)</th>
<th>Formula: ( y = a + bx )</th>
<th>R square</th>
<th>P value</th>
<th>Standard Error</th>
<th>Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right Forearm (cm)</td>
<td>( Y = 68.45 + 8.13X )</td>
<td>0.62</td>
<td>&lt;0.001</td>
<td>0.31</td>
<td>2.82 – 4.08</td>
</tr>
<tr>
<td>Left Forearm (cm)</td>
<td>( Y = 64.88 + 3.60X )</td>
<td>0.62</td>
<td>&lt;0.001</td>
<td>0.33</td>
<td>2.93 – 4.26</td>
</tr>
</tbody>
</table>

**Discussion**

A Cross sectional study on estimation of stature from forearm length was conducted in the region of Telangana, in the age group of 18 to 25 years in both male and female population, study results found a significant correlation between the length of forearm and height of the person. Statistical analysis results R square 0.74, standard error 0.2, p value <0.001 and confidence interval of 3.78 – 4.58 for right forearm and R square 0.73, standard error 0.2, p value <0.001 and confidence interval 3.79 to 4.60 considered as statistically significant. Studies conducted in India and other countries also showed similar results.

A separate linear regression formula for both right and left forearm revealed better results compared to the total study population. In our study we also observed that the left forearm length is showing more accurate results. The height of the individual is about 6.1 times of forearm length.

Study conducted by Bikramjeet Singh\(^4\), et al, on estimation of stature from forearm length in north Indian population in the age group of 17–25 years showed similar results they also found highly significant correlation between forearm length and height of a person.

A study on estimation of stature from combined length of forearm and hand in Jammu region of India by Shahnaz choudary\(^5\), et al, has shown highly significant results on correlation.

A study in Chennai, Tamilnadu was conducted by Sandhya\(^6\) in the study group of 300 members also found positive correlation between the height of the individuals and forearm length.

Study conducted outside India by Kannika Song-in\(^7\), et. al on Estimation of Stature from Forearm Length in Thai Children in the ages between 5 to 19 years unlike our study age group also found significant results.

In another study conducted by Saeyed Hassan\(^8\), et al, in Iran on estimation of height from Forearm Length in Natives of Kerman in the age group similar like our study also found significant correlation between the length of forearm and stature.

Study conducted in the age group of 21 to 35 years by Samreen Panjakash\(^9\) et. al, in North Karnataka also found significant correlation.

Similar significant correlation between forearm length and stature was observed in several studies conducted in India by Ashutosh B. Potdar et. al\(^10\), at Maharashtra. Manoj Balachandran et. al\(^11\), in Kerala. Biswa bhushan mohanty et al, in Eastern India.

**Conclusion**

Cross sectional study on physical anthropometric measurement of forearm arm length in relation to the height of an individual conducted in the population of Telangana found significant correlation. Linear regression formula developed in this region is more accurate to measure the length of an individual from the fragmented body part forearm. We observed a separate linear regression formula for both right and left hand is more significant, we also found left forearm length is more accurate. A ± 5 difference was observed in most of our study group. Several studies conducted in India and abroad also revealed similar significant correlation.

**Acknowledgement:** we thank to Dr Eenakshi ganguli Professor of Community Medicine for great support in statistical analysis.

**Conflict of interest:** Nil

**Ethical clearance:** Yes

**Financial support:** self
Reference


An Accidental Overspill: A Case Report of Fatal Paraquat Poisoning by Dermal Absorption

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Abstract

Introduction: Paraquat dichloride is a widely used, highly toxic herbicide which is sold as a brownish concentrated liquid of 10–30% strength. Due to easy availability and no specific antidote, numerous fatal cases of paraquat poisoning by ingestion have been reported accidentally or with a suicidal intent.

Case description: A 30 year old alcoholic man who unintentionally consumed poison in an inebriated state leading to spillage over the unhealed skin lesions over neck and chest area, who presented to hospital emergency 5 days after the exposure with complaints of breathlessness and reddish skin lesions and subsequently died over the course of treatment.

Discussion: The findings of this case highlight fatal systemic effects caused by accidental spillage over dermal lesions leading to respiratory and renal failure. This signifies the magnitude of dermal absorption and the measures to avoid a minimal exposure turning into a hazardous event.

Keywords: Paraquat, pulmonary fibrosis, skin absorption, herbicide, renal failure, respiratory insufficiency.

Introduction

Plant Protection Ltd. introduced paraquat (dichloride 1, 1-dimethyl-4, 4-bipyridylium) to the market in 1958, and it is today the third most extensively used herbicide. The chemical is mostly safe when used as directed, however misuse has resulted in a considerable number of deaths.¹ The Central Insecticide Board and Registration Committee (CIBRC) in India has approved a 24 percent formulation that is labelled as highly dangerous and sold under the name Gramoxone. It is mostly consumed in four Indian states: Punjab, Goa, Maharashtra, and Kerala.² It’s extremely dangerous to people, and it’s been linked to a number of incidents of acute poisoning. Pulmonary fibrosis owing to lipid peroxidation is a primary symptom of its poisoning, and the majority of cases occur from accidental or intentional intake, resulting in death³. However, a few reported cases show that paraquat may be absorbed through skin lesions and this dermal route can cause systemic toxicity.⁴ ⁵

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We hereby present such a case brought for autopsy with an alleged history of unknown poison intake. The poison was later discovered to be paraquat after proper history taking and as per circumstantial evidence.

**Case Presentation**

A 29-year-old man, farmer by profession and a known alcoholic presented to the hospital casualty with complain of breathlessness since four days along with multiple episodes of blood stained vomit since one day. On initial examination pulse rate was 102/min, Blood pressure 130/80 mmhg, Respiratory rate 53/min, SpO₂ on admission was 60% on room air and 85% on oxygen. Pallor and icterus were highly evident. Bilateral lungs showed crepitation. Abdomen was not distended but showed mild tenderness in epigastric region with normal bowel sounds.

Patient was wearing a foul-smelling shirt. Removal of the shirt exhibited ulcerated skin lesions over the front of neck and chest with minor lesions over the lower lip. Some areas of the body showed multiple whitish lesions with a central clearing particularly over the groin area and inner aspects of thighs. Chest X-ray showed findings compatible with ARDS and the clinical findings in hospital record was documented as acute lung injury, acute kidney injury and leucocytosis. Patient was managed in intensive care unit, he was intubated on arrival and was on Intravenous hydration and Intravenous antibiotics but he expired three days later during the course of treatment. As the case was of unnatural death, autopsy was performed.

External examination showed multiple ulcers with a leathery base and greenish mucoid deposit at the margins of ulcers, present over the chest, neck and near the right angle of mouth involving the both lips. Other areas of body showed whitish excoriated lesions. Yellowish discolouration of sclera and mucus membranes of oral cavity was seen. Internally both pleural cavities were filled with about one litre of straw coloured fluid. Both lungs were adherent to chest wall and congested. The heart was enlarged, liver was grossly congested, and both kidneys exhibited congestion with distorted cortico-medullary anatomy and stomach wall revealed congestion.

On scrutiny of these suspicious skin findings, the relatives were questioned again who stated that: the patient was a chronic alcoholic and one night attempted to drink paraquat that was used in their farm which accidentally spilled on himself in a considerable amount. Following the incident, he stayed at home for four days lying on his bed wearing the same clothes, with mild complaints of chest pain and breathlessness and when symptoms worsened he was rushed to the hospital. The whitish skin lesions were explained to be present since a long time for which he was taking herbal medications. The police later acquired the bottle of Gramoxone from the site of alleged incidence.

![Figure 1: Chest X-ray on admission: Scattered non homogeneous](image-url)
Discussion

Paraquat poisoning remains a significant worldwide cause of morbidity and mortality. Experiments have shown low percutaneous absorption of the paraquat through intact skin; this is the reason absorption of paraquat is scant with unblemished skin however instances of serious harmfulness have been seen in subjects with widespread skin damage. Therefore, for significant absorption of the paraquat, damaged skin is essential. It can also aggravate itself any previous skin lesions present on the body. Even than few cases of death and extensive systematic involvement have been reported by authors and they have suggested low percutaneous permeability of the parquet through the intact skin and a damaged skin is essential for significant paraquat absorption. Previous skin lesions can be aggravated by paraquat itself. A case has been described in 1978 involving exposure of abraded skin to parquat that eventually results in respiratory failure.

Although the exact cause of paraquat poisoning is unknown, it is usually assumed to be linked to oxidative stress and inflammation. When paraquat is absorbed via the skin, significant quantities of nicotinamide adenine dinucleotide phosphate (NADPH) are consumed, resulting in production of superoxide anions (O$_2^-$), hydroxyl free radicals (HO–), and hydrogen peroxide (H$_2$O$_2$) and other
reactive oxygen species (ROS) inducing lipid peroxidation, this restricts breathing, and the normal electron transport chain, which results in mitochondrial damage and cell death. The effector cells produce a significant number of inflammatory factors as paraquat enters the lungs, amplifying the inflammatory response through the “cytokine cascade effect”. The histopathological findings vary from extensive pulmonary fibrosis in fatal cases to congestion, oedema, and haemorrhage in non-fatal cases. Diffuse consolidation of lungs is the initial pulmonary manifestation, and this progresses into cystic lesions after several days with very high mortality.

In our case, the patient remained in contact to paraquat through the humid clothes with underlying broken skin. Oral ingestion was less as compared to the skin spillage which added to the delayed systemic toxicity. Major effects of poisoning are corrosion of the gastrointestinal tract, renal tubular necrosis, hepatic necrosis, and pulmonary fibrosis and death is likely within several hours to a couple of days with features of vomiting, diarrhoea, fluid loss, shock, coma, convulsions, cardiac, hepatic and renal failure, and typically, pulmonary oedema. If hepato-renal failure does not cause quick death, gradual lung damage may cause mortality within the next two weeks. Subacute poisoning occurs when the pulmonary involvement occurs 24–48 hours after consumption. In conclusion, it is clear that even minimal dermal exposure to paraquat can be fatal, especially when associated with high concentrations of the substance and/or significant delay in treatment initiation.

### Conclusion

Delayed contact with paraquat solutions even at low concentrations can cause systemic poisoning which may be fatal. The skin should be damaged in some way in order to allow adequate absorption of poison to be fatal. Although gastric lavage, haemodialysis and immunosuppressive agents are treatment choices but one has to note the importance of washing the contaminated skin, including hair and nails, vigorously, with soap following a dermal exposure. These deadly effects of the poison even on minimal exposure highlight the importance of use of protective clothing like PPE by the farmers. A thorough history taking is also essential along with examination by the caregivers to identify the poison and route of absorption to minimise the damage efficiently.

**Ethical clearance:** Not required.

**Source of funding:** Self.

**Conflict of Interest:** Nil.

**Abbreviations:**

ARD5: Acute Respiratory Distress syndrome.

PPE: Personal Protective Equipment.

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3. Paraquat poisoning by skin absorption: Two case reports and a literature review [Internet]. [cited 2022 Feb 23]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3829725/


Variants of the COVID Virus before the Onset of Omicron

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Abstract

COVID-19 belongs to a group of Coronavirus diseases that SARS-CoV-2 causes. The virus spreads from one person to another via the respiratory droplets from an infected individual produced when such an individual coughs, talks, or sneezes. The symptoms of the diseases range from mild to severe, and individuals at age extremities, that are, very old (from 65 years of age), are highly exposed to severe complications. The symptoms manifest from the second day, fourteen days after exposure to the virus. COVID strains keep on changing as a result of mutations in the viral genomic composition. Different variants of COVID-19 exist; these variants vary in severity, as reported by the World Health Organization. There are thousands of variants of COVID in the world; the virus mutates all the time, making the changes inconsequential. Some of the mutations make the virus more infectious, and some mutated viral strains tend to be dominant. Variants of concern include those that have the most potentially concerning changes. India is among the nations where the virus strains have been reported to dominate and spread to other nations. The virus is claiming the lives of many individuals, with every strain spreading from one country to another. The article will address the research review on types of COVID variants and COVID-19 epidemiology in the world.

Keywords: Variants, Pandemic, epidemiology

Introduction

Different strains of concern are reported across the world. These strains tend to spread from one country to another, claiming many lives. These strains mutate; those of concern are under the closest monitoring to ensure that the health of individuals is protected from the viral strains. Such variants of concern across the world include the following: India or the Delta variant. This strain was first reported in India and is also referred to as the B.1.617.2. According to, the strain has spread to other countries in the world⁵. According to the WHO, Delta variant cases are reported in the UK in about 75000 cases⁶. This strain is reported to have mutations in the gene that encodes the SAR-CoV-2 spike protein—mutations in the gene result in substitutions in T478K, P681R, and L452R.

The UK, Alpha, or the Kent variant is another strain reported in the United Kingdom. It is also referred to as the B.1.1.7, and it is reported to be
prevalent in Britain, with more than 200,000 cases reported. This strain has been shown to undergo mutations and has spread to more than 50 countries globally. According to the research conducted by the CDC in London, it was found out that this variant was 40%-90% easily transmissible than the pre-existing strains in the UK.3

South Africa or Beta variant, also called the B.1.135, was first identified in South Africa and is reported to have been spared to about 20 more countries with the United Kingdom included. The prevalence of this strain is reported to be higher among the younger population with no underlying health condition.5 The strain causes serious complications compared to other strains; it spread faster than other strains reported by the South African health department.

Brazil or the Gamma variant was first detected in Japan by the National Institute of Infectious Disease. It was identified among individuals who had traveled from Brazil, and it is reported to have been spared to about ten more countries with the UK included.5 The variant is also referred to as lineage P1. It results from genetic mutations in which amino acids in the genomic composition are substituted in the spike protein.

Epidemiology

The Gamma, the Alpha, the Beta, and the Delta variants are reported to spread globally and claim large numbers of lives in different countries. Characterization of the epidemiological characteristics of these variants is essential as it supports the public in health planning and the development of an inference system that can estimate different variations in transmission and immune escape of every strain of the virus.5 The B.1.1.7 variant is reported to have a 46.6% increase in its transmissibility, B.1.351 has a 32.4% increase in its transmissibility with a 61.3% immune escape, and P.1 has a 43.3% increase in the transmissibility and 52.5% immune escape.3 According to the model simulation, variants B.1.351 and P.1 are likely to supplant B.1.1.7 dominate and increase infections.

Geographical distribution and case count-Globally, COVID-19 cases have been reported to be over 150 million, according to the WHO and European CDC.2 With the first case reported in Wuhan City in China, the disease has spread globally, and many individuals have lost their lives to the disease, and the transmission is mainly through person-to-person. Transmissions of the disease are highest in household and congregate settings, with frequent cases reported in social or work gatherings.

The Problem Statement

The global overview- according to WHO, the numbers of new COVID-19 cases and deaths have shown a slight decrease; however, the case and the death incidence is still high since beginning of the pandemic. Weekly cases have decreased in Eastern Mediterranean and European regions, with the South East Asia region recording an increase in the number of newly affected cases. The death incidence has also risen in South East Asia and the Western Pacific regions. India has an upward trajectory in the number of new cases and deaths, with a 95% of cases and 93% deaths. India accounts for 50% of the total cases globally, and 30% of the total deaths reported globally. Currently, the delta strain in India is the major variant causing a rise in the number of individuals contacting the infection. Outside India, in the UK, the highest numbers of reported cases are sequenced as B.1.617.2 variant. According to the WHO world statistics updates, the number of cases per million people on June 29, 2021, was reported as follows: the United Kingdom 260.07, United States 37.50, India 29.83, Canada 16.83, Japan 11.83, and Germany 6.98. In India, the total number of COVID-19 cases since the first case include 30,362,848, with deaths accounting for 398,484, as updated on June 30, 2021.7

Different measures have been put in place globally to reduce the rise in the number of cases of COVID-19 infections. These measures include the use of pre and post-exposure prophylaxis measures where approaches other than vaccination are under investigation to prevent and control of COVID-19 infection. Infection control measures in healthcare settings have been put in place to prevent community transmission of the infection, personal preventive measures such as wearing of face masks in the community, the practice of social distancing, and quarantine of individuals suspected to have the virus.4
There are general measures recommended to prevent the transmission of COVID infection, including diligent hand washing using the hand sanitizer with at least 60% alcohol. Respiratory hygiene measures include covering the mouth while sneezing or coughing, avoiding touching the face, nose, eyes, and mouth as recommended by the American Academy of Ophthalmology. Cleaning and disinfection of objects and surfaces are frequently touched, especially in the public and home setting, as recommended by the United States Centre for Disease Control and Prevention. Adequate ventilation of the indoor spaces is also among the recommended measures to prevent the spread of the infection. These measures should be practiced by all individuals, including where there is a risk of community transmission of the SARS-CoV-2.

Source of funding: Not Required

Conflict of Interest: None

Ethical Clearance: Taken from the committee

References

6. Torjesen I. Covid-19: Delta variant is now UK’s most dominant strain and spreading through schools. https://www.bmj.com/content/373/bmj.n1445.full.
Medical, Legal and Social Problems Faced by Medical Officers Working in Primary Health Centers

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Abstract

Healthcare facilities are getting costly, and becoming non-accessible for the poor. The medical officers working in primary health centers are facing medical, legal and social problems by lack of resources and infrastructure. This study was conducted on 150 primary health centers during the period of six months from July -December 2021 in and around Srikakulam district of Andhra Pradesh. Medical professionals are facing problems mostly due to primary health centers are located far away from headquarters. Improving primary health care facilities in rural areas may give good results in health care system.

Key words: Primary health centers, Autopsy, Medico legal, Social.

Introduction

Every profession has several obligations and responsibilities. Medical profession is unique and different from other professions because of need of scientific knowledge, technical skills and understanding of human being for providing necessary patient care. Medical negligence is a concern, but it is obvious that no doctor wishes to harm their patient. Today, those involved in saving in other’s life themselves at risk because of increasing rates of assaults on medical professionals or litigations and some doctors have faced violence at the workplace.

Annually there is 110% increase in the number of medical negligence cases in India. In medical profession, success cannot be guaranteed in every case, as it depends on several factors which are beyond doctor’s control. Moreover, medicine is ever changing science. There can awareness about rights of the patient, vulnerability of medical profession for litigation is increasing day by day. Successful clinical
practice is a balance between medico-legal requirements and excellence in patient care. Moreover, healthcare professionals have many ethical, moral and legal obligations. Medical Negligence, Disaster in patient-physician relationship, Inadequate documentation, Lapses in data storage and Method of consent process are some of Medico-Legal aspects. A survey among doctors from different specialties showed knowledge gaps in obtaining informed consent. 

Good medical practice requires that medical graduates can demonstrate in practice knowledge and understanding of the law. Public health infrastructure has been referred to as “the nerve center of the public health system.” According to a report as of 2019 there was an estimated shortage of 600000 doctors and 2 million nurses in the country. The report also stated that in India there is 1 bed for 2239 persons, which is way lesser that WHO recommendation of 3 beds per 1000 population.

The healthcare facilities are highly skewed towards urban areas observed that while about 70% of India’s population lives in rural areas, only 20% of hospital beds are located in rural areas. Primary Health Centers are the bedrock of rural health services and play a prominent role in the production of healthcare services in rural areas of the country.

One of the biggest problems in rural India is that the public health facilities are facing difficulty in attracting and holding on the presence of quality and trained medical professionals may be due to lack of basic facilities in rural areas. Even if the manpower is there, their participation level in healthcare production is lower than the required due to deficient supply, insufficient equipment, poor monitoring, etc. as a result the major proportion of India’s population, even the poor, choose expensive healthcare services largely provided by unregulated private sector.

Every doctor under law has to serve his patient and cannot refuse treatment making the health care services more complicated. Also as defined under law, every doctor has to fulfill certain legal requirements during his service by compulsion or voluntarily. Apart from routine and usual “clinical” cases, a doctor will come across certain „Medico-Legal-problems at one time or the other during the practice of his/her profession.

The main problem in primary health centers to execute medico legal issues is not possible because they are not recognized as medico legal centers.

### Material and Methods

A cross sectional questionnaire based survey was conducted in Primary Health Centers in Srikakulam district in the state of Andhrapradesh. All the medical officers participated. The information collected included Age, Sex, Workplace, Recreation facilities, Interpersonal relationships, Medical facilities, Basic amenities, Medicolegal issues, Preventive measures taking for medical negligence were recorded in this study. Approval of institution ethics committee was obtained prior to the study.

<p>| Table 1: Perception of participants about recreational facilities at headquarter town (n = 150) |</p>
<table>
<thead>
<tr>
<th>S. No</th>
<th>Recreation</th>
<th>Satisfactory (%)</th>
<th>Unsatisfactory (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Garden, gymnasium</td>
<td>71(47.2%)</td>
<td>79 (52.8%)</td>
</tr>
<tr>
<td>2</td>
<td>Schools</td>
<td>49(32.8%)</td>
<td>101(67.2%)</td>
</tr>
<tr>
<td>3</td>
<td>Travel time from head quarter</td>
<td>&lt;2 hrs 84(55.7%)</td>
<td>&gt;2hrs 66(44.3%)</td>
</tr>
</tbody>
</table>

<p>| Table 2: Basic Amenities and medico legal facilities in Primary health centers |</p>
<table>
<thead>
<tr>
<th>S. No</th>
<th>Factors</th>
<th>Yes (%)</th>
<th>No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Provision for triage in mass disaster</td>
<td>99(65.71%)</td>
<td>51(34.28%)</td>
</tr>
<tr>
<td>2</td>
<td>Regular Biomedical waste collection</td>
<td>114(75.71%)</td>
<td>36(24.28%)</td>
</tr>
<tr>
<td>3</td>
<td>Availability of Color coding bin for biomedical waste</td>
<td>135(90%)</td>
<td>15(10%)</td>
</tr>
<tr>
<td>4</td>
<td>Specialist doctors</td>
<td>25(17%)</td>
<td>125(83%)</td>
</tr>
<tr>
<td>S. No</td>
<td>Factors</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>-------</td>
<td>---------</td>
<td>--------------</td>
<td>---------------</td>
</tr>
<tr>
<td>5</td>
<td>Blood bank distance</td>
<td>&lt;10 KM 121(81%)</td>
<td>&gt; 10 KM 29(19%)</td>
</tr>
<tr>
<td>6</td>
<td>Tertiary care centre</td>
<td>&lt;10 KM 117(78%)</td>
<td>&gt;10 KM 33(22%)</td>
</tr>
<tr>
<td>7</td>
<td>Ambulance services</td>
<td>130(87%)</td>
<td>20(13%)</td>
</tr>
<tr>
<td>8</td>
<td>Equipped Ambulance</td>
<td>105(81%)</td>
<td>25(19%)</td>
</tr>
<tr>
<td>9</td>
<td>Autopsy center</td>
<td>&gt;10 km 135(90%)</td>
<td>&lt;10 km 15(10%)</td>
</tr>
<tr>
<td>10</td>
<td>Preservation facilities for ML evidences, Skin Marking pencils</td>
<td>51(34.28%)</td>
<td>99(65.71%)</td>
</tr>
</tbody>
</table>

Table 3: Medico legal issues and availability of medico legal certificates

<table>
<thead>
<tr>
<th>S. No</th>
<th>Factors</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Domestic violence + Child abuse + Poison cases</td>
<td>90(60%)</td>
<td>60(40%)</td>
</tr>
<tr>
<td>2</td>
<td>Cooperation of patients for Consent in medico legal cases</td>
<td>116(77%)</td>
<td>34(22.85%)</td>
</tr>
<tr>
<td>3</td>
<td>Recording of Dying Declaration</td>
<td>30(20%)</td>
<td>120(80%)</td>
</tr>
<tr>
<td>4</td>
<td>Issuing of Drunkenness Certificate</td>
<td>15(10%)</td>
<td>135(90%)</td>
</tr>
<tr>
<td>5</td>
<td>Availability of Certificates for willing and unwilling Autopsy, Specialized form for PLWH, proformas for sexual offences</td>
<td>24(15.78%)</td>
<td>126(84.28%)</td>
</tr>
<tr>
<td>6</td>
<td>Availability of Card for referral center, MCCD Certificate</td>
<td>129(86%)</td>
<td>21(14%)</td>
</tr>
<tr>
<td>7</td>
<td>Maintaining of medico legal Registers</td>
<td>109(72.85%)</td>
<td>49(27.14%)</td>
</tr>
</tbody>
</table>

Table 4: Medical negligence and preventive measures

<table>
<thead>
<tr>
<th>S. No</th>
<th>Factors</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Primary health centers recognized as medico legal centers</td>
<td>0(0%)</td>
<td>150(100%)</td>
</tr>
<tr>
<td>2</td>
<td>Violent against medical negligence</td>
<td>24(15.71%)</td>
<td>126(84.28%)</td>
</tr>
<tr>
<td>3</td>
<td>Security guard at Primary health centers</td>
<td>105(70%)</td>
<td>45(30%)</td>
</tr>
<tr>
<td>4</td>
<td>Medical Indemnity Insurance</td>
<td>34(22.85%)</td>
<td>116(77.14%)</td>
</tr>
<tr>
<td>5</td>
<td>In brought dead cases: -Refer to higher center+ ECG + Death declaration + Police intimation + MLC registration</td>
<td>60(40%)</td>
<td>90(60%)</td>
</tr>
<tr>
<td>6</td>
<td>Patient satisfactory survey</td>
<td>62(41.42%)</td>
<td>88(58.57%)</td>
</tr>
</tbody>
</table>

Table 5: Measures taking to prevent medico legal problems

| S. No | Factors | |
|-------|---------||
| COPRA : (consumer protection act) | Education, Communication, Relations with patients | 64(42.85%) |
|       | Not taking Measures | 86(57.14%) |
| MTP : (Medical termination of pregnancy) | Conducting 58(38.57%) | Not Conducting 92(61.42%) |
|       | Measures taken 49(85.18%) | Measures Not taken 9(14.82%) |
| HOTA: (Human organ transplant act) | Registered 3(2%) | Not Registered 147(98%) |
Observations and Discussion

General characteristics of study participants:

In present study, 103(68.5%) participants were more than 35 years of age out of which 84(56%) of participants were male and 66 (44%) of participants were female.

Majority of participants 79(52.8%) complained about lack of recreation facilities like malls, gardens, gymnasiums at their headquarter town. Majority of participants 101(67.2%) felt that schools were of not satisfactory standards at their head quarter town.

Most of the participants 100(67%) felt that they had satisfactory social interaction in their neighborhood. Majority of participants 120(80%) felt that they have satisfactory relationship with Lower cadre employees like ANM, MSW, UDC etc. whereas 60(40%) participants complained of unsatisfactory relations with local politicians. 92(61.4%) participants complained of political pressure while discharging duties. This pressure was in the form of command for priority in treatment, prompt and fast treatment, pressurizing for giving fitness certificates, age certificates etc. 67(44.3%) of participants had to travel more than 2 hours per day to reach their head quarters.

Regarding storage of medical record, almost (n=135)90% doctors believed that the records of medico-legal cases must be maintained till the judgment of the case. But these results vary slightly from the study on medico-legal awareness amongst health professionals in Sudan where 94.5% agreed that hospitals must maintain records until the case is judged.5 legally, physician written records carry more weight than patient’s recollections. According to Medical Council of India medical records should maintain 3 years from commencement of treatment.6 Record Storage facility is absent in (n=81)54% of primary health centers where 63% of medico legal records are unsafe among stored. This may be due to fewer funds provided by the government for the healthcare system to maintain.7

In our study n=114 (75.71%) of primary health centers maintain biomedical waste collection and 90% among them are having color coding bins for bio medical waste. This study contrast with Divya Rao et.al8 where 50% of doctors only have enough knowledge for biomedical waste management. The waste generation rate ranges between 0.5 and 2.0 kg bed-1 day-1. At many places, authorities are failing to install appropriate systems for a variety of reasons, such as non-availability of appropriate technologies, inadequate financial resources and absence of professional training on waste management.9

Specialists are not available in (n=125)% 83% of primary health centers. Now irrespective of the ability to pay people in India increasingly seek private healthcare even for minor illness like cold fever diarrhea due to non availability of doctors.10,3

Only (n=15)10% of primary health centers located very near to blood banks and tertiary care centers. (n=130)87% primary health centers have ambulance services where 81% are well equipped for Basic Life Support and Advanced Life Support. This may be due to 108 ambulance services provided by the government. Basic Life Support ambulances have an oxygen cylinder, blood pressure apparatus and a stethoscope. In Advanced Life Support ambulances, in addition to the above equipment, there is a defibrillator monitor, electrocardiogram, syringe pump, pulse ox meter, resuscitation kit, suction machine, and nebulizer.11

Among medico legal cases 60 %( n=90) cases are like domestic violence, child abuse and poison cases, and (n=116)77.14% of doctors believed that patients are very much cooperative for giving consent.

Majority (n=120)80% of medical officers are not recording dying declarations. That means 80% of medical officers was not aware of this concept. A dying declaration forms a basis for conviction without any corroborative evidence if it is reliable and truthful. A dying declaration recorded by a competent magistrate has a significant reliability or acceptability than oral evidence or a dying declaration recorded by the investigating officer. This study contrasts with padmakumar.K et. al. which shows 32.50 per cent graduates were not aware of this concept.12

Drunkenness certificates are not being issued in (n=135)90% of primary health centers may be due to ethanol testing becomes a medico legal issue, special documentation and specimen handling are required.
and the clinical laboratory often does not have firsthand knowledge that the ethanol test result will become a medico legal issue.

Very few (n=15) 10% of primary health centers are located less than ten kilometers distance to the autopsy centers. In (n=114) 75.71% of primary health centers there is no facilities to preserve medico legal evidences and skin marking pencils. Government has to put efforts to increase facilities in primary health centers.

In (n=126) 84.28% of primary health centers there are no medico legal certificates like consent for autopsy, forms for PLWH and proformas for sexual offence cases. But in (n=129) 85.71% of primary health centers there is card for referral center MCCD certificates and medico legal registers are maintained by (n=109) 72.85% of primary health centers.

Violence against medical negligence is very less (n=24) 15.71%. This may be because (n=105) 70% of primary health centers there is good security guards. This study contrast with Richa singh et. al. where 75% of doctors face violence at workplace.

In (n=116) 77.14% of medical officers working in primary health centers they do not know about medical indemnity insurance.

As the supreme court the services provided by the medical profession under consumer protection act (COPRA) the doctors should follow preventive measures but only (n=64) 42.85% doctors following and under MTP act 1971 trained doctors can conduct MTP in primary health centers 14 but only (n=58) 38.57% doctors are conducting MTPs and most of them are following (85.18%) measures under MTP act. Most of the doctors n=90 (60%) are not following measures for brought dead cases only 40% doctors following measures like taking ECG, informing police. and most of the medical officers in primary health centers s(58.57%) patient satisfactory survey not done.

Under the human organ transplantation act 1994 the registration of hospitals must for the removal of organs.14 but only n=3 (2%) of primary health centers registered and n=147 (98%) are not registered for this.

All primary centers 100% (n=150) primary health centers are not recognized as medico legal centers.

Conclusions

1. Majority of Primary health centers are located far away from Head quarters.
2. Most of the doctors working in Primary health centers are facing problems with lack of recreation facilities and high standard schools.
3. Majority of the doctors in Primary health centers have unsatisfactory relations with local politicians.
4. Most of the Primary health centers are maintaining Medico legal registers but lack proper record storage facility.
5. Satisfactory number of Primary health centers are following proper waste disposal under the guidelines of Biomedical waste management
6. All most all Primary health centers have ambulance services but very few have Specialists doctors.
7. As no Primary health center is recognized as a Medico legal center there is no facilities to preserve medico legal Evidences and lack Medico legal certificates & proformas and not issuing drunkenness certificates.
8. Most of the doctors are unaware of medical indemnity insurance and the concept of recording Dying declaration.
9. Most of the Primary health centers are located very far to autopsy centre and blood bank facilities.
10. Hardly 2% of Primary health centers are registered under Human Organ Transplant Act [HOTA].

Suggestions and Recommendations

1. The Government should make some principals to decrease the pressure on medical officers by politicians
2. Training program need to focus on empowering the health care professionals on biomedical waste management with broad scope and practical knowledge in all aspects.
3. Health facilities should be developed in rural sector by establishing labs blood banks and appointing specialist doctors for better quality of medical professional to serve masses.
4. A coordinate approach between national rural health mission and state medical services may give good results.15

5. Urgent need to educate the medical professionals on medical indemnity insurance by conducting seminars.

6. Government should recognize primary health centers as medico legal centers to prevent medico legal problems.

7. Organ donation awareness programs should be conducted to register primary health centers under HOTAs.

Acknowledgement: I am thankful to Dr. Kulakarni, Professor Department of social and preventive medicine, NRIIMS, for giving valuable suggestions during the study period.

Conflict of interest: None

Source of Funding: Self

Ethical Clearance: Taken from Ethical committee, NRIIMS.

References


4. Naseel N. Gurupur et. al. Challenges faced in handling the medico-legal cases in a selected teaching hospital


Toxicological and Pharmacokinetic Analysis by Insilico Bioinformatics Tools of an Ancient Medicine Compound Cantharidin Derived from Spanish fly

K. Jyothi Prasad¹, K.R. Subash²

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Abstract

Background: Cantharidin, a vesicant released by spanish fly belongs to species beetles of zoological order Coleoptera. It has a long history in both folk and traditional medicine use for topical cantharidin to treat warts and molluscum and as an aphrodisiac. They are also known to cause poisoning by the presence of toxic principle.

Aims and Objectives: The aims of the study were to study possible drug likeness, pharmacokinetic and toxicological profiling of known toxicological active principle cantharidin by insilico 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 structures are then screened for molecular properties with Simplified Molecular Input Line Entry System, followed by absorption, distribution, metabolism, elimination, and toxicity through SWISS ADME software web based tool. The reports are analyzed and predicted for drug likeness, pharmacokinetic and toxicity characters of cantharidin.

Results: The compounds screened cantharidin for drug likeness had a Log P score of >4.15, Bioavailability Score of 0.55, +ve blood brain barrier +ve Intestinal penetration and -ve to Cytochrome P450 enzyme induction and inhibition. The toxic hazard classification by Cramer resulted as HIGH –CLASS III with Skin Sensitization score of 0.861

Conclusion: The insilico analysis predicts cantharidin belongs to class III Toxic hazard classification but potential as drug like candidate for topical administration due to its high skin sensitivity and irritation ability as a vesicant in treatment of warts and molluscum.

Keywords: cantharidin, Toxicology, Spanish fly, bioinformatics

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Introduction

Cantharidin, commonly known as “Spanish fly,” in Asian continents, cantharidin topically was used historically for furuncles and piles, ulcers, venomous worms, and tuberculous scrofuloderma.\(^1,2\) and for abdominal masses\(^3\) and rabies, as well as an abortifacient\(^4\) it was used oral preparation.

It has been used in folklore as a sexual stimulant. Cantharidin poisoning is reported by cutaneous exposure, unintentional inoculation, and inadvertent ingestion of the beetle itself.

Blister beetles are plant-eating insects that exude a blistering agent. They can be found in the eastern United States, southern Europe, Africa, and Asia. When the beetle senses danger, it exudes cantharidin by filling its breathing tubes with air, closing its breathing pores, and building up body fluid pressure until fluid is pushed out through one or more leg joints\(^5\).

Cantharidin is readily absorbed from all surfaces, including the skin and topically on application to the skin, redness and burning pain are produced which is followed by formation of vesicles.

Cantharidin on oral ingestion, there is burning sensation of mouth, throat and abdomen followed by nausea and vomiting of blood stained material, pain in abdomen, severe thirst, tenesmus and difficulty in swallowing and speech. Later, a dull pain is felt in the loins, desire to micturate, but urine is scanty and bloodstained. Priapism in males and abortion in pregnant females may occur. The patient becomes prostrated with convulsions and coma preceding death at a fatal dose of 15-30 mg of cantharidin or 1.5 g of powder with a fatal period of 24hr\(^6\).

Cantharidin in a collodion vehicle has been used by dermatologists as a treatment for molluscum contagiosum. The present study explores toxicological aspects and pharmacokinetic predictions of cantharidin using \textit{insilico} bioinformatics tools.

Aims and Objectives

The aims of the study were to study possible drug likeness, pharmacokinetic and toxicological profiling of known active principle cantharidin by \textit{insilico} analysis and prediction tools.

Materials and Methods

Hardware and Software The selected compounds molecular properties of chemical structure from Spanish fly active principle cantharidin are carried out in Hewlett Packard PC 32-b0390 in 2021 Model installed with windows 11 java enabled with updated plugins.

Data Set The chemical structures of cantharidin from Spanish fly with two-dimensional (2D) pictures were collected from accredited indexed published journals and other sources such as PubChem, Chembank, 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\(^7\).

Virtual Screening

Drug likeness, Pharmacokinetic and Toxicological Prediction

The 3D structures developed are explored virtually using online prediction software\(^8\). Results from \textit{insilico} analysis of compounds on drug likeness, pharmacokinetics 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 January 2022 to March 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 \textit{insilico} bioinformatics tools.

Results and Discussion

The present study is designed to study and analyze Toxicological properties of cantharidin followed by a prediction of pharmacokinetic parameters based on results obtained by bioinformatics experimental models. In Table 1 the 2D chemical structure of
cantharidin were retrieved from PubChem online compound database. The 2D structures converted to 3D structure by ChemSketch software. The 3D structures are processed with MOLSOFT L.L.C Software, and the molecular properties are predicted (Table 1).

**Table 1: Spanish fly compounds 2D, 3D structure & Physiochemical properties**

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Characters 2D Structure</th>
<th>Chemical Formula</th>
<th>Molecular weight</th>
<th>Num. Hbond acceptors</th>
<th>Num. Hbond donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cantharidin</td>
<td>C10 H12 O4</td>
<td>196.2 g/mole</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>3D Structure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Chemical Formula</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Molecular weight</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Num. Hbond acceptors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Num. Hbond donors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

H-bond-Hydrogen bond
The molecular weight was 196.2 g/mole with high Hydrogen bond acceptor site of 4 bonds and zero donor bonds. This combined with high gastrointestinal absorption and Log Kp value of -7.09 cm/s and bioavailability score 0.55 is predicted as drug like candidate with zero violations as per Lipinski’s rule\textsuperscript{10}. Lipophilicity plays a significant role in drug discovery and compound design. The lipophilicity of an organic compound can be described by a partition coefficient, log P. (Table 2)

Table 2: ADME predicted profile of Spanish fly active compounds

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Pharmacokinetic Parameters</th>
<th>cantharidin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>G.I Absorption</td>
<td>High</td>
</tr>
<tr>
<td>2.</td>
<td>BBB</td>
<td>Yes</td>
</tr>
<tr>
<td>3.</td>
<td>P-gp Substrate</td>
<td>No</td>
</tr>
<tr>
<td>4.</td>
<td>CYP450 3A4 Inhibitor</td>
<td>No</td>
</tr>
<tr>
<td>5.</td>
<td>Log Kp</td>
<td>-7.09 cm/s</td>
</tr>
<tr>
<td>6.</td>
<td>Druglikeness</td>
<td>Yes; 0 violation: MLOGP&gt;4.15</td>
</tr>
<tr>
<td>7.</td>
<td>Bioavailability Score</td>
<td>0.55</td>
</tr>
</tbody>
</table>

MLOGP -Moriguchi octanol-water partition coefficient, log kp - Human skin permeability coefficients

Lethal Dose value is the amount of a solid or liquid material that it takes to kill 50% of test animals in one dose. It is also called the median lethal dose. The Toxicological prediction over lethal dose 50 values on various routes in mouse model revealed LD 50 value of 0.37 mg/kg/d in subcutaneous route with low reliability index and other routes predicted with borderline prediction reliability index as shown in Table 3A.

Table 3A: Toxicology prediction profile of Spanish fly active compounds

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Species</th>
<th>LD50(mg/Kg/d)</th>
<th>Reliability Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Mouse Oral</td>
<td>770</td>
<td>0.4</td>
</tr>
<tr>
<td>2.</td>
<td>Mouse Intraperitoneal</td>
<td>4.42</td>
<td>0.46</td>
</tr>
<tr>
<td>3.</td>
<td>Mouse Intravenous</td>
<td>36.38</td>
<td>0.48</td>
</tr>
<tr>
<td>4.</td>
<td>Mouse Subcutaneous</td>
<td>0.37</td>
<td>0.28</td>
</tr>
</tbody>
</table>

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

The Cramer classification scheme is an guideline to predict and estimate the Threshold of Toxicological Concern (TTC) for a chemical substance based on its chemical structure. Cantharidin chemical structure is predicted with class III representing the most severe toxic hazard. The Table 3B results predict cantharidin with positive prediction to cause skin sensitization with a value of 0.861 and allergic contact dermatitis positive prediction. The predictions results revealed positive potential towards carcinogenicity and negative teratogenic potential.

Table 3B: Toxicology prediction profile of Spanish fly active compounds

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Toxicology Profile</th>
<th>Cantharidin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Toxic hazard classification by Cramer</td>
<td>HIGH -CLASS III</td>
</tr>
<tr>
<td>2.</td>
<td>Skin Sensitization</td>
<td>0.861-POS-IN</td>
</tr>
<tr>
<td>3.</td>
<td>Allergic contact dermatitis(human &amp; guinea Pig)</td>
<td>POS-IN</td>
</tr>
<tr>
<td>4.</td>
<td>Respiratory Sensitization</td>
<td>POS-OUT</td>
</tr>
</tbody>
</table>
Cantharidin in a collodion vehicle has been used by dermatologists as a treatment for molluscum contagiosum and warts since the 1950s\textsuperscript{11,12}. High skin sensitization and allergic dermatitis may suggest cantharidin possibly inhibits protein phosphatase activity resulting in endothelial permeability by elevated albumin flux and dysfunction of barrier\textsuperscript{6}. The clinical effects can mostly be attributed to the irritative effects on the exposed organ systems. The secretions cause an urticarial dermatitis that is manifested several hours later by burns, blisters, or vesiculobullae. Symptoms may be immediate or delayed over several hours.

In addition to the local effects, predicted results as class III -Toxic hazard classification by Cramer (Table-3B) cantharidin can cause systemic toxicity with diaphoresis, tachycardia, hematuria, and oliguria from an extensive dermal exposure. When ingested, severe GI disturbances and hematuria can occur. Initially patient complaints may include burning of the oropharynx, dysphagia, abdominal cramping, vomiting, and hematemesis followed by lower GI tract symptoms of hematochezia and tenesmus. The aphrodisiac properties are related to cantharidin’s ability to cause vascular engorgement and inflammation of the genitourinary tract leading to priapism and pelvic organ engorgement\textsuperscript{13}.

### Conclusion

Cantharidin historically as ancient medicine used by asian continents as vesicant and used by dermatologists as a treatment for molluscum contagiosum and warts. Studies over cantharidin have exhibited vasoconstrictor activity and positive inotrope effect in guinea pig and human cardiac tissue in vitro\textsuperscript{14,15}. Although the current study predicts cantharidin is too toxic belonging to class III toxic hazardous substance to administer systemically, it is possible to develop drug like candidate for its ionotropic effects on heart and topical application with safer derivatives as the druglikeness score is within applicability domain.

**Conflict of Interest:** None declared.

**Ethical clearance:** No Humans or animals involved in study.

**References**


Pattern of Suicides during COVID-19 Pandemic among Autopsies Conducted at Tirupati, Andhra Pradesh: A One-Year Retrospective Study

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Abstract

Background: COVID-19 pandemic is a global challenge. It’s just not limited to physical impact but also has serious mental, social and economic impacts all over the world. Self-harm and suicides are its extreme effects.

Aim: To study and analyze the patterns of suicide during the COVID-19 pandemic.

Materials and Methods: A retrospective autopsy-based analysis of suicidal deaths occurred during March 2020 and February 2021 was conducted in the department of Forensic medicine, Sri Venkateshwara Medical College, Tirupati. A total of 897 autopsies were conducted, out of which 248 were suicides.

Results: 248 cases of suicide were studied among them 182 were male and 66 were female. Majority of the deaths were due to hanging (94, 37.90%) followed by poisoning (61, 24.59%). The most important contributing factor for suicide was domestic conflict/violence (85, 34.27%) followed by financial loss or loss of income (81, 32.66%).

Conclusion: This study reemphasizes the need of proactive responses to psychological health especially during events of stressful conditions like lock down and pandemic. Social, economic and public health response is necessary to prevent suicidal behavior.

Keywords: Suicide, COVID 19 pandemic, Lockdown, Hanging, Domestic conflicts.

Introduction:

The coronavirus pandemic is a global challenge. It had damaging effects on health, social and economic aspect of life all over the world.

The increasing number of infections and uncertainty induces substantial fear and concerns leading to stress and the anxiety, which was superimposed by lockdown restrictions, financial breakdown, lack of physical contact with other family members and friends.¹ There is a pervasive awareness of uncertainty over the future and an understanding that the pandemic is far from over. The psychological sequelae of pandemic will probably persist for

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months and years to come. These psychological sequelae often spread their ugly wings in all aspects with suicides being the final outcome.

Many people are migrating to villages because of absence of work and wages in cities.

Suicides and self-harm are a serious public health problem; however, it is preventable with timely, evidence based and often low-cost interventions. Multiple cases of COVID-19 related suicides in the USA, UK, Italy, Germany come up Bangladesh, India and other countries have been reported in mass media and psychiatric literature.

Globally, 7,03,000 people die by suicide every year and more than one in every 100 deaths (1.3%) in 2019 were the result of suicide\(^2\). In the Indian context, NCRB data suggest that 1,53,052 suicides were reported in 2020 as compared to 1,39,123 suicides in 2019. The rate of suicides have increased by 8.7% during 2020 over 2019\(^3\).

The objective of this autopsy-based study is to provide an overview of prevalence and analyze the pattern of suicide during COVID-19 pandemic in order to help devise strategies to support the vulnerable groups. It will also help in taking concrete measures to prevent such unnatural and untimely deaths in the future.

**Materials and Methods**

This is a retrospective postmortem-based study conducted in the department of Forensic medicine and Toxicology, Sri Venkateswara Medical College, Tirupati. Data in relation to the deaths occurred during March 2020 and February 2021 was collected from inquest reports, hospital case sheets, autopsy reports and toxicology reports. Detailed history and information regarding the deaths was also gathered from deceased’s close relatives’ friends and police.

A total of 897 postmortem examinations were conducted during this period, out of which 248 deaths were suicidal and included in this study.

To ensure consistency and uniformity, data was collected in a predesigned standard proforma. It was tabulated, analyzed meticulously.

**Results**

A total of 248 cases were studied and among them 182 (73.38%) were male and 66 (26.61%) were female. Majority of the study population belonged to 21 to 30 years age group (126, 50.8%)(Table 1). Of the study population, 236 (95.16%) were known and 12 (4.83%) were unknown. Marital status revealed that 196 (79.03%) were married and 40 (16.12%) were unmarried. Marital status was not known in 12 (4.83%) of cases (Table 2). Majority of the deaths occurred at home (99, 39.9%) followed by farm/field (73, 29.43%) (Table 3). Hanging (94, 37.90%) was the most common method of suicide followed by poisoning (61 24.59%) and drowning (42, 16.93%) (Table 4). Laborers/daily wage workers (79, 31.58%) were the most affected followed by self-employed people (50, 20.16%). The most important contributing factor for suicide was domestic conflict/ violence (85,34.27%) followed by financial loss or loss of income (81, 32.66%). Probable reason was not known in 12 cases (4.83%) (Table 5).

**Table 1: Age wise distribution of suicidal cases.**

<table>
<thead>
<tr>
<th>Age group</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11-20</td>
<td>3</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>21-30</td>
<td>93</td>
<td>33</td>
<td>126</td>
</tr>
<tr>
<td>31-40</td>
<td>47</td>
<td>16</td>
<td>63</td>
</tr>
<tr>
<td>41-50</td>
<td>19</td>
<td>6</td>
<td>25</td>
</tr>
<tr>
<td>51-60</td>
<td>11</td>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td>61-70</td>
<td>8</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>&gt; 70</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>182</td>
<td>66</td>
<td>248</td>
</tr>
</tbody>
</table>

**Table 2: Distribution of cases according to Identity and Marital status**

<table>
<thead>
<tr>
<th>Identity status</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Known</td>
<td>173</td>
<td>63</td>
<td>236</td>
</tr>
<tr>
<td>Unknown</td>
<td>9</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>182</td>
<td>66</td>
<td>248</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Marital status</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Married</td>
<td>145</td>
<td>51</td>
<td>196</td>
</tr>
<tr>
<td>Unmarried</td>
<td>29</td>
<td>11</td>
<td>40</td>
</tr>
<tr>
<td>Not known</td>
<td>8</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>182</td>
<td>66</td>
<td>248</td>
</tr>
</tbody>
</table>
**Table 3: Distribution of cases according to place of suicide**

<table>
<thead>
<tr>
<th>Place of Suicide</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Home</td>
<td>63</td>
<td>36</td>
<td>99 (39.90%)</td>
</tr>
<tr>
<td>Water bodies</td>
<td>31</td>
<td>11</td>
<td>42 (16.93%)</td>
</tr>
<tr>
<td>Farm/fields</td>
<td>57</td>
<td>16</td>
<td>73 (29.43%)</td>
</tr>
<tr>
<td>Railway tracks</td>
<td>22</td>
<td>3</td>
<td>25 (10.08%)</td>
</tr>
<tr>
<td>Forest</td>
<td>9</td>
<td>0</td>
<td>9 (3.62%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>182</td>
<td>66</td>
<td>248 (100%)</td>
</tr>
</tbody>
</table>

**Table 4: Distribution of cases according to cause of death**

<table>
<thead>
<tr>
<th>Cause of Death</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hanging</td>
<td>68</td>
<td>26</td>
<td>94 (37.90%)</td>
</tr>
<tr>
<td>Poisoning</td>
<td>44</td>
<td>17</td>
<td>61 (24.59%)</td>
</tr>
<tr>
<td>Drowning</td>
<td>31</td>
<td>11</td>
<td>42 (16.93%)</td>
</tr>
<tr>
<td>Burns</td>
<td>17</td>
<td>9</td>
<td>26 (10.48%)</td>
</tr>
<tr>
<td>Railway accidents</td>
<td>22</td>
<td>3</td>
<td>25 (10.08%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>182</td>
<td>66</td>
<td>248 (100%)</td>
</tr>
</tbody>
</table>

**Table 5: Distribution of cases according to occupation and probable reason for suicide**

<table>
<thead>
<tr>
<th>Occupation</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farmer</td>
<td>26</td>
<td>9</td>
<td>35 (14.11%)</td>
</tr>
<tr>
<td>Labourer/daily wage worker</td>
<td>57</td>
<td>22</td>
<td>79 (31.58%)</td>
</tr>
<tr>
<td>Service men/ Government employee</td>
<td>23</td>
<td>6</td>
<td>29 (11.69%)</td>
</tr>
<tr>
<td>Self employed</td>
<td>39</td>
<td>11</td>
<td>50 (20.16%)</td>
</tr>
<tr>
<td>Students</td>
<td>9</td>
<td>3</td>
<td>12 (4.83%)</td>
</tr>
<tr>
<td>Housewife</td>
<td>--</td>
<td>7</td>
<td>7 (2.82%)</td>
</tr>
<tr>
<td>Unemployed</td>
<td>17</td>
<td>2</td>
<td>19 (7.66%)</td>
</tr>
<tr>
<td>Domestic help</td>
<td>3</td>
<td>2</td>
<td>5 (2.01%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>8</td>
<td>4</td>
<td>12 (4.83%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>182</td>
<td>66</td>
<td>248</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Probable reason</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Financial / Job loss</td>
<td>73</td>
<td>8</td>
<td>81 (32.66%)</td>
</tr>
<tr>
<td>Domestic conflict/Violence</td>
<td>47</td>
<td>38</td>
<td>85 (34.27%)</td>
</tr>
<tr>
<td>Poverty and Hunger</td>
<td>13</td>
<td>3</td>
<td>16 (6.45%)</td>
</tr>
<tr>
<td>Anxiety and Depression</td>
<td>40</td>
<td>14</td>
<td>54 (21.77%)</td>
</tr>
<tr>
<td>Not Known</td>
<td>9</td>
<td>3</td>
<td>12 (4.83%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>182</td>
<td>66</td>
<td>248 (100%)</td>
</tr>
</tbody>
</table>

**Discussion**

Suicide is a preventable loss that affects families, communities, and the entire country. Mostly suicides worldwide are related to depression and psychiatric diseases.

WHO did predict the rise in the number of mental health problems due to the global pandemic and has addressed it through various modes like messages and publications related to mental health awareness and prevention.\(^4\)

In the present study out of 897 postmortem examinations 248 cases were suicides. In this 73.38% were males and 26.61% were females. Similar proportion of sex wise distribution was seen in the studies of Panigrahi et. al\(^5\) and Pathare et. al\(^6\).

Most of the subjects committing suicides were in the middle age group (21 to 40 years) with 76.20%. The underlying reason may be that productive adults/working adults and people on whom family burden is the most, bore the maximum grunt and stress related to the pandemic. The stress of supporting the family, family issues, job and financial stresses compounded the already existing pandemic stress. This is in concurrence with the study of Shrivastava et. al\(^7\).
Hanging is the preferred method of suicide in 94 (37.90%) cases followed by poisoning in 61 (24.59%) cases, drowning in 42 (16.93%) cases, burns in 26 (10.48%) cases and railway accidents in 25 (10.08%) cases. The findings are similar to the studies by Mamun et. al. and Goyal et. al. This preponderance of hanging can be due to repeated lockdowns and loss of work/jobs making the victims stay indoors/at home for longer duration. Moreover, materials and space required for hanging is easily accessible to most people.

Majority of deaths were at home (99, 39.90%), followed by farms/fields (73, 29.43%), water bodies (42, 16.93%), railway track (25, 10.08%) and forest (9, 3.62%). Similar findings were noted in the study by Ashok S J. Social and emotional isolation contributes to increase in psychological disorders and suicidal behaviour.

Daily wage workers/labourers and self-employed were badly affected by the pandemic (129, 51.74%). Repeated lockdowns, restrictions over activities, lack of opportunities of livelihood lead to migration. The uncertainty, unemployment, closure of small stall on the roadside etc. lead to severe impact on daily wage workers and self-employed people. Study by Richa et. al. revealed similar results.

Domestic violence/conflicts (85, 34.27%) were the predominant factor for suicides, followed by financial/job loss (81, 32.66%), anxiety and depression (54, 21.77%) and poverty/hunger (16, 6.45%). Cause for suicide was not known in few cases (12, 4.83%). Frequent lockdowns have led to increased domestic abuse/conflicts as a greater number of women and children are trapped in their homes with the abusers. Lack of employment and closure of self-employment avenues also led to men being at homes for longer durations and venting their anger and frustration over family members. Similar results were seen in study by Shrestha R et. al.

Conclusion

Preventing suicides during COVID-19 era is a challenging and unavoidable task. There should be continuous traditional and social media campaigns, awareness programs to promote mental health and reduce distress. Our study findings reemphasize the need of proactive responses to address the mental health issues and economic issues of the pandemic.

Risk of suicide often increases with rising unemployment and related strains, interpersonal violence, substance abuse, financial loses. Effective prevention efforts will be required to comprehensively deal with the full spectrum of mental health disorders and issues. Active outreach is necessary, especially for people with history of psychiatric disorders, COVID-19 survivors, and older adults.

The efforts should be concentrated and of both short and long-term in nature to prevent these deaths. Government should consider providing financial grants for food and unemployment support. The print, television and social media has an immensely vital role to play. Let’s hope that the efforts of clinicians, researchers, policy makers, media and public in general will reduce COVID-19 related suicides.

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: It is a retrospective study and identity of the deceased is nowhere disclosed. Therefore, the approval of Institutional Ethics Committee is not required.

References


The Scenario of Abdominal Wound Victims, Treated and Autopsied at Al-Ameen Medical College Hospital and District Hospital, Vijayapur- Karnataka

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Abstract

Introduction: Even in 21st century in spite of advances in medical sciences threat to human life by abdominal wounds is a challenge to medical man. In an attempt to save the life in medical emergencies, assessment of extent and depth of intra abdominal wounds is not easy.

Materials and Methods: All the cases of abdominal wounds that are treated admitted and autopsies held on the dead bodies at Al-Ameen medical college hospital and District hospital Vijayapur during the period from January 2009 to 31st December 2009 were included in the study. Demographic details, pattern of death and types of injuries were studied.

Results: Out of 101 cases of victims of abdominal wounds, 79 cases (78.21%) were males & 22 cases (21.78%) were females. Most common age group affected was 21-40 years & the main cause of injury was due to road traffic accidents. Liver was the most common organ injured followed by combination of intestines & mesentery. Haemorrhagic shock was the main cause of death.

Conclusion: First 6 hours is the most crucial period as most fatal cases are recorded in that time, which needs prompt transport & improved treatment infrastructure for survival of abdominal injury cases.

Key words: abdominal wounds, fatal, liver, road traffic accident, shock.

Introduction

Trauma-- one of the leading preventable causes of death in developing countries is a major health and social problem. Trauma affects generally the young people and accounts for loss of more years of life, than lost due to cancer and heart diseases put together¹.

According to the world health organisation by the year 2020, trauma will become the first or second
leading cause of death over all ages\(^2\). Abdominal injury is a significant cause of morbidity and mortality; expedient diagnosis and treatment of intra abdominal injuries are essential to prevent morbidity and death\(^3\).

The objective of the study was to know: Age & sex of victims, manner of abdominal injuries, pattern & type of injury, common organ involved, associated injuries, and cause of death & duration of survival in abdominal injuries.

**Materials and Methods**

The present study was conducted at Al Ameen medical college, Hospital Vijayapur, Karnataka from January 2009 to 31\(^{st}\) December 2009. Total 101 cases were studied, which included brought dead, treated, survived and died while giving treatment. Clinical history and other relevant data were collected from hospital case sheets and summaries. Detailed description about the pattern, nature of injuries, complications, cause of death and mechanism of death was taken. All the cases other than abdominal injuries were excluded from the study.

**Statistical analysis**: The data was entered in the Microsoft excel sheet and the statistical analysis was done by using SPSS Software version 21.

**Results**

In the present study, out of 101 cases studied was in the age group of 21-40 years only 4 cases in age group >60 years & only 1 case in <10 years age group [Graph:1]. Out of 101 victims 79 (78.92%) cases were males & 22 (21.78%) cases were females [Table:1]. Accidental cases 68 in number are more than assault/homicidal 33 cases and suicidal cases [Graph:2]

Out of 101 cases studied 88(87.13%) cases were blunt injury & 13(12.87%) were penetrating type injury. Liver is the most common organ involved 31% followed by combination of intestines, mesentery (18%) and Spleen (12%).

Table 2: shows head is the common single site involvement associated with abdominal wounds in the study 20 (19.80%) cases. However, multiple site involvement was commonest. Table 3: shows commonest cause of death in abdominal wound as haemorrhagic shock in 54 (87.09%) cases, infection 7 (11.29%) cases. Only 1 case died due to neurogenic shock.

Table 4: shows 14 males and 3 females died on the spot, 5 males & 4 females died within 1 hr, 11 males died within 1-6 hr after reaching hospital. Only 1 survived for more than one week. Out of 101 cases 28 males and 11 females got cured and survived showing the fatal phase of abdominal wounds.

**Graph 1: Shows the number of abdominal wound cases in relation to Age & Sex.**
Graph 2: Shows number of cases of abdominal wounds in relation to manner.

Table 1: Depicts the number of males and females cases with abdominal injury

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>No of Cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>79</td>
<td>78.22%</td>
</tr>
<tr>
<td>Female</td>
<td>22</td>
<td>21.78%</td>
</tr>
<tr>
<td>Total</td>
<td>101</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 2: Shows other body regions associated with abdominal wounds.

<table>
<thead>
<tr>
<th>Body region associated with abdominal injury</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head</td>
<td>20</td>
<td>19.80%</td>
</tr>
<tr>
<td>Chest</td>
<td>16</td>
<td>15.84%</td>
</tr>
<tr>
<td>Limb &amp; Extremities</td>
<td>16</td>
<td>15.84%</td>
</tr>
<tr>
<td>Only Abdominal</td>
<td>25</td>
<td>24.75%</td>
</tr>
<tr>
<td>Multiple</td>
<td>24</td>
<td>23.76%</td>
</tr>
</tbody>
</table>

Table 3: Shows commonest cause of death in abdominal wound

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Cause</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Neurogenic shock</td>
<td>01</td>
<td>1.61%</td>
</tr>
<tr>
<td>2.</td>
<td>Haemorrhage</td>
<td>54</td>
<td>87.09%</td>
</tr>
<tr>
<td>3.</td>
<td>Infection</td>
<td>07</td>
<td>11.29%</td>
</tr>
</tbody>
</table>

Table 4: Shows duration of survival in abdominal wound cases in relation to sex.

<table>
<thead>
<tr>
<th>Duration of survival</th>
<th>Male number</th>
<th>Percentage</th>
<th>Female number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spot death</td>
<td>14</td>
<td>13.86%</td>
<td>3</td>
<td>2.97%</td>
</tr>
<tr>
<td>Within 1 hr</td>
<td>5</td>
<td>4.95%</td>
<td>4</td>
<td>3.96%</td>
</tr>
<tr>
<td>1-6 hrs</td>
<td>11</td>
<td>10.89%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6-12 hrs</td>
<td>3</td>
<td>2.97%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12-24 hrs</td>
<td>6</td>
<td>5.94%</td>
<td>1</td>
<td>0.99%</td>
</tr>
<tr>
<td>1-2 days</td>
<td>6</td>
<td>5.94%</td>
<td>1</td>
<td>0.99%</td>
</tr>
<tr>
<td>2-7 days</td>
<td>6</td>
<td>5.94%</td>
<td>1</td>
<td>0.99%</td>
</tr>
<tr>
<td>More than 1 week</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.99%</td>
</tr>
<tr>
<td>Survived</td>
<td>8</td>
<td>27.72%</td>
<td>11</td>
<td>10.89%</td>
</tr>
</tbody>
</table>
Discussion

In the present study, total 101 cases were studied, 39 cases survived, 16 cases were brought dead and 46 cases died while on treatment.

Out of these 101 cases, males 49(48.51%) cases and females 12(11.88%) belong to the age group of 21-40 years. This age between 21-40 years, subjects lead a more active life & are at the peak of their creativity having the tendency to take risk, thereby subjecting themselves to the dangers of accidents and injuries. The findings consistent with present study were reported by M A B Siddique et. al.4, Akash Janjhi5, M Sugatha6 & B.V.S.Ananda Rao7.

In our study out of 101 cases, 79(78.21%) cases were males and 22 (21.78%) cases were females. Indicating that males are more vulnerable than females, probably due to the fact that males are usually the earning members of the families make them more vulnerable to the accidents, industrial mishaps & also risk of violence as compared to females who indulge in household activities. These findings are almost similar to M Sugatha6, Ananda Rao BVS7, Khajuria et. al8& Numan Husaini et. al9.

In this study 68(67.32%) cases were accidental accounting for more than 2/3rd of total cases, out of 68 cases, 63(92.64%) were due to road traffic accidents & 3 cases due to fall from height & 2 cases for other reasons. Devi.T.H.Meera et. al10, John F.Ferry et. al11 & Rashid Nehal Khan12 reported similar findings.

In our study out of 101 cases studied, 88(87.12%) cases are of blunt type and 13(12.88%) cases are of penetrating type. Blunt type is almost more than 4/5th of total cases. Similar studies were done by Rashid Nehal Khan12, Bowley D & Boffard K13.

In our study, 31% cases Liver was the most common organ injured followed by intestines, mesentery in 18% cases & 12% Spleen. Many studies found liver as the most common organ involved. Probably, because of its large size extending from 4th intercostals space down to iliac crest and placed more anteriorly as compared to other solid organ & with less morbidity. This matches with other abdominal trauma studies done by Solanki14 and Naik BV15.

Out of 101 cases studied, most common single site involved with abdominal injuries was head in 20(19.80%), multiple site involvement was found in 24(23.76%) cases, 25 cases had only abdominal injury. These findings are similar to other studies by Krauss et. al.16, Shubendu K17 & Bordoni P H C et. al.18.

Out of 62 fatal cases, Haemorrhagic shock was the main cause of death in 54(87.09%) cases. Similar findings were observed by Frank Hildebrand et al19& Kunjan modi20.

Out of 62 fatal cases, 17(27.41%) cases were brought dead. 34.65% of patients died within 6 hours of injury. Almost similar findings are observed by Numan Husaini et. al.9. Probably delay in transport, non-availability of blood and treatment within golden period was cause for this.

Conclusion

In the present study, majority of victims of abdominal wounds, both in fatal & non-fatal cases were males. Maximum numbers of victims were in the age group of 21-40 years. Majority of cases were due to accidents i.e to road traffic accidents. Blunt type injury was common than penetrating type. The most common organ involved was liver followed by combination of intestines & mesentery. Deaths were due to haemorrhagic shock followed by infection.

Head injury was the most common associated wound in abdominal wound cases. First 6 hours is the most crucial period as most fatal cases recorded in that time, which needs prompt transport & improved treatment infrastructure for survival of abdominal injury cases. This indicates the need of improvement in vehicular design and usage, transport of wounded as soon as possible and upliftment of infrastructure at tertiary level hospitals.

Conflict of interest: None

Source of Support: Nil

Ethical clearance: Approved by Institutional ethical committee.

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4. M A B Siddique et al; Study on abdominal injury; an analysis of 50 cases; *TheJournalofTeachersAssociation*, Dec 2004; vol-17, Number 2.


19. Hildebrand F et al; Blunt abdominal trauma in polytraumatised patients; *EuropeanJournalofTrauma*, 2006, no;5 pp: 430-436.

Patterns of Cardiac Rupture in Blunt Cardiac Injuries and Coronary Artery Disease: A Retrospective Autopsy Study

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Abstract

Cardiac rupture is the most severe form of blunt chest injury and coronary artery disease and that leads to a devastating medical emergency, Becker and colleagues identified three types of myocardial rupture, in Govt Stanley medical college and hospital, 2017-2019 cases with the postmortem findings and hospital records suggestive of cardiac rupture, were studied retrospectively and the Information were collected and analyzed. In the retrospective study population 426 cases had blunt chest trauma in that cardiac rupture seen in 28 cases amongst Becker type I rupture seen in 26 cases and type II rupture seen in 2 cases. Amongst 792 cases of coronary artery disease with myocardial infarction 13 cases had cardiac rupture in that Beckers type I rupture noted in 5 cases and type II were noted in 8 cases. Beckers type III rupture cases were not presented in the retrospective study population. Male cases were predominantly seen in both blunt chest injuries and in coronary artery disease and most of the cases were in the age group of 31-60 years. Among blunt chest injury traffic accidents were most common cause for cardiac rupture and in coronary artery disease left anterior descending artery occlusion was the most common cause for cardiac rupture, In the present study it has been proved that a diseased heart is more susceptible to traumatic injury than a normal one. The diseased heart is more likely to rupture at the fragile myocardium sites ,The presence of fatal cardiac trauma in head on collision cases emphasizes the utility of safety belts while driving. The present study is undertaken to identify the exact pathophysiology and bio mechanism of cardiac rupture.

Key words: cardiac rupture, blunt cardiac injury, coronary artery disease, beckers classification, fragile myocardium.

Introduction:

Cardiac rupture is a devasting cardiovascular emergency that leads to immediate haemodynamic collapse and death. It may result from a variety of aetiologies, of which blunt chest injuries, penetrating chest injuries and coronary artery disease are most often reported. The review on road safety published by the World Health Organization (WHO) in 2015 providing information from 180 countries indicates that worldwide the total number of road traffic deaths has plateaued at 1.25 million per year.¹ Of which most of the blunt cardiac injuries were unnoticed in poly trauma cases and that lead to a fatal outcome and unexplained deaths in traffic accident cases.

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Blunt cardiac injury (BCI) refers to injury sustained due to blunt trauma to the heart. The cardinal manifestations of such injury ranges from clinically silent to deadly cardiac wall rupture in contrast to the penetrating cardiac injuries, they most often reported with external injuries. Blunt chest trauma may produce great vessel and cardiac injuries, including the pericardium, myocardium, coronary arteries, and cardiac conduction system. Blunt cardiac injury is the most clinically under diagnosed traumatic injury in the adult and pediatric population, this kind of injuries significantly increases morbidity in poly trauma patients and in many cases, it leads to unexpected death.

In coronary artery disease cardiac rupture is most common complication of acute myocardial infarction, which include free-wall rupture (FWR) and ventricular septal rupture (VSR). They are considered to be a major lethal complication of coronary artery disease with uncertainty.

Rupture of the left ventricular (LV) free wall during acute myocardial infarction (AMI) is nearly always fatal, and a higher frequency has been reported in hypertensive compared with normotensive patients, and during the first AMI ventricular ruptures were most frequent than compared with second or third infarcts due to dense fibroblast and collagen accumulation over the infarct site.

Becker and colleagues identified 3 types of myocardial rupture. Type 1 rupture is characterized as an abrupt, slit-like myocardial tear and corresponds to the acute phase of MI (<24 hours). In type 2 rupture, an area of myocardial erosion is evident, indicating a slowly progressive tear. Type 3 rupture has marked thinning of the myocardium and perforation in the central portion of aneurysm, which typically occurs during the late phase of MI (>7 days).

The exact incidence and pattern of cardiac rupture after blunt chest trauma and coronary artery disease is unknown as rates reported vary significantly in the literature, and there is a significant number of cases with atypical presentation hence the present study is undertaken to identify the exact pathophysiology and bio mechanism of cardiac rupture.

**Aims and Objectives**

1. To estimate most common site of cardiac rupture in relation to blunt cardiac injury and in coronary artery disease.
2. To estimate most common biomechanics involved in cardiac rupture.
3. To estimate most common etiology involved in cardiac rupture amongst blunt cardiac injury and in coronary artery disease.

**Study Design**: Retrospective Cross Sectional Study done in cases with the postmortem findings and hospital records suggestive of cardiac rupture, subjected for post-mortem examination in Govt Stanley medical college and hospital, in the year 2017-2019.

**Inclusion criteria**:

Post-mortem records of death individuals with the cause of death as cardiac rupture, over the past three years.

**Exclusion criteria**:

1. Post-mortem records of individuals with cardiac disease other than rupture.
2. Cases with penetrating cardiac injuries, and blunt cardiac injuries with pericardial and cardiac contusions, coronary vessel dissections.
3. Iatrogenic cardiac rupture due to resuscitation and cardiac catheterization were excluded from the study group.

**Material and Methodology**

The incidence and clinical characteristics of Cardiac rupture in blunt cardiac injury and coronary artery disease will be documented and classified based on autopsy reports and hospital case sheet records for a period of 3 years from 2017 to 2019 and they will be analyzed retrospectively in Department of forensic medicine, Government Stanley medical college and hospital, Chennai using a standardized proforma based on previous studies.

The Etiology, biomechanics, anatomical location and type of myocardial rupture based on Becker’s
classification will be identified and documented in the standardized study proforma based on the autopsy files and by the available clinical records.

Results and Discussion

In the retrospective study population a total of 1326 cases was died due to traffic accidents, fall from height, assault cases, in that 426 cases had a medical history and autopsy findings suggestive of blunt cardiac injury. And 1058 cases was died due to sudden cardiac arrest in that 792 cases had autopsy findings and histopathological findings suggestive of coronary artery disease.

Of 426 cases of blunt chest trauma 28 cases had cardiac rupture, amongst Becker type I rupture seen in 26 cases and type II rupture seen in 2 cases due to delayed cardiac rupture (Image 1&2). Amongst 792 cases of coronary artery disease with myocardial infarction 13 cases had cardiac rupture in that Beckers type I rupture noted in 5 cases and type II were noted in 8 cases. Beckers type III rupture were not presented in the retrospective study population (Table 1 & 2)

### Table 1: Shows number cases with cardiac rupture among study group

<table>
<thead>
<tr>
<th>Study group</th>
<th>Total number of cases</th>
<th>Cases with cardiac rupture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blunt chest trauma (traffic accidents, fall from height, assault)</td>
<td>426</td>
<td>28</td>
</tr>
<tr>
<td>Coronary artery disease with myocardial infarction</td>
<td>792</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>1218</td>
<td>41</td>
</tr>
</tbody>
</table>

### Table 2: Shows Beckers classification of cardiac rupture among study group

<table>
<thead>
<tr>
<th>Study group</th>
<th>Type I rupture</th>
<th>Type II rupture</th>
<th>Type III rupture</th>
<th>Total cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blunt chest trauma (traffic accidents, fall from height, assault)</td>
<td>26</td>
<td>2</td>
<td>-</td>
<td>28</td>
</tr>
<tr>
<td>Coronary artery disease with myocardial infarction</td>
<td>5</td>
<td>8</td>
<td>-</td>
<td>13</td>
</tr>
</tbody>
</table>

Image 1 and 2: Shows backers type I and II cardiac rupture
Table 3: Shows various type of biomechanical force involved in blunt chest injuries and coronary artery disease with myocardial infarction.

<table>
<thead>
<tr>
<th>Types of biomechanical force</th>
<th>No of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sudden acceleration/ Deceleration</td>
<td>11</td>
</tr>
<tr>
<td>Direct impact</td>
<td>4</td>
</tr>
<tr>
<td>Compressive force by sternum and dorsal vertebraes</td>
<td>6</td>
</tr>
<tr>
<td>Concussion</td>
<td>3</td>
</tr>
<tr>
<td>High ram effect</td>
<td>2</td>
</tr>
<tr>
<td>Low velocity injuries to the chest wall</td>
<td>2</td>
</tr>
<tr>
<td>Hypertension</td>
<td>13</td>
</tr>
</tbody>
</table>

Image 3: Shows occlusion of left anterior descending artery.

In the study group, Male cases were predominantly seen in both blunt chest injuries and in coronary artery disease and most of the cases were in the age group of 31-60 years. And there is a significant association were noted between middle aged male and hypertension, in the study group hypertension was demonstrated in the form of clinical records, concentric left ventricular hypertrophy and by sclerosed blood vessels.

Amongst 426 cases of blunt chest trauma 298 cases were due to road traffic accident, 97 cases were due to fall from height and 31 cases were due to assault. Of 792 cases of coronary artery disease cases atheromatous occlusion of left anterior descending artery (Image 3) were most commonly involved in 345 cases, next most commonly involved vessels were right coronary (254 cases) and left circumflex (143 cases) and , and in 50 cases there are involvement of both right and left coronary artery.

In cases of cardiac rupture due to blunt chest injuries 12 cases had rupture over right ventricle, 9 cases had rupture in left ventricle, 4 cases had rupture in interventricular septum, 2 cases at the level of arch of aorta and 1 case had rupture over annular margin of atrio ventricular valve. The most common site for cardiac rupture in blunt chest injuries were right ventricle and next most common sites were left ventricle, and interventricular septum. in blunt chest injuries no atrial ruptures were noted in the study group.

In blunt cardiac injury various pattern of thoracic injuries were identified in that rib fractures were most commonly noted in 266 cases, sternum fracture were seen in 81 cases, thoracic vertebrae fracture was seen in 62 cases and minimal soft tissue injury seen in 13 cases.

In a study conducted by Waele J.J.D. et. al.,^6 noticed that blunt cardiac injury was common after sternum fracture and the severity of the fracture was an indicator of possible myocardial damage. In the present retrospective study left side rib fractures was the most common thoracic Injury associated with cardiac rupture. 2 in 13 cases had no significant high velocity injuries like fractures and tissue lacerations. But in these cases the minimal blunt force chest injuries produces cardiac rupture in left ventricular free wall due to the pre existing cardiac disease and in sum cases delayed rupture were noted in concussion cardiac injuries due to delayed transmural necrosis of myocardium.

Furthermore, cardiac rupture may happen rarely in cardiac resuscitative procedure due to severe compressive injury to the thoracic cage and these injuries should be overlooked and excluded in the study population^7. In some studie the authors did not exclude the influence of CPR or other iatrogenic factors during the process of cardiac rupture, in our study we excluded these cases in study population.
In coronary artery disease with myocardial infarction 11 cases had rupture in left ventricle free wall and 2 cases had rupture in interventricular septum. and in The present study showed that first MI, anterior infarct, male sex, and aged >40 years remain significant risk factors for Cardiac rupture and they are consistent with previous study findings done by Figueras J, Alcalde O et. al\(^8\) High blood pressure could play an important role in the development of Cardiac rupture since it dramatically increases intracavitary pressures and shear stress force against the necrotic area during myocardial contraction, leading to a tear.

In a study conducted by Bates RJ, Beutlers.. et. al\(^9\) the incidence of myocardial hemorrhage in infarcted areas and the proportion of Becker type 1 and 2 ruptures were higher in patients undergoing cardiac catheterization and fibrinolysis than in those who did not receive reperfusion therapy, and our study findings were inconsistent with the above mentioned study.

The most common biomechanics noted in blunt chest injuries are due to traffic accidents were sudden deceleration and acceleration noted in 11 cases, compressive force by sternum and dorsal vertebrae’s in 6 cases, concussion injury based on pascals law seen in 3 cases, low velocity injuries in 2 cases and high ram effect in fall from height cases were seen in 2 cases.

In a study conducted by Qi Wang, Lingyun Yang et. al demonstrated more than one mechanism were involved in cardiac rupture. Ruptures can be sporadic or multiple and occur in atriums, ventricles, interatrial septum, interventricular septum, heart valves, blood vessels and papillary muscles.\(^{10}\) In our retrospective study also attributed multiple biomechanical factors were involved in cardiac rupture and in coronary artery disease hypertension is the most common biomechanical factor involved in cardiac rupture. (Table 3)

**Conclusion**

Cardiac rupture is the most severe form of blunt chest injury. as most of the cardiac rupture have associated with left side ribs and sternal fractures, a proper monitoring of these victims is desired in blunt thoracic trauma cases. Injuries associated with heart rupture, like rib fractures, brain injury, contusions and etc, are used to judge the magnitude level, the direction and the characteristic of the force applied on the body. In the present study it has been proved that a diseased heart is more susceptible to traumatic injury than a normal one. The diseased heart is more likely to rupture at the fragile myocardium sites, Therefore, even a minor impact may be the triggering mechanism leading to cardiac rupture, and the lesion on the body surface may be minimal. In rare conditions, cardiac rupture may caused by a combination of the two mechanism like myocardial infraction and blunt chest injury and making the clinical picture confusing and misleading. And the forensic pathologists should define whether the victim had diseased heart or not by the comprehensive anatomy and by the microscopic examinations to avoid medico legal issues in the respected cases. The presence of fatal cardiac trauma in head on collision cases emphasizes the utility of safety belts while driving and the Forensic pathologists should be aware of the characteristic of CPR-related cardiac rupture.

**Limitations**

In the present retrospective study impact of Cardiac cycle on rupture has been not madeout clearly and The complexity of cardiac structure and the variety of external forces makes it hard to explain the various mechanisms of cardiac rupture. Due to minimal autopsy based studies on biomechanics and pathophysiology. The Cardiac rupture remains a puzzle and needs to be decoded by further prospective studies.

**Ethical clearance:** Taken from medical education committee of Government Stanley medical college and hospital

**Source of funding:** Self

**Conflict of interest:** NIL

**References**


Fatal Injuries Associated with Road Traffic Accidents: 
An Autopsy Based Retrospective Observational Study on 
Brought Dead Cases in Jaipur, Rajasthan

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Abstract

Background and Objectives: The vast majority of trauma centers OPD is a result of RTA. Understanding and 
dealing with varied patterns of fatal injury in road traffic accident cases is critical to a trauma center’s effectiveness. 
Road traffic crashes claim the lives of approximately 1.3 million individuals each year¹. Pedestrians, cyclists, and 
motorcyclists made up about half of the total². The primary goal of this research is to examine the patterns of fatal 
injuries and to assess the needs and approaches at trauma centers.

Methods: The current study was conducted at the forensic medicine department of SMS Medical College and 
Attached Hospitals, Jaipur, among brought dead road traffic accident cases brought to SMS MC Trauma centre. 
This cross-sectional study was conducted to shed light on various fatal bodily injuries that cause a person to die 
so quickly. In this study, The study included cases from January 1st, 2021 to December 31st, 2021. All information 
gathered from postmortem reports, panchanamma, and record register.

Results: 369 cases were brought dead RTA cases at trauma centre which involved in this study. Male victims 
accounted for 85.90 percent of instances (n=317), while female victims accounted for 14.9 percent (n=52). The 
majority of the victims were between the ages of 21 and 40. 20.86 percent (n=77) of instances occurred between 
6:00 and 9:00 p.m., followed by 20.05 percent (n=74) of cases occurring between 3:00 and 6:00 p.m., 70.46 percent 
(n=260) of the victims were two-wheeler drivers or passengers. RTA was somewhat more common on the highway 
(52.3%) (n=192) than in the city (47.96%) (177). Coma was the most common cause of death, accounting for 57.18 
percent of all deaths (n=211). The most common postmortem findings were bone fracture 85.90 percent (n=317) 
and injury to a key organ 88.88 percent (n=328).

Conclusions: The study reveals the distribution of fatal injuries, which gives us an indication of the necessity for 
advanced trauma centre facilities. This study will assist us in gaining a better understanding of the fatality rate of 
various bodily traumas. It could be a step forward in helping us preserve a precious life by indicating the focus of 
attention during the assessment of badly traumatized RTA patients.

Key words: road traffic accident, fatal injuries, trauma centre, brought dead

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Introduction

In India, accidents kill more people than terrorism or natural disasters, yet we never talk about it. Mr. Nitin Gadkari, India’s surface transport minister. Every day, 1214 traffic accidents occur in India. Two-wheelers account for 25% of all fatalities on the road. Every day, 377 people die, the equivalent of a jumbo plane crash. Accidents are a major non-communicable illness epidemic in the twenty-first century. It makes road traffic accidents a serious public health problem that must be addressed.

Epidemiological studies have been undertaken around the world using data extracted from emergency rooms, websites, ambulance records, and police files. Medico-legal autopsies, on the other hand, have been highlighted as critical in the epidemiological assessment of RTA cases. RTA cases have been assessed using medical-legal autopsy in investigations in Manipal and Bengaluru (India), Iran, Bangui (Central African Republic), Australia, Mthatha and Transkei (South Africa), Sri Lanka, and Kazakhstan.

Medico-legal autopsies are used to determine the precise cause and method of death, as well as the time since death and the circumstances surrounding death. We can greatly minimize deaths by understanding the elements that determine the death rate due to RTA and executing it.

Age and gender are two of the most important risk factors to be considered when assessing RTAs. Of all age groups and genders, young males have been reported to be more prone to experiencing RTAs. Numbers of Cases were also influenced by the clock time. Head injury and vascular injury was the foremost reason behind running out of time to save a patient. This provides us good Information about alarming sign of serious patient who need prompt attention in trauma emergency.

The number of RTA instances fell in May and June, perhaps as a result of the second lockdown, which was imposed worldwide at the time because to the COVID-19 pandemic. Large and small businesses, schools, and colleges were all closed down immediately at the time. This was a preventive strategy that helped to reduce the number of deaths caused by RTA at the time, but it was also destructive in other ways. A detailed examination of the traffic data reveals that the number of fatalities per kilometer travelled has risen. Higher speeds due to decreased traffic volumes, a lack of rule enforcement, and a higher proportion of walkers and motorcyclists who are more vulnerable than other road users, could all are contributing factors. As a result, lockdown hasn’t shown to be highly successful in terms of deaths.

Numerous RTA-related postmortems have been conducted at SMS MC. It accounts for nearly half of the daily number of postmortem examinations. It got my attention. We intend to get epidemiological attribution of RTA causalities in this study by getting data from medico legal autopsies of brought dead RTA victims at SMS Medical College and Attached Hospital, Jaipur, Rajasthan. Our main focus is the distribution of serious and fatal injuries, such as bone fractures, injuries to key thoracic and abdominal organs, brain traumas, and death causes. We also paid close attention to details such as the location of the incident, the time, the vehicle involved, and the driver or occupant.

Materials and Methods

Our study is a retrospective observational record based study. All of the information gathered by our department. The key to obtaining needed information was an office copy of the autopsy report, panchnamma, and record register. The study was conducted over a one-year period. All of the road traffic accident victims who were brought dead at the SMSMC Jaipur trauma centre were placed on the table. Between January 1st and December 31st, 2021, 369 cases were scrutinized by Age, sex, shifting time, month of occurrence, day of occurrence, and time of occurrence were all used to separate the data. We look at the incidence location, the wounded body part, and the body’s important organs. It will enable us to investigate the causes of early death following an RTA. Exclusion criteria in this study were an unknown deceased body, a shift time of more than 6 hours, and treated referred cases.

All pertinent data was collected and recorded into a Microsoft Excel data sheet. Data was entered into SPSS using Microsoft Excel. The mean and standard deviation for categorical data were used. Data that is not categorical is expressed in percentages and proportions. The chi square test and other appropriate
statistical tests for significance were used to examine the significance of the derived results. A statistically significant P value of <.05 was used.

**Observations and Discussion**

During the study period, 3579 postmortems were conducted, with 1691 (47.24%) of those cases being related to road traffic accidents. 369 (21.82%) were brought dead at trauma emergency within 6 hours of the event. Depict in Figure no. 1.

The study included 369 cases, of which 317 (85.90 percent) were male and 52 (14.09 percent) were female, similar to Tanuj Kanchan et. al\(^7\); male of 89.8% and female of 10.2 %, Deepak Sharma et al\(^{20}\); male of 77.30% and female of 22.69%, and Arvind Kumar et. al\(^{21}\); male of 88.22% and female accounted for 11.77%. The majority of the males who died were between the ages of 21 and 40, with 220 deaths (59.62%). Deepak Sharma et. al\(^{20}\) (63.91%), Arvind Kumar et. al\(^{21}\) (54.25%), Aiman Kurshid et. al\(^{22}\) (54.5%), and N. Bayapa Reddy et. al\(^{10}\) (50%) are all in agreement with these findings (2014). The age group of 41 to 60 years old came in second, with 86 people in that category (23.30%). In this study, the male to female ratio was 6.09:1. This is in line with the findings of Aiman Kurshid et. al\(^{22}\) (6:03:1), Arvind Kumar et al\(^{21}\) (7:49:1), N. Bayapa Reddy et. al\(^{10}\) (11:5:1), and Deepak Sharma et. al\(^{20}\) (3:4:1).

Women 19 (36.53%), 16 (30.76%), and males 201 (63.40%), 70 (22.08%) were tied for first and second place in both age categories. This is shown in table number 1.

<table>
<thead>
<tr>
<th>Age group (yr)</th>
<th>Male (%)</th>
<th>Female (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-20</td>
<td>30</td>
<td>9</td>
<td>39 (10.56%)</td>
</tr>
<tr>
<td>21-40</td>
<td>201</td>
<td>19</td>
<td>220 (59.62%)</td>
</tr>
<tr>
<td>41-60</td>
<td>70</td>
<td>16</td>
<td>86 (23.30%)</td>
</tr>
<tr>
<td>&gt;60</td>
<td>16</td>
<td>8</td>
<td>24 (6.50%)</td>
</tr>
<tr>
<td>total</td>
<td>317(85.9)</td>
<td>52 (14.0)</td>
<td>369</td>
</tr>
</tbody>
</table>

The chi-square statistic is 16.7957
The p-value is .000779
The result is significant at p < .05
Mean, μ: 34.86
Median = 32
Standard Deviation, σ: 14.986144860831

Among the occupant and passenger, 212 (57.45%) cases are of two wheeler driver, followed by two wheeler occupant, which are 48 (13%). Maximum no. of two wheeler users were died, 260(70.46%) followed by pedestrian, 49 (13.27%) which is consistent with findings of Patil et.al\(^{25}\) (2008), Tanuj Kanchan et. Al\(^9\) and Gururaj et. al\(^{24}\). Number of two wheeler driver of total consistent with Patil et. al\(^{25}\) (61.2%), Deepak Sharma et. al\(^{20}\)(75.0%), but contradicted with findings of Archna et. al\(^{26}\); they observed that pedestrians were maximum number of fatalities (35.79%) followed by two wheeler (30.5%). In 44 (11.92%) cases, people walking on foot have been trampled by four wheelers, which is consistent.
with Jha et. al.\textsuperscript{22} and 22 (5.96\%) people are those who were riding in four wheelers which was contrast with findings of Patil et. al.\textsuperscript{25}. Depict in Figure no. 2.

![Figure 3: Line graphical representation of per month cases](image)

This Figure no. 3 shows the number of cases occurring month wise. There was a decline in the number of postmortems in 2 special months when India was locked down due to 2\textsuperscript{nd} wave of COVID. In these two months, the number of cases in May and June were 21(5.69\%) and 20(5.42\%) respectively.

The second thing that is worth seeing is that it is at its maximum feet in winter. These four months of winter, January in the beginning of the year in which the number of cases was 37 (10.02\%) and 36 (9.75\%), 38(10.29\%), 38(10.29\%) cases were found at the end of the year in month of October, November and December respectively. I found only one paper Aiman Khurshid et. al.\textsuperscript{22} (Karachi, Pakistan) whose graphical representation was so much similar in distribution of accident death throughout the year.

During this study, we have found variation in the number of accidents at different time of the day. The cause of more than average accidents in one particular time zone of the day must be the number of vehicles on the road, congestion and hurry to go their destinations, all together in that moment. In 52.03\% (n=192) of received dead cases, the location of the incident was the highway and in 47.96\% (n=177) of the cases, it happened in the periphery of the city. Which is in contrast with N. Bayapa Reddy et. al.\textsuperscript{10} in which 30\% cases were reported from highways and 70\% cases were reported from city roads. Maximum no. of cases occurred 6:01 PM to 9:00 PM of window amounting 20.8\% cases (77) followed by 03:01 PM to 06:00 PM midnight was 20\% of cases(74). These findings are consistent with Aiman Khurshid et. al.\textsuperscript{22} in which maximum percentage of cases (52.8\%) and N. Bayapa Reddy et. al.\textsuperscript{10} (69\%) occurred during 12:00 PM to 12:00 AM. Depict in Table no.2.

<table>
<thead>
<tr>
<th>Time Interval</th>
<th>Highway</th>
<th>In City</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>00:01 AM TO 03:00 AM</td>
<td>15</td>
<td>14</td>
<td>29(7.8)</td>
</tr>
<tr>
<td>03:01 AM TO 06:00 AM</td>
<td>13</td>
<td>4</td>
<td>17(4.6)</td>
</tr>
<tr>
<td>06:01 AM TO 09:00 AM</td>
<td>12</td>
<td>15</td>
<td>27(7.3)</td>
</tr>
<tr>
<td>09:01 AM TO 12:00 PM</td>
<td>16</td>
<td>24</td>
<td>40(10.8)</td>
</tr>
<tr>
<td>12:01 PM TO 03:00 PM</td>
<td>25</td>
<td>27</td>
<td>52(14.0)</td>
</tr>
<tr>
<td>03:01 PM TO 06:00 PM</td>
<td>31</td>
<td>43</td>
<td>74(20.0)</td>
</tr>
<tr>
<td>06:01 PM TO 09:00 PM</td>
<td>46</td>
<td>31</td>
<td>77(20.8)</td>
</tr>
<tr>
<td>09:01 PM TO 12:00 AM</td>
<td>34</td>
<td>19</td>
<td>53(14.3)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>192</td>
<td>177</td>
<td>369(100)</td>
</tr>
</tbody>
</table>

Chi- square value: 15.338341768528
Degrees of freedom: 7
P value: 0.031898821641
P value is <.05, place of accidental death and the time interval of occurrence are dependent.
We found that coma (head injury) accounted for the highest number of deaths, which was 57.45% percent of the total. This finding is consistent with Aiman Khurshid et al (nearly 75%), Arvind Kumar et al (68.73%) Gahlot Shribbagwan et al (57.5%), Vinay Kumar M. S. et. al (50%) (2017) and Murali Mohan et al this finding contradict with N. Bapaya reddy et al; in that study most common injured part was thorax (73%) followed by abdomen (49%) then on third position was of head injury (47%). after that the number of cases of hemorrhagic shock was significant, which was 40.37% of total. These findings consistent with Murad Zafar Marri et. al in that study head injury (42.20%) and hemorrhagic shock (32.60%) are leading cause of death. Very few died due to spinal shock, chemical peritonitis and traumatic asphyxia, whose percentage share was 1.08%, 0.54% and 0.54% respectively.

Table 3: Distribution based on fractured bone

<table>
<thead>
<tr>
<th>Fractured bone</th>
<th>No. of cases</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any of bone</td>
<td>317</td>
<td>85.90%</td>
</tr>
<tr>
<td>Skull</td>
<td>181</td>
<td>49.05%</td>
</tr>
<tr>
<td>Limbs</td>
<td>151</td>
<td>40.92%</td>
</tr>
<tr>
<td>Ribs</td>
<td>122</td>
<td>33.06%</td>
</tr>
<tr>
<td>Face</td>
<td>43</td>
<td>11.65%</td>
</tr>
<tr>
<td>Pelvis</td>
<td>33</td>
<td>8.94%</td>
</tr>
<tr>
<td>Spine</td>
<td>11</td>
<td>2.98%</td>
</tr>
</tbody>
</table>

In the above Table no. 3, distribution of the fracture of bone has shown. Out of 369 cases, there were 317 (85.90%) such cases in which at least one bone was found to be fractured, this was a large proportion. Skull bone fracture was found in 181 cases (49.05%) out of 369 cases. These finding consistent with as most common fractured bone is skull in Aiman Khursid et al (53%), Arvind et. al. (69.63%), Murali Mohan et al (75.07%) and Chandra Hasini B.R. (70.7%) and it contradict with N. Bapaya Reddy et. al in that study most common fracture was involve to ribs (63.3%).

Table 4: Distribution based on injury to vital organ

<table>
<thead>
<tr>
<th>Injured Vital Organ</th>
<th>No. of Cases</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any of vital organ</td>
<td>329</td>
<td>89.15%</td>
</tr>
<tr>
<td>Brain</td>
<td>244</td>
<td>66.12%</td>
</tr>
</tbody>
</table>

Injury to vital organ is found in 329 cases (89.15%) of the total cases shown above. In 244 (66.12%) cases out of 369, brain injuries have been found in the form of contusions, lacerations or hemorrhages. 189 cases were of Intra cranial hemorrhages. Most common intracranial hemorrhage was subarachnoid hemorrhage (129 cases, 34.95%) followed by sub Dural hemorrhage (123, 33.33%) in our study which is consistent with Murali Mohan et al (SAH;53.52% and SDH;19.13%) and contradict with Vinay Kumar M.S. et al (SDH;69.5%) and Chandra Hasini B.R. (SDH;92.6%, SAH;82.4%) Liver was the most common organ injured thoraco-abdominally which has shown in 102 cases (27.64%) of total. Consistent with the findings of N. Bapaya Reddy et al (32.65%), contradict lung with Chandra Hasini B.R. in which were most commonly affected (29.9%) followed by liver (25%). In our study Lung injuries found in 70 cases (18.97%). Depict in table no. 4

Conclusions

The government can set laws and enforce them, but we are the ones who drive on the road in the end. Accidents are an issue that may be avoided, and it is critical to intervene before they occur. We require a trauma centre that is well-equipped and trained, as well as a secure and quick ambulance service. RTAs have a high fatality rate. This mostly results in the loss of society’s most capable members, and it must be halted. This research will help us learn more about the fatality rates of various physical traumas. This study will assist us in gaining a better understanding of the fatality rate of various bodily traumas. It could be a step forward in helping us save a precious life. The government’s project “Good Samaritan” is a great one. There is a training programme at SMS MC Jaipur to teach the general public how to perform CPR. There is a well-equipped teaching lab that
is also useful for doctors. The road transportation department provided the entire fund for it. The key to preventing it is raising awareness. At the end, “late by accident.”

**Ethical clearance:** Taken from institutional ethics committee of SMS Medical College Jaipur

**Source of funding:** Self

**Conflict of Interest:** RTA is now established problem of society; we need to work on it to decrease the death toll.

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The Current Practice and Medico Legal Aspects of Informed Consent in Obstetrics and Gynaecology in a Teritary Care Hospital, Can We Improve?: An Interventional Study

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Abstract

The Informed Consent plays major role in both Patients and the Doctors to carry out various aspects in the surgical procedures. This paper was an interventional study it was conducted in the Department of Forensic Medicine and Toxicology, Sri Manakula Vinayaga Medical College and Hospital, Madagadipet, Puducherry to audit and to improve it was conducted in the Department of Obstetrics and Gynaecology. The deficiencies were identified and it was analysed. The results of both pre-interventional and post-interventional were recorded, which showed the significant improvement in the consent form of the major and minor procedures. This study results will give importance on documenting the Informed Consent day to day life practice.

Keywords: Informed Consent, Documenting, Analysing and Intervention

Introduction

Informed Consent suggests that an agreement, compliance or permission given voluntarily with none compulsion.¹ It may be outlined as “the voluntary and revocable agreement of competent individual to participate during a therapeutic or analysis procedure, supported an adequate understanding of its nature, purpose and implications”.² consent is currently accepted because the cornerstone of practice.³,⁴ All medical procedures, as well as examinations, diagnostic procedures and medical analysis on patients within the absence of consent represent assault (IPC 351) that he’s liable in damages.⁵ Consent plays monumental role in way of life of a Doctor. it’s necessary to simply accept that seeking consent for surgical intervention goes on the far side getting a signed and completed consent form: it’s the method of providing the knowledge that permits the patient to form a choice to endure a selected treatment.⁶ Patients should perceive the character of the wellness, edges of surgery, Risk of surgery, different treatments, sort of physiological condition, Complications of surgery and consequences of surgery. The most aim of our audit was to judge the method of consent within the
surgical procedures within the busy regional medical specialty and medical specialty unit. It absolutely was aimed to judge post interventional observe of consent for surgery.

**Materials and Methods**

This study was done at Department of Forensic Medicine and Toxicology, Sri Manakula Vinayaga Medical College and Hospital, after obtaining approval from Institutional Ethics Committee (IEC No: 98/2016). It was a hospital based Interventional study conducted in the period of 24 months from October 2016 to October 2018. The informed consent form of the major and minor cases was taken in the Department of Obstetrics and Gynaecology. It was divided in to three category of A, B & C. Already the category of A was published with the checklist of the preliminary variables prepared from the informed consent form. Now the category of B the Procedure variables of Nature of the disease, Benefits of surgery, Risk of surgery, Alternative treatments, Type of anaesthesia, Complications of surgery and consequences of surgery were analysed. Sampling method was by using systemized random sampling method. The total sample was 690 consent forms using “Epi info Software Version 7.2 2.6”, taking in to the account the improvement in practice of documenting Informed Consent form 34% based on previous study with 95% confidence interval and 90% power. 690 consent forms were taken and it is analysed in which 345 consent forms were analysed in Pre – Interventional and 345 in Post – Interventional. A check list Proforma of the informed consent form contains 18 variables which was prepared after going through the guidelines of MCI, Royal college of Obstetricians and Gynaecologist of London. The 18 variables were securitized and validated by the Department of Forensic Medicine and Toxicology, in these it was divided in to 3 categories. In which categories B (Procedural variables) 345 consent form analysed in the period of pre – intervention. After identifying the deficiencies we kept the interventional workshop for the faculty of Department of Obstetrics and Gynaecology aiming is to emphasise the importance of documenting the informed consent forms. Post – interventional of checklist of 345 consent forms were again analysed, “Student T test” was used to compare the data obtained from the Pre and Post – interventional period. This is to find the effect of documentation of the Informed Consent forms.

**Checklist with variables prepared from informed consent form**

**Category B : Procedure Variables**

1. Nature of the disease
2. Benefits of surgery
3. Risks of surgery
4. Alternative treatments
5. Type of anaesthesia
6. Complications of surgery
7. Consequences of surgery

**Results**

**Procedure variables of pre-interventional (n=345) and post-interventional (n=345)**

<table>
<thead>
<tr>
<th>List of procedure variables</th>
<th>n (%) pre-interventional</th>
<th>n (%) post-interventional</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nature of the disease</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td>Benefits of operation</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td>Risk of the operation</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td>Alternative course of treatment</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td>Type of anaesthesia</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td>Complication of surgery</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td>Post- surgical consequences</td>
<td>0%</td>
<td>100%</td>
</tr>
</tbody>
</table>
The study results showed that, on analysing the 345 informed consent forms obtained during the pre-interventional period under category B variables (Preliminary variables) almost all the variables been documented completely. Procedural variables in the pre-interventional informed consent forms used for the patients who underwent major and minor elective surgeries didn’t have the printed information about the details of the procedural variables like Nature of the disease, Benefits of surgery, Risk of surgery, Alternative treatments, Type of anaesthesia, Complications of surgery and consequences of surgery but it was asked orally by the check list prepared.

Pre-Interventional

Table 1: Patients details regarding operated procedure information Yes response (n=345)

<table>
<thead>
<tr>
<th>Serial number</th>
<th>Questions</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Type of surgery / operation informed</td>
<td>295(85.51%)</td>
</tr>
<tr>
<td>2.</td>
<td>Benefits of surgery / operation informed</td>
<td>238(68.99%)</td>
</tr>
<tr>
<td>3.</td>
<td>Type of anaesthesia informed</td>
<td>276(80.00%)</td>
</tr>
<tr>
<td>4.</td>
<td>Complication of surgery / procedure informed</td>
<td>220(63.77%)</td>
</tr>
<tr>
<td>5.</td>
<td>Relative chances of success or failure informed</td>
<td>285(82.61%)</td>
</tr>
<tr>
<td>6.</td>
<td>Informed about the risk of surgery / procedure</td>
<td>301(87.25%)</td>
</tr>
<tr>
<td>7.</td>
<td>Details of alternative courses of treatment that are available were informed</td>
<td>253(73.33%)</td>
</tr>
</tbody>
</table>

Later during the intervention workshop, post graduate was assigned the role of developing customized informed consent forms including the details of the procedural variables for the commonly done major and minor surgeries in their department, under the guidance of the faculty members. Later during post-intervention analysis of the informed consent the results were analysed.

Post – Interventional

Table 2: Patients details regarding operated procedure information Yes response (n=345)

<table>
<thead>
<tr>
<th>Serial number</th>
<th>Questions</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Type of surgery / operation informed</td>
<td>335(97.10%)</td>
</tr>
<tr>
<td>2.</td>
<td>Benefits of surgery / operation informed</td>
<td>290(84.06%)</td>
</tr>
<tr>
<td>3.</td>
<td>Type of anaesthesia informed</td>
<td>329(95.36%)</td>
</tr>
<tr>
<td>4.</td>
<td>Complication of surgery / procedure informed</td>
<td>319(92.46%)</td>
</tr>
<tr>
<td>5.</td>
<td>Relative chances of success or failure informed</td>
<td>300(86.96%)</td>
</tr>
<tr>
<td>6.</td>
<td>Informed about the risk of surgery / procedure</td>
<td>337(97.68%)</td>
</tr>
<tr>
<td>7.</td>
<td>Details of alternative courses of treatment that are available were informed</td>
<td>315(91.30%)</td>
</tr>
</tbody>
</table>

Discussion

In this study, consent forms were analysed within the pre and post intervention amount with an intervention mode, however there was a study done by O.C Osime et. al., titled “Current practices and medico-legal aspects of pre-operative consent” at University of Republic of Benin teaching hospital, Benin city, wherever they need done analysis of 133 educated and consent forms and additionally interviewed all the patients with any intervention and post intervention analysis. A study conducted by Catherine Leng and Kavita sharma, titled “associate degree audit cycle of consent kind completion: A useful gizmo to boost junior doctor coaching, out of seventy four consent forms ninety one of forms had edges of the procedure documented within the year of 2013 and once the intervention 100 percent within the year of 2014. whereas in our study total 345 consent forms in this 238(68.99%) educated regarding the Benefits of the operation, once the intervention 290 (84.06%) were educated by the doctors. The quality of consent obtained is another
aspect of informed consent. A study conducted by O.C.Osime et al, titled “Current practices and medico-legal aspects of pre-operative consent” at University of Republic of Benin teaching hospital, Benin city, wherever they need done analysis of 133 educated and consent forms, in this only 92 patients (69.2%) were told the benefits when compared our study total 345 consent forms in this 238 educated regarding the Benefits of the operation, once the intervention 290(84.06%) were educated by the doctors.

The importance of correct documentation of the consent method has been highlighted by in a very review by Bhattacharyya et. al on the medico-legal aspects of consent in orthopaedic surgery. They found that measures, together with correct documentation and filling of consent in patients notes, were related to a reduced indemnity risk.

When it involves documentation of complications of the surgery, during this study it had been found that in the pre and post interventional amount, 0% and 92.46% severally, was the proportion of documentation, however in comparison study done by Jennifer Isherwood, titled “Documenting informed consent in elective hip replacement surgery: a simple change in practice”, the proportion of documenting the procedure specific complication was eight six out of one hundred consent forms analyses.

**Conclusion**

The study have recorded and analysed the existing standards of documenting informed consent forms and the deficiencies were shared during the intervention workshop, which increased the awareness of the faculties on proper documentation, as evident from the post intervention. The study has improved the consent practices related to surgical procedures in Department of Obstetrics and Gynaecology of our institute by developing a structured informed consent form considering the national and international guidelines in both English and Tamil languages, which was later approved and implemented in our institute. Such studies have to be done in other specialties also with regular awareness programs and analysis of the consent form documentation, which in- turn benefits the patient by making them aware on the surgery details and the doctors by acting as a better defence against negligence suits.

**Ethical Clearance:** Sri Manakula Vinayagar Medical College and Hospital, after obtaining approval from Institutional Ethics Committee (IEC No: 98/2016)

**Conflict of Interest:** Nil

**Source of Funding:** Nil

**References**

5. Indian contracts act, act number 9, 1872.
Prima Facie Principle in Resolving the Ethical Dilemma of Twisted Ovarian Cyst Cases in Pregnant Women 7-8 Weeks with a History of Primary Infertility

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Abstract

Background: Resolving the ethical dilemma in a case of 7-8 weeks pregnant women with a history of primary infertility with twisted ovarian cysts.

Method: A case report.

Finding: a 25-year-old patient, presents with severe right lower abdominal pain due to a twisted cyst in the right ovary. The patient is also currently 7-8 weeks pregnant with an intra uterine pregnancy with a history of primary infertility. The ethical dilemma that arises in this case is lifting the ovary (oophorectomy) with the risk of reducing the chances of subsequent pregnancy or maintains the ovary with cysts with the risk of infection and severe pain. For this reason, an analysis of solving ethical dilemmas is carried out by using the prima facie principle. By prioritizing the principle of non-maleficence over autonomy, patients are encouraged to undergo exploratory laparotomy operations to oophorectomy (removal of the ovaries) by maintaining a 7 - 8 week pregnancy.

Conclusion: The prima facie principle guides clinicians to respect the patient’s autonomy but must not make the patient worse. By using the prima facie principle, the patient’s medical problems can be resolved while still prioritizing the values of medical ethics.

Keywords: ethical dilemma, prima facie, primary infertility, twisted ovarian cyst.

Introduction

Ovarian cysts in pregnancy at this time occur a lot, there are incidents in pregnancy with ovarian cysts as much as 0.5-1% in pregnancy. Most of these cysts are benign or malignant cysts.1 But in some cases twisted cysts can occur in pregnant women which can cause acute abdominal pain, distension, nausea, vomiting caused by rupture or twisting of
ovarian cysts. The incidence of twisted cysts 5 per 10,000 pregnancies.\textsuperscript{2} The incidence of twisted cysts in early pregnancy is 0.2%. Patients undergoing twisted ovarian cyst surgery in pregnancy at a young age are at risk of failure in their current pregnancy, and can also make a 45\% reduction factor in fertility in subsequent pregnancies. Acute abdominal pain is an indication for emergency measures for pregnant women.\textsuperscript{3} The ideal time for an operative pregnancy with an ovarian cyst> 5 cm is performed at the age of the second trimester of pregnancy, but if there are twisted cysts then an emergency surgery is performed.\textsuperscript{4}

The principle of operation must be re-emphasized to reduce the rate of postoperative adhesion and subfertility. The number of follicles that develop naturally and after ovulation induction is significantly less so it is necessary to prevent the formation of postoperative adhesions in diseased ovaries.\textsuperscript{4} An interesting case concerns a patient who experienced acute abdominal pain due to a twisted cyst. The main problem in handling these patients is saving lives because twisted cysts can cause necrotic ovarian tissue and can cause sepsis to patients, but there is another problem, the patient’s condition is now 7-8 weeks pregnant, which is the first pregnancy after waiting for 7 years (primary infertile). In general, infertile conditions in married couples greatly affect psychological, economic, social and medical aspects. This condition can trigger psychological stress such as anxiety, depression, and loss of confidence from the patient. This condition becomes medically dilemmatic in its decision making so an in-depth ethical analysis that covers these 4 important aspects is often called the application of clinical ethics. One of the applications of clinical ethics is by using basic ethical principles, including using the prima facie principle.\textsuperscript{5,6,7,8,9}

**Method**

The method of this study was case report.

**Finding**

Patient Mrs. M, 25 years old, came with complaints of severe pain in the lower right abdomen that was felt by the patient since 3 hours before entering the hospital, from the results of the patient’s history also complained of nausea and vomiting, physical examination and supporting ultrasound examination found a diagnosis of patients with intra-uterine pregnancy. and a twisted cyst in the right ovary with a cyst size of 7.48 cm x 6.25 cm x 6.16 cm with twisting (figure 1). Previous menstrual history no complaints. The patient also complained of vomiting 5 times and made stomach pain that was increasingly uncomfortable. Patients are advised to undergo exploratory laparotomy surgery up to salpingo oophorectomy (removal of the ovaries) by maintaining a pregnancy age of 7-8 weeks.
This case shows an ethical dilemma, in which the doctor is faced with two conditions, namely helping a patient who is sick, in this case abdominal pain due to twisted ovarian cysts or maintaining the current pregnancy (which the patient and her husband are waiting for after 7 years) and must also maintain the patient’s organs. In cases like this, a doctor can use ethical considerations in making decisions, and is supported by the medicolegal aspects that follow.

The condition of the patient and her husband have experienced primary infertility for 7 years since marriage. But now the patient has successfully conceived 7-8 weeks, while there are complications of pregnancy in the form of twisted ovarian cysts that cause severe abdominal pain. The ethical dilemma that arises is whether or not the ovary is removed. Both of these difficult choices each have consequences. If the ovary is removed, the patient is worried that she will experience an abortion threat to her pregnancy and can further affect the decline in fertility in the next pregnancy. However, if the ovary is not removed, the severe pain felt by the patient can threaten the life of the patient, so that the doctor still recommends the operation to remove the ovary because it is considered an emergency condition and requires immediate surgery.

Discussion

Medical ethics in general is made to increase professionalism, knowledge, understanding, appreciation, practice of basic principles of bioethics and medical ethics in his profession as a doctor. Medical ethics is formulated to maintain the nobleness of the profession, reduce ethics-legal conflict, deterrence of unethical behaviour, and maintain the relationship between doctor and patient as a relationship of trust. Ethics is the study of moral principles, while ethics is a set of principles or values related to morals as in the code of ethics. The term ethical is used to express an attitude / view that is acceptable (ethically acceptable) or not (unacceptable). Doctors must uphold the ethical values contained in the Indonesian Medical Ethics Code/ Kode etik kedokteran Indonesia (KODEKI). Article 2 of KODEKI states that a doctor must always try to carry out his profession in accordance with the highest professional standards. The article shows that professional decisions taken by doctors are aimed at attitudes, actions and behaviour. The decision must be taken by prioritizing consistent good intentions, earnestly and providing solutions with scientific integrity, moral integrity and intellectual honesty according to professional standards.

In handling patients in the clinic, in addition to medical indications that do have an effect on the initial side, but the management of patients will also be determined by the “art” based on moral values in the basic ethical principles valid in simple concrete cases. If in concrete cases as complex as in this case which leads to ethical dilemmas, then the prima facie principle is applied among the four basic ethical principles in applying its ethical handling. The prima facie principle will simply require a valid new context in the patient or family when in the process of medical treatment (a process that coincides with clinical judgment, which comes from the authority of clinical privileges that doctors have). In general it has been understood that in the medical practice there are 4 basic ethical principles namely beneficence, non-maleficence, justice and autonomy. In this case, the patient’s position falls into the category of non-maleficence because it involves the safety of the patient’s soul. In the context of non-maleficence, the principle of prima facie is when the patient (turns into or in a state of emergency) where a medical intervention is needed in order to save his life.

In this case there is a desire of the patient to maintain the ovary for fear of reducing the chances of subsequent pregnancy because the patient has experienced primary infertility. This desire is indeed the patient’s autonomy right to self-determination. But at the same time the patient experiences severe abdominal pain due to twisted ovarian cysts. Here there is a contradiction the basic ethical principles between autonomy and non-maleficence. In the process of identifying which ethical principles are most relevant in certain concrete cases, the “collision between ethical principles” is so strong that it remains difficult to be convinced which ethical principles are the most dominant.

The patient’s request to keep her ovaries of course at risk is great pain that the patient will continue to
feel, even threatening his life. Meanwhile the choice to remove an ovary with a twisted cyst is also at risk because the chances of getting pregnant for patients become smaller. The doctor’s job is to ensure the patient’s condition is better, although both choices are equally difficult to decide, there is a principle that can be used as a guideline, namely the minus mallum principle, which is to make decisions with the least risk of harm. Of course these risks are equally severe for patients and doctors, according to the minus mallum principle, the smallest risk is lifting the ovaries. 6,7,8

The best choice for solving ethical dilemmas is ethical adjustment or compromise between autonomy and non-maleficence. This principle is referred to as prima facie, namely the selection of one basic ethical code is “valid” according to the context based on data or concrete situations, so here the basic ethical principle of autonomy changes to non-maleficence.7

The team of doctors provides an explanation to patients about the medical indications, risks, and alternative therapies before surgery. The medical indication for this patient is to save the patient’s life. Surgical management with laparoscopic cystectomy is often performed to prevent rupture and malignancy as well as to maintain optimal fertility.14 The risk of the procedure must also be explained to the patient and his family. Both short-term and long-term risks must be discussed and understood by the patient and her husband. Long-term risks are usually only in theory but can occur even though the possibility is small, but if not delivered at the time of giving informed consent can be a problem later on.15 Treatment options must be fully beneficial for patients, or at least not cause harm, in decision making, win-win solution or shared decision making should be made between the team of doctors and patients and their families.

After several discussions with the patient, the patient and her husband finally agreed to remove the cyst as well as their ovaries. Management of pregnant patients with twisted ovarian cysts can be done with conservative observation by conducting regular ultrasound monitoring and operative measures can be performed on patients with consideration of complications in pregnancy namely severe acute abdominal pain. Based on basic ethical principles, doctors perform the principles of beneficience, non-malficence and autonomy. In principle, eliminating the function and organs of patients is basically something that is prohibited because it will weaken the patient, but it is permissible if to save the patient. Informed consent is needed in every medical procedures, one of its contents is about the risk if the patient undergoes an invasive medical procedures.6,7,8,9

Ovarian cyst patients with pregnancy must undergo treatment for fertility maintenance using the hormone progesterone preparation. This shows that if the patient will continue to experience decreased fertility even though he did not undergo surgery, the patient’s condition in pain requires the doctor to rescue the patient. Intraoperatively, the right ovarian cyst mass was twisted with a size of 7.48 cm x 6.25 cm x 6.16 cm, so removal of the right cyst and ovarian mass should be taken (figure 2). Postoperative diagnosis found necrotic ovaries due to twisting. After it was decided to remove the cyst mass then the cyst was sent to anatomic pathology for further examination to assess whether there were signs of malignancy from the cyst (Figure 3). After the patient is undergoing emergency surgery, the foetal heart rate is positive. Furthermore, patients are advised to exercise routine control during their current pregnancy.

Figure 2. Intraoperative images of twisted ovarian cysts
Figure 3. Specimen of a cyst that has been removed
Conclusion

In making medical decisions, it is not always about the medical aspects but also always accompanied by ethical decisions that balance the benefits and risks of medical procedures to be performed. In this case using the prima facie principle, the patient’s goodness becomes greater. The choice of removal of the ovary is not the best choice but at least a little smaller risk than if the ovary is not removed because it can be fatal for the patient.

**Ethical approval:** This is a retrospective case report without the use of any samples from human subject, so ethical approval can be waived.

**Competing interest:** The authors declare that there are no competing interests related to the study

**Funding:** This case report did not require funding

**References**

Autopsy Study of Renal Lesions in Snake Bite Cases

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Abstract

Background: Snake bite is the significant health problem in India, particularly in many rural regions. In general, the bites of snakes are due to the lower epidemiological profile and they are caused by non-poisonous snake bites, dry bites and envenomation. Venom is the saliva of snake ejected during the act of biting from the modified parotid glands. It can be neurotoxic, vasculotoxic in action.

Methods: A descriptive study was carried out in 107 patients conducted at Madurai Medical College & Hospital, Madurai from the period of April 2017 to March 2018. Out of 107 patients proportionate samples was selected based on the inclusion and exclusion criteria.

Findings: Out of the 107 patients, 49 (45%) were males and 58 (54.3%) were females. Similarly, deaths were reported to the hospital, who failed to receive ASV and with those patients who reported late to the hospital with a mortality rate, 81%. Other findings like local findings like pain and swelling 76 (43%), cellulitis, 76 (43%), gross findings and histopathological findings were recorded.

Conclusion: The rationalisation of anti-snake venom therapy can be done to prevent ASV related to kidney injury. Incidence of renal lesions in neurotoxic snake bites suggests a strict monitoring of renal parameters and timely intervention of treatment measures such as dialysis even in neurotoxic snake bite.

Keywords: Snake bite, renal lesions, neurotoxicity, Acute Kidney Injury.

Introduction

Considerable morbidity and mortality in tropical regions, is a significant public health problem caused by snake bite. Snake bite is known from ancient age, referring to ancient medical writings¹. Based on WHO reports, about 5 million people each year are affected by poisonous snake bite, which resulted 2.5 million envenomation, greater than 1 million deaths². Among the rural population predominant high risk is seen in Asia and Sub-Saharan African regions. According to India, the mortality rate due to venomous snake bite cases is estimated to be 35000 to 50000 per year and recorded to be the highest among other countries. Increase mortality in India is due to various factors like social, economic and cultural reasons³.

Snake venom is a rich bio-resource of biologically...
active components, but only one percent of these molecules have been characterised. Identification and characterisation of toxic components present in snake venom are the main step not only to understand the pathophysiological changes observed after bites, but also to improve the treatment after snake bites. Snake venom is composed of 90% of proteins, polypeptides, many other organic and inorganic substances. Most snakes have 6-12% of enzymes in their venom. No venomous snake has all components few of which that are present are ATPase, Cardiotoxin, Hylaurodinase, Neurotoxins, Ophanin, Phosphotidase etc.

Clinical manifestations mainly depend on the dose of venom injected, bite to needle time, potency and adequacy of anti-venom given to the patients. Envenomation causes certain complex effects, like insignificant lesions caused at the bite site to necrosis. Most of the life threatening effects of envenomation are disseminated intravascular coagulation, acute kidney injury, sepsis and sudden cardiac death from arrhythmia. Snake venom has isoenzyme, phospholipase PLA2 which is responsible for the manifestations of systemic functions such as local capillary damage, tissue necrosis and anticoagulation action. This kind of clinical syndrome is distinguished by hypotension, kidney injuries and pituitary failure.

The present study aims to know the epidemiological profile of snake bite cases, spectrum of histopathological changes, lesions in association with post-mortem and mortality, major causes and long term changes in kidney function that follows hemotoxic envenomation.

Materials and Methods

Descriptive study was conducted by collecting data from the medico-legal autopsies of snake bite cases conducted at the mortuary of Madurai Medical College & Hospital, Madurai from the period of April 2017 to March 2018 after getting ethical clearance from Institutional Ethics Committee. One hundred and seven subjects were considered for this study out of which 49 were male subjects and 58 were female subjects, aged between 0 to >50 years. There are certain exclusion criteria included in this study are: Bite from other reptiles or animals, obscure histories, decomposed bodies and patients with pre-existing chronic renal illness.

During post-mortem examination, the kidneys were collected and preserved using 10% formalin. After processing the tissue of 2-3 micron, sections were subjected to macroscopic and microscopic studies. The dissected tissues of kidneys were stained with haematoxylin and eosin. The slides were then subjected for study under light microscope to detect changes.

Statistical analysis

The collected data were stored using computer program. The data which is continuously exhibited is expressed as mean±SD. The mean calculated from the study is compared using unpaired t test. P value < 0.05 was considered to be statistically significant. All statistics were carried out using Graphpad Instat, Version 3.06.

Results and Discussion

Table 1: First aid prior to anti snake venom

<table>
<thead>
<tr>
<th>First aid</th>
<th>No.of cases</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Total (n)</td>
<td>Percentage (%)</td>
<td></td>
</tr>
<tr>
<td>No first aid</td>
<td>43</td>
<td>49</td>
<td>92</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>Tourniquet</td>
<td>3</td>
<td>4</td>
<td>7</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Incision</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Application of herbal medicine</td>
<td>3</td>
<td>5</td>
<td>8</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Suction of venom form the bite site</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
Table 2: Bite to ASV time

<table>
<thead>
<tr>
<th>Bite to ASV time</th>
<th>No.of cases</th>
<th>Male</th>
<th>Female</th>
<th>Total (n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1 Hour</td>
<td></td>
<td>5</td>
<td>10</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>1-6 Hours</td>
<td></td>
<td>5</td>
<td>12</td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td>6-24 Hours</td>
<td></td>
<td>30</td>
<td>20</td>
<td>50</td>
<td>46</td>
</tr>
<tr>
<td>&gt; 24 Hours</td>
<td></td>
<td>9</td>
<td>16</td>
<td>25</td>
<td>23</td>
</tr>
</tbody>
</table>

Table 3: Local findings

<table>
<thead>
<tr>
<th>Local finding</th>
<th>No.of cases</th>
<th>Male</th>
<th>Female</th>
<th>Total (n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edema</td>
<td></td>
<td>36</td>
<td>40</td>
<td>76</td>
<td>43</td>
</tr>
<tr>
<td>Blisters</td>
<td></td>
<td>9</td>
<td>3</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>Ulceration</td>
<td></td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Cellulitis</td>
<td></td>
<td>36</td>
<td>40</td>
<td>76</td>
<td>43</td>
</tr>
<tr>
<td>Gangrene</td>
<td></td>
<td>5</td>
<td>6</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>Regional lymphadenopathy</td>
<td></td>
<td>17</td>
<td>10</td>
<td>27</td>
<td>15</td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td>9</td>
<td>14</td>
<td>23</td>
<td>13</td>
</tr>
</tbody>
</table>

Table 4: Histopathological findings in kidney

<table>
<thead>
<tr>
<th>Glomeruli</th>
<th>No.of cases in Krait</th>
<th>No.of cases in Viper</th>
<th>No.of cases in Cobra</th>
<th>No.of cases in undetermined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congested capillary loops</td>
<td>8</td>
<td>13</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Thickened capillary loops</td>
<td>0</td>
<td>6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Fibrin thrombi</td>
<td>1</td>
<td>7</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Renal infarction and cortical</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Necrosis</td>
<td>8</td>
<td>4</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Tubules</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffuse acute tubular necrosis</td>
<td>10</td>
<td>12</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Focal acute tubular necrosis</td>
<td>6</td>
<td>25</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Interstitium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Congestion</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Mild</td>
<td>3</td>
<td>13</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Moderate</td>
<td>4</td>
<td>5</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Severe</td>
<td>6</td>
<td>16</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>blood vessels</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other ancillary findings(IHC/IF)</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Age group most commonly involved in the study is 50 years and above (n=39,36%). This percentile differs from other studies such as Chugh et al. where mean age of subjects was 33±10 years. In our study percentage of males affected 45% (n=49) and percentage of females affected is 54.3% (n=58). More number of working women in Madurai district can be a reason for such discordance.
Snake bite during activity (n=92, 86%) predominated the snake bite during sleep (n=15, 14%). This is in contrast with Rupinder Sharma et al. who demonstrated bite during sleep was more compared to bite during activity. The difference in numbers can be explained by the fact that neurotoxic bites especially Krait is most common during night and at indoors and it is predominant in North India. Lower limb was the commonest site of snake bite in our study followed by upper limb and abdomen. People accidentally step onto the snake during walking.

In most number of cases in our study, no first aid was given before ASV (n=92, 86%), tourniquet was inserted in 7% (n=7) of cases and herbal medicines was applied in 7% (n=8) of cases. Nonmedical treatment was not taken in a majority of cases. This is in discordance with Singh RR et al. in which 43% took non-medical treatment before ASV. Though most of our study group are uneducated, sensitisations among people even in rural areas have prevented them from taking non-medical measures.

Bite to ASV time is more than 6 hours (late SAV) in 70% (n=75) cases and less than 6 hours (early SAV) in 30% (n=32) cases. Bite to needle time is higher in our study due to the delay in reporting of patients. This is in concordance with Sharma N et al. 8. Local pain and swelling is present in 43% (n=76) cases. Rubina Naqir et al. 9 had a similar findings. This was partly due to late presentation of patients in our study leading to settlement of local symptoms. Cellulitis was also present in majority of cases in our study (n=76, 43%). Harshavardhan et al. 10 reported that 96% of cases had cellulitis.

In our study, major gross findings noted was swelling and edema (n=79, 74%) followed by congestion (n=28, 26%) on outer surface. On cut section, medullary congestion was noted in 52% cases (n=55) and Petechial haemorrhages in cortico-medullary junction noted in 23% (n=25) cases. This is in unison with Yogesh C et al. 11 who observed congestion in majority of cases. Findings of Petechial haemorrhage are also consistent with Alakesh Halden et al. 12 in which 21% cases reported Petechial haemorrhage. Pale cortex and congested medulla is seen in 55 cases (n=52%) in our study.

Among the Viper cases reported (n=49, 46%). Glomerular changes noted in 26 cases 53% (congested capillary loops in 13 cases, thickened capillary loop in 6 cases and fibrin thrombi in 7 cases). Cortical necrosis in 4 cases (4%). Tubular changes in 37 cases (76%) (acute diffuse tubular necrosis in 12 cases, acute focal tubular necrosis in 25 cases). Interstitial changes in 34 cases (69%) (mild interstitial inflammation in 13 cases, moderate interstitial inflammation is 5 cases, severe interstitial inflammation in 16 cases). Among the krait cases reported, (n=24, 22%) histopathological changes noted in kidney are as follows: Glomerular changes in 9 cases (congested capillary loops in 8 cases & fibrin thrombi in 1 case). Tubular changes in 16 cases (focal ATN in 6 cases and diffuse ATN in 10 cases) and interstitial changes in 12 cases. Among the cases of Cobra bite reported in our study (n=21 cases). Glomerular lesions noted in 10 cases (congested capillary loops in 7 cases, fibrin thrombi in 2 cases and thickened capillary loop in 1 case). Tubular lesions noted in 10 cases (acute focal necrosis in 6 cases and diffuse type in 4 cases). Interstitial changes noted in 11 cases and renal cortical necrosis noted in 3 cases.

**Conclusion**

In the present study, out of 107 cases of snake bite, age group commonly involved is 50 years and above, most affected being agricultural labourer, maximum fatality occurred at winter and during activity. Lower limb was the commonest site involved and majority of cases received anti snake venom, even though bite to ASV time is more. Among the local findings, local pain and swelling predominated. Histopathological study of kidney revealed that tubular lesions (acute focal tubular necrosis) predominant in Viperid bites, tubular (diffuse ATN) and interstitial changes (severe inflammation) in Krait bite and glomerular (congested capillary loop) and interstitial (severe inflammation) changes in Cobra bite. Prevalence of acute kidney injury in our study was 79.5% which is very high compared to previous studies. Further monitoring of renal parameters even in neurotoxic snake bite and prompt dialysis should be done. This indicates the need for further large scale investigations with detailed pathology of neurotoxic snake bite with ultra-structural and molecular studies. Advanced age and increased time interval from snake bite to anti-
venom administration were independent risk factors for snake bite induced AKI. Special investigation into the biochemical and immunologic components of Cobra and Krait venom should also be considered. Rationalisation of anti-snake venom therapy to be done to prevent ASV related kidney injury.

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: Obtained from Institutional Ethics Committee, Madurai Medical College.

References
Analysis of Mass Number of Samples and Links Evidences Using DNA Finger Printing: Concludes act of Mob Lynching

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Abstract

Mob lynching is the heinous crime in the world by which a group of people kills the criminal or suspects of crime. In 2018 forensic science team facing the challenge of such horrible crime. Forensic Science Laboratory received a mass number of exhibits and analysis of this large number of exhibits was critical, complex and time-consuming. Analysis of crime scene evidences recovered from suspects and biological samples of deceased along with their clothes were performed. Step by step analysis of these exhibits carried out by the detection of blood which minimizes the complexity of analysis and DNA profiling of these blood detected exhibits decreases the time cycle and increases the truthiness of analysis. DNA profiles obtained from a mass number of crime scene evidence and evidence recovered from suspects linked with DNA profiles of the deceased. This concludes the crime of mob lynching, uniqueness of the location and presence of a suspect at that time with the same location.

Keywords: Mob lynching, crime scene evidence, DNA fingerprinting, mass exhibits, DNA profiling, PCR, Mixed DNA.

Introduction

Mob lynching is a major issue in the world since the 18th century¹. Lynching is unlawful mob violence by a group of people to kill the individual that is a crook or suspected of a criminal offence against the community². There are various types of mob justice such as murder, burning, hanging, maiming, beating and having varied reasons like faux news, illiteracy, failure of the criminal justice system, social media and so on³. Many lynching cases recorded in the world in recent centuries and the collection and analysis of evidence from such a complex crime is the biggest challenge in front of forensic experts. Step by step analysis of many evidence and convert them into proof against such heinous crime by finding links between criminals with a crime is the major challenge of a forensic scientist. Using modern techniques in forensic science and the tremendous efforts of a forensic team make it possible to find links in such a complex crime. DNA fingerprinting is one of the powerful and sensitive techniques used to prove crime against victims⁴,⁸.
In India, DNA technology is generally used for the paternity and maternity testing, establishing identity in sexual offence cases, identification of deceased, concealment of birth cases, identification of accused in murder cases and wildlife species identification. DNA profiling of blood, hair and saliva by performing STR-based analysis is the important evidence to solve the crime. Short Tandem Repeat (STR) markers are rich in the human genome and characterized by the high level of polymorphism used in DNA technology for the identification. Blood is one of the vital evidence in most of the crime such as murder or rape. Individual’s gender and genetic information were provided by the DNA profiling of their biological evidence. Nowadays various advanced instruments and new methods of DNA extraction having different principles are available which removes the substances interfering with PCR and give a pure sufficient quantity of DNA. Step by step analysis performed by the detection of body fluids on exhibits, determine species origin by cross-over electrophoresis (COE), extraction of DNA and their quantification, amplification using polymerase chain reaction (PCR) and electrophoretic separation.

Material and Methods

1. PrepFiler Express™ Forensic DNA Extraction kit.
2. AmpFISTR™ Identifiler™ PCR Amplification Kit.
3. GeneScan™ 600 LIZ™ Size Standard v2.0.
4. Hi-Di™ Formamide.
5. Quantifiler® Human DNA Quantification Kit.

Instruments

6. AutoMate Express™ Forensic DNA Extraction System Catlog number: 4441763
7. This Instrument is compatible with the PrepFiler Express™ and PrepFiler Express BTA™ Forensic DNA Extraction kits.

Table 1: Source and number of evidences received in Forensic Science Laboratory

<table>
<thead>
<tr>
<th>Sources of evidence</th>
<th>Crime Scene</th>
<th>Suspects</th>
<th>Medical Officer</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of evidences</td>
<td>35</td>
<td>63</td>
<td>24</td>
<td>122</td>
</tr>
</tbody>
</table>
Instead of the above evidence, fifty-six blood samples of suspects also received for comparison. Total hundred and twenty-two evidence contain weapons, biological samples of deceased, pairs of chappal, earth samples and clothes (Table-2). Weapons are generally wooden sticks, bamboo sticks, metallic rods, metallic plank, metallic pipes, metallic sheets, stones, bricks and plastic pieces.

**Table 2: Nature and number of evidences received in Forensic Science Laboratory**

<table>
<thead>
<tr>
<th>Nature of evidences</th>
<th>Weapons</th>
<th>Clothes</th>
<th>Biological samples</th>
<th>Pairs of Chappal</th>
<th>Earth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of evidences</td>
<td>24</td>
<td>84</td>
<td>10</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Detection of blood on the weapons and clothes:

Kastle-Meyer test also known as phenolphthalein test was performed for the detection of bloodstains on the weapons, chappals and garments. Out of these hundred and twenty-two pieces of evidence, detection of blood on hundred and twelve-piece of evidence was performed. Blood was not detected on forty-six pieces of evidence. Results of the blood detection are as follows:

**Table 3: Detection of blood on evidences**

<table>
<thead>
<tr>
<th>Nature of evidences</th>
<th>Weapons</th>
<th>Clothes</th>
<th>Pair of Chappal</th>
<th>Earth</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood detected on evidences</td>
<td>24</td>
<td>39</td>
<td>1</td>
<td>2</td>
<td>66</td>
</tr>
</tbody>
</table>

After the detection of blood, species origin of detected bloodstains was determined using cross-over electrophoresis.

Total fifty-five samples were performed for the DNA analysis.

**Extraction of DNA:**

DNA was extracted from the bloodstains and nail clippings using PrepFiler Express Forensic DNA Extraction Kit. Bloodstain detected on the surface edge of the plastic pipe, wooden log, wooden sticks, wooden planks, metallic pipe, metal rod, and metallic sheets were swabbed with a sterilized wet cloth piece. Extraction of bloodstains and nail clipping was performed by adding 500 µL PrepFiler™ Lysis Buffer to the LySep™ Column/tube assembly and the mixture was incubated for 40 min at 70 °C and 750 rpm in a Thermo shaker. After the cell lysis centrifuged the column/tube assembly for 2 minutes at 10,000 rpm. The collected lysate was placed for automated extraction run using the PrepFiler Express instrument protocol on the Automate Express Forensic DNA extraction system. The isolated, purified DNA was stored at 4°C.

**Quantification:**

Extracted DNA used to be quantified by using Quantifiler® Human DNA Quantification Kit (Applied Biosystems, Foster City, CA) on an Applied Biosystems 7500 Real-Time PCR System as per the protocols endorsed by the manufacturer.

**PCR based STR Analysis:**

The quantified DNA used to be processed for STR (Short Tandem Repeat) profiling using AmpFiSTR® Identifiler PCR Amplification Kit with the assist of VeritiTM 96-Well Thermal Cycler according to the protocols endorsed through the producer [Fig-1]. This kit includes PCR Reaction mix, Primer set and Taq Gold Polymerase enzyme. A total of 1 ng of DNA was required for the amplification. Based on the quantitation, the volume of DNA was added up to 10 µL.
Capillary electrophoresis was performed on the 3500 Genetic Analyzer system (Applied Biosystems) by adding 1 µL/sample of PCR product to the mixture of Hi-Di Formamide (8.5 µL/sample) and GeneScan 600 LIZ Size Standard v2.0 (0.5 µL/sample). After the completion of electrophoresis, the sample was analysed using GeneMapper® ID-X. Different fragments of DNA molecules were separated based on their size. Interpretation of DNA profiles was done by comparing with reference samples.

**Results**

The extracted DNA was typed at 15 STR Loci and gender-specific Amelogenin locus using the PCR amplification technique.

1. A mixed DNA profile was obtained from blood detected on the crime scene evidence of weapon-1 (Plastic pipe) matched with the DNA profile of Deceased-3 and Deceased-5.

2. A DNA profile was obtained from blood detected on the crime scene evidence of weapon-3 (wooden log) and weapon-12 (metallic rod) are identical and from the same source of male origin and matched with the DNA profile of Deceased-1.

3. A DNA profile was obtained from blood detected on crime scene evidence of weapon-5 (wooden stick), weapon-7 (wooden plank), weapon-8 (metallic pipe), weapon-15 (metallic sheet), weapon-16 (metallic sheet), Pancha and cloth piece are identical and from the same source of male origin and matched with the DNA profile of Deceased-5.

4. A DNA profile was obtained from blood detected on the crime scene evidence of weapon-9 (metallic pipe) is of male origin and matched with the DNA profile of Deceased-4.

5. A DNA profile obtained from blood detected on the evidence recovered from Suspect-1, towel and full shirt and evidence recovered from Suspect-2, full shirt and wooden stick are identical and from the same source of male origin and matched with the DNA profile of Deceased-3.

6. Mixed DNA profiles obtained from the knicker of deceased-1 and full pant of deceased-2 matched with the DNA profiles of deceased-1 and deceased-5.

**Discussion**

In this case, the crime scene investigation team collects a huge number of evidence including a large number of weapons. Out of the hundred and twelve exhibits, blood was detected on sixty-six exhibit. Total fifty-five exhibit were performed for the DNA analysis from which DNA profiles are obtained from the twenty-seven sample. Control samples of the deceased was not available because the nail clippings of deceased collected by medical officer was soaked with blood. This is one of the major challenges of analysis which was overcome by cleaning the nail clippings with plenty of water multiple times and after the analysis single control DNA profiles of each deceased obtained. Out of the crime scene evidence, the DNA profiles of the eleven...
number of evidence are linked with deceased profiles of which nine are weapons. Out of the evidence recovered from the suspects, the DNA profiles of the four piece of evidence link with the deceased profile of which one of them is a weapon. Out of the twenty-eight suspects identified by police officer two are directly linked with this crime by the DNA profiles of the evidence recovered from them matched with deceased profile. This is one of the examples of DNA fingerprinting which proves that it is the strongest evidence against criminals for the justice of deceased and their relatives.

**Conclusion**

Mob lynching is one of the complex and sensitive crime and time-bound analysis with conclusive results is the important aspect while dealing with a large number of exhibits. Detection of blood on mass number of exhibits minimise the number of exhibits for further analysis and decreases cost and time of analysis. Analysis of a large number of shreds of evidence showed the profiles obtained from suspects, deceased and crime scene exhibits links between them enhances truthiness of the result. Links between the DNA profiles of weapons and the deceased confirm the weapons used in crime. Links between the DNA profiles of exhibits recovered from suspects and deceased proves the involvement of suspects in this crime. Links between mixed DNA profiles of clothes of the deceased concludes the all the deceased were suffered at the same time with the same location. Overall results justify the act of mob lynching, their unique location and the presence of suspect on that location.

**Conflict of interests:** Nil.

**Funding:** The authors declare that there is no funding taken from any agency all the expenses are borne by authors.

**Ethical Clearance:** No involvement of human or animal in this research.

**References**


Prevalence of Acute Pancreatitis and Hyperamylasemia in Acute Organophosphorus poisoning in Kashmir valley (North India)

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Abstract

Organophosphorus poisoning is soaring due to easy availability in rural settings of developing countries. There are many reports of Acute Pancreatitis associated with OP poisoning and a casual relationship has been demonstrated in animal models. This study was therefore designed to determine the prevalence of hyperamylasemia and acute pancreatitis in organophosphate poisoning in our setup and to reduce mortality and hospital stay by its early detection. This is a cross sectional hospital based study carried out in SKIMS Medical College, Srinagar, J&K which is tertiary care facility. All patients included were above 18 years of age with a recent history of organophosphate ingestion, cutaneous absorption or inhalation with clinical signs and symptoms of organophosphate poisoning. Confounding factors like drugs causing pancreatitis, ERCP within 24 hrs, previous history of pancreatitis, cholelithiasis and or any other local pathology were excluded. Serum Amylase and Lipase were measured. Imaging like ultrasonography and CT scan Abdomen was ordered. BISAP scoring was used to assess severity of acute pancreatitis. 50 patients with ingestion of organophosphates were evaluated. 23 patients developed abdominal pain, among which 11 patients had hyperamylasemia while 7 developed Clinical acute pancreatitis with high lipase and imaging findings. 2/3 of patients who developed pancreatitis had mild severity while other 1/3 had severe severity. Pancreatitis in Organophosphorus is not uncommon and needs high clinical suspicion for early diagnosis and management.

Keywords: Acute Pancreatitis, Hyperamylasemia, Organophosphorus Poisoning.

Introduction

Incidence of organophosphorus poisoning is soaring due to easy availability in rural settings of developing countries where its use as pesticide is quite high[1]. Worldwide 3 million people are exposed each year with 300000 deaths attributed to them[2-3]. Organophosphates common in use are parathion, malathion, fenthion, diazianon, chlorpyrifos while carbamates are methomyl and aldicarb. Nerve agents like sarin, tabun, and soman...
are organophosphorus compounds. There are many reports of Acute pancreatitis associated with OP Poisoning\textsuperscript{[4-7]}\textsuperscript{[4-7]}. And a causal relationship has been demonstrated in animal models\textsuperscript{[8]}. Cholinergic stimulation of the pancreas and the Sphincter of Oddi (SO) results in both increased pancreatic secretion and increased SO activity in animal models. It has been shown that excessive cholinergic stimulation using an acetelcholine agonist can result in acute pancreatitis. Organophosphate used as an insecticide irreversibly inhibits cholinesterase resulting in delayed breakdown of synaptic acetylcholine and has been noted to cause acute pancreatitis in humans. In animal models organophosphates results in acute pancreatitis associated with raised pancreatic duct pressure. This is thought to be secondary to “obstruction” at the SO level coupled with cholinergic stimulation of pancreatic secretion. This study was therefore designed to determine the prevalence of hyperamylasemia and acute pancreatitis in Organophosphate poisoning in our setup and to reduce mortality and hospital stay by its early detection.

**Materials and Methods**

This is a cross sectional hospital based study carried out in SKIMS MEDICAL College, Srinagar, Jammu and Kashmir which is tertiary care facility catering for acute and sub-acute emergencies. The study was conducted over a period of 2 years from November 2017 to October 2019. A total of 50 patients (41 females and 9 males) of acute poisoning with organophosphate insecticide were studied. All patients including both sexes and ages above 18 years with a recent history of Organophosphate ingestion, cutaneous absorption or organophosphate poisoning were included in the study. Confirmation of the poison was done by seeing the container brought bought by the patient’s family members and composition printed over them. Those patients with history of ingestion of any other material along with organophosphates, who were addicted to alcohol or had a history of gallstones or gallstones seen on ultrasound or any other history of gastric ulcer or burning epigastric pain in past were excluded from the study. Also those who had gone through ERCP (endoscopic retrograde cholangiopancreatography) in previous 24 hours or had a positive history of drug intake like azathioprine, mercaptopurine, asparaginase, valporic acid, pentamidine, estrogens etc. were not included in the study.

Serum amylase and lipase levels were measured with a Hitachi-911 autoanalyser via enzymatic and colorimetric assay. Serum amylase levels between 0 and 96 U/L and serum lipase levels between 0 and 60U/L were accepted as normal. Any two of the following criteria were used to diagnose acute pancreatitis: typical abdominal pain, threefold or greater elevation in serum amylase and/or lipase level, and/or confirmatory findings on cross-sectional abdominal imaging. All patients diagnosed to have acute pancreatitis also underwent an ultrasonographic study (by experienced radiologists) and computerized tomography, if necessary. Also the serum levels of Bilirubin, SGPT, SGOT, LDH, CPK, creatinine, Blood urea nitrogen, lipid profile and leukocytes counts were measured.

Data Collection Procedure: A special Proforma was designed to enter all the collected data containing the basic information about the patient, history of recent event and the past history, physical examination and lab investigations including complete blood count, random blood sugar, alanine aminotransferase (ALT), lactate dehydrogenase, serum amylase and lipase, ultrasonography abdomen and Computed Tomography Abdomen.

Data Analysis: The collected data was analyzed statistically using SPSS version-10 on computer.

Descriptive statistics like frequency and percentage of qualitative variables like sex, history , presenting complaints, clinical findings and causes of pancreatitis including gallstones, alcohol addiction, drug intake etc and quantitative variables like age, weight, amount of pesticide ingestion, time of ingestion and stomach wash.

**Results**

During period of study 50 patients with ingestion of organophosphates were evaluated. 45 among 50 patients intended to commit suicide (90.0%), whereas 05 patients, (10.0%) were exposed to organophosphates due to accidental event. None
came with a homicidal attempt. All 50 patients (100.0%) were exposed to organophosphates through gastrointestinal route, none via inhalation or cutaneous absorption. Among 50 patients, sex distribution showed 41 patients (82.0%) were females and 9 patients (18.0%) were males. Male to female ration was 0.23:1. Among 41 females 39 (96%) had intended suicidal attempt and 02 (4%) gave statement of accidental intake of organophosphates. Among 09 males, 07 (77.78%) males were exposed to organophosphates as suicidal attempt and 02 (22.22%) were accidental. Mean age at presentation was 22.5 years. The youngest was 18 years old whereas the oldest was 80 years. Through the 2 year period, 11 of 50 patients (22%) were found to have hyperamylasemia among whom 7 patients (14.0%) had more than threefold elevated amylase levels. Highest level of serum amylase was 1035 U/L. SGPT was elevated in 3 (6.0%) patients. Among the patients with normal amylase levels, isolated elevated leucocyte count was noted in 6 patients (12.0%) isolated raised LDH was found in 2 patients (4.0%) raised CPK seen in (2.0%) patient and raised SGPT was seen in 0 patients (0.0%). Among 50 patients, the most common clinical presentation was excessive salivation seen in 42 (84.0%) of patients while vomiting was seen in 23 (46.0%) of patients. Abdominal pain was seen in 23 patients (46.0%) out of these 11 had hyperamylasemia and 7 had acute pancreatitis proven by concomitant raised lipase levels and ultrasound findings. Confusion and drowsiness was noted in 30 patients (60.0%). One patient of pancreatitis (2%) had developed necrosis on CT Scan.

The frequently observed clinical findings among 50 patients with a positive history of ingestion of organophosphates were constructed pupils seen in 43 patients (86.0%). Fasciculations were noted in 23 patients (46.0%). Chest crepitations were heard in 20 patients (40.0%). Convulsions were seen in 2 (4.0%). Mild Acute Pancreatitis with BISAP score of 0-1 was seen in 5 (10%) patients while severe acute pancreatitis with BISAP score of >=3 was seen in 2 (4.0%) patients. Pancreatic necrosis on CT Scan was seen in only 1 patient (2%). No patient died during the study.

Table 1: Biochemical markers in organophosphorus poisoning patients, patients Pancreatitis vs patients without pancreatitis

<table>
<thead>
<tr>
<th></th>
<th>Mean TLC (10X10^3/mm^3)</th>
<th>Mean SGOT (U/L)</th>
<th>Mean SGPT (U/L)</th>
<th>Mean ALP (U/L)</th>
<th>Mean Amylase (U/L)</th>
<th>Mean Lipase (U/L)</th>
<th>Mean LDH (U/L)</th>
<th>Mean Bilirubin (mg/dL)</th>
<th>Mean BUN (mg/dL)</th>
<th>Mean Creatinine (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>7.6</td>
<td>24</td>
<td>22</td>
<td>101</td>
<td>132</td>
<td>78</td>
<td>149</td>
<td>0.82</td>
<td>18</td>
<td>0.71</td>
</tr>
<tr>
<td>Pancreatitis Patients</td>
<td>9.6</td>
<td>41</td>
<td>47</td>
<td>193</td>
<td>547</td>
<td>211</td>
<td>137</td>
<td>1.13</td>
<td>22.5</td>
<td>0.93</td>
</tr>
</tbody>
</table>

SGPT- serum glutamic-pyruvic transaminase,  
SGOT- serum glutamic-oxaloacetate transaminase,  
LDH-Serum Lactate Dehydrogenase,  
ALP- Alkaline phosphate,  
TLC- Total Leucocyte count,  
BUN-Blood urea nitrogen

Discussion

Cases with acute pancreatitis as a complication of organophosphate exposure have been reported in the literature[9]. Ahmed Arshia et al.[10] conducted a descriptive study at the Medicine Department, Abbasi Shaheed Hospital Karachi during the period of six months from 16th June 2003 to December 2006. All patients of both sexes and ages above 15 years admitted with a positive history of organophosphate poisoning (OP) were included in the study. Among 90 patients, hyperamylasemia was found in 28(31%) patients. Hyperlipasemia was seen in nine (10%) patients and pancreatitis was seen in two (20%) patients. Concluded that hyperamylasemia is more frequently seen in organophosphate poisoning while two patients proved to have acute pancreatitis as a complication. S Singh et al[11] carried out a prospective study in PGIMER, Chandigarh, India between June 2001-June 2005, to find the incidence of hyperamylasemia and acute pancreatitis in patients with OP poisoning. Of the 79 patients studied,
serum amylase was found to be elevated (> 200 S.U) in 37 patients (46.95%), among them in three patients it was 800 S.U. One of them showed swollen pancreas on ultrasonography which was confirmed by Computerized Tomography. In other two patients, evidence of pancreatitis was not observed. There was no significant correlation between the nature of compounds (OP or carbamates), duration and severity of cholinergic syndrome and increase in serum Amylase. It has been concluded that mild elevation of serum Amylase is common in patients with OP poisoning, however acute pancreatitis is rare. Isahin et. al.\cite{12} conducted a prospective study in the Department of Internal Medicine, University of Yuzuncu Yil, Medical faculty, Van, Turkey in 2002, to find the prevalence of pancreatitis in OP poisoning. Four of the total 47 patients with acute OP poisoning had obviously elevated Amylase and Lipase levels (Amylase>300 U/L ; Lipase >60 U/L). Only two of the patients with Amylase levels between 100 and 300 U/L had elevated levels of Lipase. None of the patients with normal Amylase levels had elevated levels of Lipase. A total of 12. 76% were diagnosed as acute pancreatitis. It was concluded that acute pancreatitis is not a rare complication of organophosphorus poisoning. In order to improve the outcome of OP poisoning early diagnosis of acute pancreatitis is important and serum Amylase and Lipase levels should be routinely considered carefully. W C Lee et. al.\cite{13} carried out a retrospective study of medical records of 121 patients with the diagnosis of OP poisoning over three years in Veterans General Hospital, National Yang-Ming University in 1998. Serum amylase, pancreatic amylase, salivary amylase, lipase and cholinesterase levels and the clinical manifestations were analyzed. It was observed that 44 patients (36%) had hyperamylasemia (Amylase>360 U/L). Lipase was measured in 28 patients with hyperamylasemia; nine of 28 had hyperlipasemia (Lipase >380 U/L). The finding of hyperamylasemia was closely related to clinical severity and presence of shock. It was concluded that hyperamylasemia is frequent in severe OP Poisoning. However, hyperamylasemia is not synonymous with acute pancreatitis and pancreatic amylase is not reliable parameter in the diagnosis of organophosphate induced pancreatitis due to its low sensitivity and specificity. Lipase assay is indicated in patients with hyperamylasemia for early diagnosis of pancreatitis. N. Matsumiya et. al.\cite{14} performed a retrospective study of OP poison in intensive care unit of analyze the incidence of respiratory failure in Department of Anesthesiology 7 Critical care medicine, Kyodo General Hospital, Ibaraki, Japan in 1996. Of the 32 OP poisoning patients, 16 developed respiratory failure and received ventilator support. An increase in plasma amylase above the normal range was found in patients who developed respiratory failure. Chatruvedi et. al.\cite{15} in their 2013 one year study, 30 of 96 patients (31.25%) were found to have hyperamylasemia. 16 patients (16.67%) had more than threefold elevated amylase levels. Among 13 patients (10%) out of 30 with hyperamylasemia had elevated lipase levels, 6 had >3Xuln lipase levels with diagnosis of pancreatitis.

However in our study, we observed frequent hyperamylasemia in 22.0% which is lower as observed in other studies. We found that elevation of serum amylase is common in patients with pesticide poisoning. However, acute pancreatitis is also not uncommon. However, acute pancreatitis is also not uncommon. We have also observed elevation in the serum levels of SGPT, LDH, CPK and TLC in most patients showing raised amylase and lipase. Limitation of our study was that serum cholinesterase levels were not done on admission because of non availability of test in our hospital lab.

### Conclusion

Acute pancreatitis and hyperamylasemia are not uncommon in organophosphate poisoning. Diagnosis of acute pancreatitis should be given importance and appropriate treatment as clinically indicated and can be life-saving.

Conflict of Interest Statement: The authors declare that there is no conflict of interest related to the contents of this article

Source of Funding: None, all investigations were done in hospital as admitted patient and were part of routine and baseline work-up.

Ethical Clearance: Was not needed as it was an analytical study which is exempted from ethical clearance as there was no financial or ethical issue involved in this analytical(retrospective) study.
References


Factors Influencing Peak Expiratory Flow Rate among Selected People in Basrah City

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Abstract

Background: In many obstructive airway diseases, including asthma, the Maximum Expiratory Flow (PEFR) measurement offers a reliable and objective technique for estimating the amount of airway blockage. Peak expiratory flow rate can be easily evaluated with a peak flow meter at home by the patient or their parents. The peak expiratory flow rate test measures how fast the air is circulating through the lungs.

Materials and Methods: The study was done in college of Dentistry, University of Basrah, we select 301 person who agree to participate of this study, an anthropometric measure were obtained from all subjects after simple interview, peak expiratory flow rate was obtained by using peak flow meter, then the result were analyzed using SPPSS.

Conclusions: Peak expiratory flow rate in males was higher than females and showed a strong positive correlation with the study participants’ age, height, weight, and body surface. The subjects’ age and body mass index had an inverse relationship with peak expiratory flow rate; however, it was not statistically significant

Keywords: PEFR, BMI, height, weight, surface area

Introduction

Knowledge of the normal function of individual organs in humans is fundamental in examining the effect of the patient’s pathological process. In many obstructive airway diseases, including asthma, the Maximum Expiratory Flow (PEFR) measurement offers a single, reliable, and objective technique for estimating the amount of airway blockage. The PEFR can be easily evaluated with a peak flow meter at home by the patient or their parents¹. The anatomy of the respiratory system is well adapted to its primary function of transporting gases (O₂) and out (CO₂) of the body. The tidal volume (TV) is the amount of air moving in and out of each respiratory cycle of the lungs. In a healthy male and a healthy female, it measures around 500 mL, and on average, 400 ml respectively. The extra volume of air that can be

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inspired after the normal, calm inspiration is reached, is inspiratory reverse volume (IRV; \( \sim 2 \) L), while the extra air volume can be expired from the lungs after the normal expiry of the tidal volume with a certain effort, is the expiratory reserve volume (ERV; \( \sim 1 \) L), and the amount of air remaining in the lungs of a person after exhalation is the residual volume. (RV; \( \sim 1.3 \) L. The total volume of air in the lungs is the lung capacity (TLC), with the greatest inspiration. The average lung capacity of healthy adults is approximately 6 liters. The different lung capacity of individuals depends on their age, gender, body composition, and ethnicity\(^1\). The inspiratory capacity (IC, \( \sim 2.5 \) L) is the maximum air volume that can be inspired by a normal quiet expiration that is the total of the tidal volume (TV) and the inspiratory reverse volume. While the functional residual capacity (FRC; \( \sim 2.5 \) L) reflects the volume of the air left in the lungs after the expiration of a normal breath (RV + ERV).\(^2\)

The pulmonary dysfunction was determined using dynamic measurements of lung volumes and capabilities. CVF is often clinically measured as an index of pulmonary function, the highest air that can be exhaled by maximum inspiration. It provides important information about the strength and other aspects of the pulmonary function of the breathing muscles. Forced expiratory volume (FEV) measures the amount of air that can be exhaled by a person during the forced breath. The air exhaled in the first (FEV1), second (FEV2), and/or third (FEV3) of the forced breath can be measured. The peak expiratory flow rate (PEFR), which originates from full lung inflation, is the maximum flow rate generated during the forceful expiration. PEFR reflects large airway flows in particular and is dependent on the patient’s voluntary work and muscular strength.\(^3\)

In order to make the PERF test useful, you need to keep records of your flow rate, except that when the flow rate is low or decreasing, you will not see the trend. These trends can help prevent worse symptoms before a complete attack on asthma. The PEFR test can help you decide if your medicine is adjusted. It can also help you to determine if pollutants or environmental factors affect your breathing. PEFR was introduced in 1942 and accepted as a spirometrically-sensitive index by Hadron as a measure of ventilatory function.\(^2\)

### Materials and Methods

We chose 301 participants at random from Basrah City and neighboring areas who met the normality criteria outlined in an earlier paper during their attendance to the outpatient-teaching clinic at College of Dentistry, Basra, Iraq. All subjects in the current study were over the age of 18 and were either nonsmokers or symptom-free smokers. The age, sex, height without shoes, and weight were recorded, the surface area was calculated using:

\[
surface\ area = \sqrt{\frac{m \times H}{3600}}
\]

Where \( m \) mass (Kg) and \( H \) height (Cm).

The use of parameters in addition to the body mass is in some cases required for a more precise scaling relationship. One example is the scatter of a person’s surface area, \( A \) (m\(^2\)). Which depends on height \( H \) (m) and mass (Kg).

\[
A = 0.202 \times m^{0.425} \times H^{0.725}
\]

All respondents' heights were measured without shoes, as well as their chest measurements following expiration (empty chest) and inspiration (full chest). The Peak flow meter correctly made a maximum effort and recorded the greatest value obtained in three consecutive attempts while standing. We normalized the data by dividing a subject’s measured PEFR by the predicted peak, because PEFR is a function of both height and age. All of the factors, as well as the PEFR, were encoded and entered into a spreadsheet (Ms. Excel), which was then employed in conjunction with the statistical package SPSS version 23.

### Results

The participants in this prospective interventional study were 301 people who met the inclusion criteria. One hundred twenty one males (40.2\%) and 180 women (59.8\%) participated in the survey. All of the people chosen are between the ages of 17 and 75, with a mean age of 27.95 and a standard deviation of \( \pm 12.4 \). The participants’ heights ranged from 141 to 197 cm, with a mean of 161.17 and SD of \( \pm 9.78 \), and their weights ranged from 41 to 155 kg, with a mean of 161.17 and SD of \( \pm 15.77 \). About 30\% of the
people chosen live in rural areas. Only approximately a quarter of the men in the study smoked (22.3%).

According to the body mass index (BMI) in this study, underweight people made up only 1.3 percent, normal people made up 54.5 percent, and overweight people made up 44.2 percent. The tables (1, 2, and 3) show the measure of EFPR in terms of smoking habits, gender, and residency participants, respectively. Men had a higher PEFR than women, and those who lived in rural regions had a higher PEFR, which was statistically significant, and as expected, it was higher among non-smokers men, although the difference was not statistically significant.

The association between PEFR and all participants’ age, height, weight, BMI, and surface area is shown in table (4). The age of the participants in this study was shown to be inversely correlated with the measured PEFR, but with no statistical significance, but the PEFR was found to be positively correlated with height, weight, and surface area, with significant statistical significance. Despite the fact that BMI has a negative correlation, it is not statistically significant.

The calculated Regression equation of both genders was:

Female: $402.679 - 0.0259 \text{Height} + 0.023 \text{weight} - 1.057 \text{age}$.

Male: $100.912 + 3.44 \text{Height} + 0.234 \text{weight} - 1.562 \text{age}$.

**Discussion**

Age of the people was a good predictor of PEFR (4). In this study, the age was associated positively with the PEFR, but with no statistical significance. The PEFR decreased with age, most likely, as it was dependent on expiratory effort, lung elastic recoil, and airway size, all of which are known to decline with age (5,6). PEFR was also significantly higher in males than females, which is consistent with earlier findings (7,4,8,9). Males with a higher PEFR score than women with a weaker body and more fat deposits are influenced by body composition, muscular strength and nutritional status (10).

The PEFR was highly statistically significant correlated/associated positively with the participants’ weight. This finding was consistent with many studies (11), in which we concluded that the PEFR was significantly lower in obese women, and in (12) which stated that the PEFR was significantly lower in overweight adolescents, and in study (6), which concluded that the PEFR of the study individuals increased as their weight increased, despite a study conducted in India in 2019 finding no correlation (8). PEFR was found to be significantly correlated with participants’ height, as found in another study that stated that the PEFR correlated well with height and weight, another study concluded that age, height, and weight were significant predictors of PEFR (4), and another study (6) stated that the PEFR was significantly correlated positively with people’s height. The PEFR had a positive relationship with the study individuals’ height, which was likely due to the taller respondents’ larger chest volume. With an increase in height, the airway channels expand and the expiratory muscle work increases (5).

All of the study participants’ residences were found to have an impact on the measured PEFR, with those who resided in rural areas having a significantly higher; this conclusion contradicted a study conducted in Bangladesh, which reported that rural areas had a significantly lower PEFR (13). Regarding body surface area was found to be positively correlated with PEFR, and this finding was consistent with other studies (9), which demonstrated an increase in body surface area resulted in an increase in the PEFR of the study subjects, as well in agreement with a study from Bangladesh (13), which found a significant positive correlation between PEFR and body surface area.

In the study individuals, the PEFR was found to be adversely linked with BMI, with a reduction in the PEFR as the BMI increased. However, there was no significant association between the PEFR and BMI (p>0.05), this finding was consistent with other study (5,8). PEFR was higher in nonsmokers solely among males, as expected, however this was not statistically significant. However, comparable findings have been observed in other studies, such as one conducted in India (2,9).

**Conclusions**

The PEFR was higher in males than females in this study, and it exhibited a strong positive correlation...
with age, height, weight, and body surface area of study participants. The subjects’ age and BMI had an inverse relationship with PEFR, however it was not statistically significant.

Table 1: PEFR measurement of the study sample according to the smoking habit

<table>
<thead>
<tr>
<th>Smoking habit</th>
<th>N</th>
<th>Mean SD. Deviation</th>
<th>SD. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non smoker</td>
<td>94</td>
<td>458.670 91.0317</td>
<td>9.3892</td>
</tr>
<tr>
<td>Smoker</td>
<td>27</td>
<td>437.358 80.4690</td>
<td>15.4863</td>
</tr>
</tbody>
</table>

P value 0.274

Table 2: PEFR measurement of the study sample according to gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>N</th>
<th>Mean SD. Deviation</th>
<th>SD. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>121</td>
<td>453.915 88.9079</td>
<td>8.0825</td>
</tr>
<tr>
<td>Female</td>
<td>180</td>
<td>337.917 69.2101</td>
<td>5.1586</td>
</tr>
</tbody>
</table>

P value 0.01

Table 3: PEFR measurement of the study sample according to residency

<table>
<thead>
<tr>
<th>Residency</th>
<th>N</th>
<th>Mean SD. Deviation</th>
<th>SD. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban</td>
<td>211</td>
<td>381.387 93.9691</td>
<td>6.4691</td>
</tr>
<tr>
<td>Rural</td>
<td>90</td>
<td>391.956 101.5724</td>
<td>10.7067</td>
</tr>
</tbody>
</table>

P value 0.384

Table 4: Correlation of PEFR with age and anthropometric measurement

<table>
<thead>
<tr>
<th>Peak expiratory flow rate</th>
<th>Age</th>
<th>Height</th>
<th>Weight</th>
<th>BMI</th>
<th>Surface area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation Coefficient</td>
<td>-.030-</td>
<td>0.491**</td>
<td>.265**</td>
<td>-.009-</td>
<td>.376**</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.602</td>
<td>.000</td>
<td>.000</td>
<td>.883</td>
<td>.000</td>
</tr>
<tr>
<td>N</td>
<td>301</td>
<td>301</td>
<td>301</td>
<td>301</td>
<td>301</td>
</tr>
</tbody>
</table>

Ethical Clearance: taken from institutional ethical committee.

Source of Funding- None funded

Conflict of Interest: Nil

References
1. Delgado BJ, Bajaj T. Physiology, lung capacity, 2019;


Species Identification of Canned Meat Products by Using DNA-Based Methods

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Abstract

Fraud among different meats became great issue concerns of economical and hygienic aspects. Canned meat products are widely adulterated for economically motivated. The objective of the current study was to test a variety of canned meat products sold on the Iraq/Basrah commercial market to assure the authenticity of meat products for quality and safety purposes. Twenty seven canned meat samples were purchased from supermarkets. They were targeting cattle, sheep, goat, horse, donkey, and chicken for the possibility of a species mixture. DNA was extracted from each sample in triplicate and tested using DNA-based method. Samples analysed in the current study were found to be mislabeled. Meat species (chicken and beef) were detected in the samples collected from supermarkets that are not declared in their labels. Overall, the mislabeling identified in the current study either due to mixing of cheaper meat species into more expensive or mixing of meat species during processing due to cross-contamination, unintentionally.

Key words: Canned meat, fraud, identification method, DNA technology.

Introduction

Meat is the muscle fiber of an animal that is consumed as food. It is rich in water (75%), protein (20%), and fat (5%). Muscle fiber of an animal is also contained carbohydrates, vitamins, and minerals. In most regions of the world, the consumption of meat products continues to elevate. Due to meat products are favored around the world, mislabeling of meat products have become common and can lead to economic deceit. Usually, customers depend on the product labeling accuracy to make right choices for public health and religious causes.

Strategies have been utilised to identify meat species in products are based on either protein or DNA measurements. Protein detection for species of meat is unable to identify between species which are close relatives compare with DNA detection. However, DNA detection for species of meat is a reliable, effective, simple and rapid method. In previous studies, identification species of meat by DNA-based technique revealed the use of a label that is incorrect for sale under the name of another product. Since becoming aware of these issues, this work aimed to detect the meat species in products.
under laboratory conditions to prevent the sale of adulterated meat products.

**Materials and Methods**

**Sample collection**

Twenty seven canned meat products were collected from different commercial markets in Basrah province/ Iraq. Following collection, canned meat products were kept at room temperature until analysed. The Commercial canned meat products analysed for authentication are shown in Table 1.

Table 1: Commercial canned meat products analysed for adulation

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Product label</th>
<th>Trademark</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Beef Luncheon Meat</td>
<td>Baidar</td>
<td>Kingdom of Saudi Arabia</td>
</tr>
<tr>
<td>2</td>
<td>Chicken Luncheon Meat</td>
<td>Baidar</td>
<td>Kingdom of Saudi Arabia</td>
</tr>
<tr>
<td>3</td>
<td>Beef Luncheon Meat</td>
<td>Hena</td>
<td>United Arab Emirates</td>
</tr>
<tr>
<td>4</td>
<td>Chicken Luncheon Meat</td>
<td>Hena</td>
<td>United Arab Emirates</td>
</tr>
<tr>
<td>5</td>
<td>Beef Hot Dog</td>
<td>AlTaghziah</td>
<td>Lebanon</td>
</tr>
<tr>
<td>6</td>
<td>Chicken Hot Dog</td>
<td>AlTaghziah</td>
<td>Lebanon</td>
</tr>
<tr>
<td>7</td>
<td>Beef Luncheon Meat</td>
<td>Ghadeer</td>
<td>Jordan</td>
</tr>
<tr>
<td>8</td>
<td>Corned Beef Loaf</td>
<td>Burdon</td>
<td>Brazil</td>
</tr>
<tr>
<td>9</td>
<td>Beef Hot Dog</td>
<td>Al Qaisar</td>
<td>Kingdom of Saudi Arabia</td>
</tr>
</tbody>
</table>

**Preparation of sample for DNA extraction**

Small pieces (2 gram) of sample were collected from each canned meat products using sterile forceps and mixed thoroughly with distal water (60 ml) in a blender (at 230 rpm for 120 sec) to homogenize. The sample was then put in microcentrifuge tube, labeled with the sample ID and stored to prevent DNA degradation at -20 °C.

**DNA extraction and detection**

DNA extraction was carried out in triplicate for all canned meat products using DNA extraction kit GsyncTMDNA (Geneaid Biotech Ltd., Taiwan). It was done as described by the manufacturer’s instruction. Briefly, the meat product sample (25 mg) was lysed with ATL buffer (200 μL) and Proteinase K (20 μl), vortexed at 30 min, and incubated overnight at 60 °C. GSB buffer (200 μl) and absolute ethanol (200 μl) were then added to each tube and the tubes were revortexed for 10 sec. The samples were then put in GC columns, washed with W1 buffer (400 μl), centrifuged (14,000 xg for 30 sec), followed by second washed with W2 buffer (600 μl), centrifuged (16,000 xg for 30 sec), and discard the flow through. To dry the column matrix, the GC column centrifuged (16,000 xg for 3 min). After that, the dried column was put in a sterile microcentrifuge tube (1.5 ml) to add elution buffer(100 μl), stand at room temperature (3 min) to allow elution buffer, and centrifuged (16,000 xg) to elute purified DNA (30 sec). DNA qualities were then checked on agarose gel (1.5%). DNA quantities were identified by spectrophotometer (Nano Drop Technologies, Wilmington, USA).

**PCR primers and amplification**

The primers described in previous study⁶ were used in the current study for PCR assay (Table 2).

Table 2: Primer sequence and target fragment for PCR assay
Polymerase Chain Reaction (PCR) assay for gene amplification

DNA from the collected samples was amplified in a total 25 μL reaction volume containing genomic DNA (1μg) of each specie, primers (1μM), Mgcl2 (2mM), dNTP (0.2mM), PCR buffer (2.5μL of 10X) and the enzyme Taq DNA polymerase (1unit). The PCR assay were applied by thermal cycler as follows: initial denaturation step (94°C for 4 min) followed by 30 cycles of: denaturation step (94°C for 30 sec), annealing step (57-64°C for 30 sec), extension step (72°C for 30 sec), and a final elongation (72°C for 30 sec).

PCR products detection

The amplified PCR product was detected on agarose gels (1.5%) prepared with agarose in Tris-borate-EDTA buffer (1x) at 100 V for 30 min, stained with dye (Ethidium Bromide), and images using gel documentation systems (UVIdoc, UK). The size of the band was determined by comparison with a standard DNA ladder (New England Biolabs).

Results and Discussion

Mitochondrial DNA fragments of cattle (84pb), sheep (121pb), goat (122pb), horse (145pb), donkey (83pb), and chicken (183bp) were amplified. PCR analysis of canned meat revealed negativity results of all samples for regarding to sheep, goat, horse, and donkey meats (Table 3, Figure 1-4). However, the current study revealed that chicken and beef meat were found in the canned meat products that are not declared in their labels (Table 3, Figure 5-6).

Adulteration by exchange or mixing undeclared species in meat products under food labeling regulations is illegal. Detecting the meat species used in a meat product is an importance issue in food industry. The current study revealed that there was mislabeling in analysed samples. This finding is in agreement with previous studies, in which mislabeling was reported in different marketed meat products. In Turkey, meat products that were declared as beef contained poultry and donkey meat 6. In addition, raw meat that was declared as beef contained mix of horse and deer meat 7. In China, meat products that were declared as buffalo contained cattle, pork, and duck meat 5. In Iran, raw burgers that were declared as beef contained poultry meat 8. In France, 18% of ground meat samples collected from local European and Asian markets revealed adulteration cases with undeclared meat species 9. In Bangladeshi, up to one third of beef-labeled products revealed adulteration cases of products with buffalo and chicken 10.

In the present study, chicken meat was detected undeclared animal species. The main reason for the exchange of chicken flesh (cheaper) in beef (more expensive) is economic. Another reason might be an accidental cross contamination can occur...
during handling, the use of shared equipment, and processing \textsuperscript{11}. The meat species exchange occurs usually in processed meats (canned meat, salami, sausages, and sucuk). The main reason for success the adulteration in the processed meats is processing technique. This technique causes invisible changes in the physical appearance (color, texture, and flavor). So, detection of adulation by visual inspection is more difficult in processed meats than in fresh meat\textsuperscript{10}.

The current study and the others found that all countries have requirements about authenticity and labeling. In general, chicken meat exchange is common in Muslim countries because of cheaper than beef meat.

### Table 3: PCR results for canned meat product

<table>
<thead>
<tr>
<th>Product label</th>
<th>Adulteration ingredients</th>
<th>PCR results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cattle</td>
<td>Sheep</td>
</tr>
<tr>
<td>Beef Luncheon Meat/Baidar</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Chicken Luncheon Meat/Baidar</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Beef Luncheon Meat/Hena</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Chicken Luncheon Meat/Hena</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Beef Hot Dog/AlTaghziah</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Chicken Hot Dog/AlTaghziah</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Beef Luncheon Meat/ Ghadeer</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Corned Beef Loaf/Burdon</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Beef Hot Dog/Al Qaisar</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

Note: (+) denotes for presence and (-) stands absence

Figure 1: PCR product with 121pb using sheep specific primer in 1.5% agarose gel electrophoresis. PCR analysis showing the negatively result of all samples for regarding to sheep meat (L1- L9) M: molecular marker; lane 1: Beef Luncheon Meat/Baidar; lane 2: Chicken Luncheon Meat/Baidar; lane 3: Beef Luncheon Meat/Hena; lane 4: Chicken Luncheon Meat/Hena; lane 5: Beef Hot Dog/AlTaghziah; lane 6: Chicken Hot Dog/AlTaghziah; lane 7: Beef Luncheon Meat/ Ghadeer; lane 8: Corned Beef Loaf/Burdon; lane 9: Beef Hot Dog/Al Qaisar.

Figure 2: PCR product with 122pb using goat specific primer in 1.5% agarose gel electrophoresis. PCR analysis showing the negatively result of all samples for regarding to goat meat (L1- L9) M: molecular marker; lane 1: Beef Luncheon Meat/Baidar; lane 2: Chicken Luncheon Meat/Baidar; lane 3: Beef Luncheon Meat/Hena; lane 4: Chicken Luncheon Meat/Hena; lane 5: Beef Hot Dog/AlTaghziah; lane 6: Chicken Hot Dog/AlTaghziah; lane 7: Beef Luncheon Meat/ Ghadeer; lane 8: Corned Beef Loaf/Burdon; lane 9: Beef Hot Dog/Al Qaisar.
Conclusion, mislabeling of canned meat with cheaper protein is a problem. To protect the consumer to make right choices when purchasing canned meat, canned meat must be regularly analysed using effective methods.

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Conflict of Interest: Nil

Source of Funding: Self-funding

Ethical Clearance: Taken from the scientific Committee, University of Basrah.
References


To Assess the Incidence of Catheter Associated Urinary Tract Infection and Evaluate the Effectiveness of Evidence based Practice Protocol for Prevention of Catheter-associated Urinary Tract Infections (CAUTI) in Adult Critical Care Patients

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Abstract

Background: UTIs are the most common type of healthcare-associated infection. Among UTIs acquired in the hospital, approximately 75% are associated with a urinary catheter, which is a tube inserted into the bladder through the urethra to drain urine. The most important risk factor for developing a CAUTI is prolonged use of the urinary catheter. Therefore, catheters should only be used for appropriate indications and should be removed as soon as they are no longer needed.

Aim: The aim of the study is to prevent CAUTIs in adult critical care patients, by using evidence based practice protocol.

Materials and Methods: A Quasi Experimental one group pretest posttest research design was used. 200 staff nurses were selected from hospitals by using purposive sampling technique. Biophysical measures like signs and symptoms of CAUTI and urine culture was done to assess the incidence of CAUTIs in adult critical care patients before administration of evidence based practice protocol.

Result: The overall incidence rate of CAUTI in adult critical care patients was 11.1%. A Comparison of Pre and Post test level of Knowledge of Staff Nurse’s regarding CAUTIs shows that 46(23.0%) had poor knowledge before intervention and it was reduced 2(1.0%) after intervention. None of the staff nurse had excellent knowledge before intervention and was increased 12(6.0%) after intervention, 23(11.5%) had pre test good knowledge and was increased to 122(61.0%) in post test. Pre test mean knowledge of staff nurse regarding CAUTIs was 13.68 whereas post test knowledge score was 16.5 and was statistically significant with p-value <0.0001.

Conclusion: This approach shows for reduction of CAUTIs in critical care settings. The same type of protocol or education programme might be adopted for the other health care associated infections.

Key words: Adult Critical Care Patients, Catheter Associated Urinary Tract Infection, Evidence Based Practice Protocol, Incidence.

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Introduction

The urinary system is one of the main routes through which the human body excretes liquid waste.1 Urinary tract infection (UTI) is a collective term that describes any infection involving any part of the urinary tract, namely the kidneys, ureters, bladder and urethra.2

CAUTI is defined by CDC as a UTI in a patient who was catheterized for more than 2 days with at least one of the following signs or symptoms: fever (>38°C); urgency; frequency; dysuria; suprapubic tenderness; costovertebral angle pain or tenderness and a positive urine culture of ≥10^5 colony-forming units (CFU)/ml with no more than 2 species of microorganisms. If an indwelling urinary catheter was in place for > 2 calendar days and then removed, the UTI criteria must be fully met on the day of discontinuation or the next day.3

Urinary tract infection (UTI) is one of the most common infections in humans, accounting for more than 150 million cases worldwide. In addition to being the most common bacterial infection, UTIs also account for 36% of all healthcare-associated infections. Of these 36% infections, 80% of them are estimated to be catheter associated. Beyond the initial urinary infection, catheter-associated UTIs (CAUTIs) can lead to complications including bacteremia, endocarditis, osteomyelitis, septic arthritis, and meningitis. These pathologies collectively result in prolonged hospital stays and increased morbidity and mortality.4

The risk of developing a CAUTI is directly related to catheter dwell time. For catheterized patients, the rate of development of catheter-associated bacteriuria is approximately 3% to 7% per day and is more common in the elderly and females. The two most important factors that lead to the development of CAUTIs and have been the main focus of quality improvement areas are unnecessary urinary catheter placement and inappropriate delay in removing a catheter when it is no longer needed. Unfortunately, 38% of attending physicians are unaware that their patients have a urinary catheter in place. Furthermore, in 20% to 50% of cases, there is no clear indication for catheter placement.5

Diagnostic testing for CAUTI includes urinalysis and urine culture.6 Prevention strategies for CAUTI include; hand hygiene steps to be followed before and after handling or manipulation of the catheter or urinary drainage bag, while maintaining a continuous sterile closed drainage system. Keeping the catheter and the peri-catheter area clean with regular use of antiseptic solutions, along with the incontinence care.7

Nurses have to be aware of CDC guidelines in carrying out procedures like urinary catheter insertion, collection of urine specimens and maintenance of indwelling catheter. Adherence of staff nurses is important in reduction of occurrence of hospital based infections.8

Catheter restriction protocols have been a common component of successful multi-modal interventions to decrease catheter use and CAUTI rates, including hospital-wide interventions such as the emergency department, inpatient units (including general medical, surgical wards and ICU), and in the peri-procedural setting.9

Undertaken study was conducted to prevent catheter associated urinary tract infections (CAUTIs) in adult critical care patients, through enhancing the knowledge and improving the quality care practice of staff nurses by using evidence based practice protocol.

Materials and Methods

An evaluative approach was used for the present study. The design adopted for the study was quasi experimental one group pretest posttest research design. The study was conducted in medical ward, surgical ward, POW, Intensive care unit and emergency ward of BLDE (Deemed to be University) Shri B M Patil MCH & RC. Total 200 staff nurses were selected by using Non Probability Purposive Sampling Technique. The study includes adults both the males and female staff nurses who fulfilled the inclusion criteria and available at the time of data collection. The data were collected on selected wards to assess the incidence of catheter associated urinary tract infection in adult critical care patients by urine culture. The next part of data collection involved the staff nurses by assessing the pretest using structured knowledge questionnaire which also includes sociodemographic profile and self reported practice questionnaire. After the pretest evidence
based practice protocol (EBPP) was inducted. Seven days after administration of EBPP the posttest was obtained. Data were analyzed by using descriptive and inferential statistics. Continuous data were expressed in terms of mean and standard deviation (SD). Comparison of mean pre test and post test knowledge scores using paired t test.

Results

A total of 200 staff nurses completed the questionnaire. The sociodemographic data represented in Table 1 that frequency and percentage distribution of adults according to socio-demographic profile such as age, gender, educational qualification, years of experiences and exposure to CNE programme. Majority of staff nurses were 36-45 years of age, 70% of participants were female, and 39% of participants completed their BSC (N) degree and most of the participants 28% from medical ward. The results reveal that majority of the staff nurses have ≥ 10 Years work experience and very few 25.5% only had attended CNE programme on CAUTIs. Table No 2, 3 and 4 shows comparison of pre and post test level of knowledge of staff nurses and paired difference between pre test and post test knowledge score of staff nurse regarding knowledge on CAUTIs. Table No 5, 6 and 7 shows comparison of pre and post test level of practice of staff nurses and paired difference between pre test and post test practice score of staff nurse regarding prevention of CAUTIs.

Table 1: Frequency and percentage distribution of adults according to Socio-demographic Profile

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Sociodemographic Profile</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
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<td><strong>Age</strong></td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>22-35</td>
<td>60</td>
<td>30.0</td>
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<td></td>
<td>36-45</td>
<td>67</td>
<td>33.5</td>
</tr>
<tr>
<td></td>
<td>46-55</td>
<td>54</td>
<td>27.0</td>
</tr>
<tr>
<td></td>
<td>55 &amp; Above</td>
<td>19</td>
<td>9.5</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>200</strong></td>
<td><strong>100.0</strong></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Male</td>
<td>59</td>
<td>29.5</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>141</td>
<td>70.5</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>200</strong></td>
<td><strong>100.0</strong></td>
</tr>
<tr>
<td><strong>Educational Qualification</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>GNM</td>
<td>62</td>
<td>31.0</td>
</tr>
<tr>
<td></td>
<td>B.Sc(N)</td>
<td>79</td>
<td>39.5</td>
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<tr>
<td></td>
<td>PB.BSc(N)</td>
<td>44</td>
<td>22.0</td>
</tr>
<tr>
<td></td>
<td>MSc(N)</td>
<td>15</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
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<td><strong>100.0</strong></td>
</tr>
<tr>
<td><strong>Specialty Area</strong></td>
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<td></td>
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</tr>
<tr>
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<td>Surgical Ward</td>
<td>54</td>
<td>27.0</td>
</tr>
<tr>
<td></td>
<td>Intensive care Unit</td>
<td>49</td>
<td>24.5</td>
</tr>
<tr>
<td></td>
<td>Post-Operative Ward</td>
<td>28</td>
<td>14.0</td>
</tr>
<tr>
<td></td>
<td>Emergency Ward</td>
<td>13</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>200</strong></td>
<td><strong>100.0</strong></td>
</tr>
<tr>
<td><strong>Work Experience</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0-3 Years</td>
<td>60</td>
<td>30.0</td>
</tr>
<tr>
<td></td>
<td>7-9 Years</td>
<td>67</td>
<td>33.5</td>
</tr>
<tr>
<td></td>
<td>≥ 10 Years</td>
<td>73</td>
<td>36.5</td>
</tr>
</tbody>
</table>
Table 1 shows that majority 67(33.5%) of the staff nurse were in the age group 36-45, 60(30.0%) were in the age group 22-35, 54(27.0%) were in the age group 46-55 and 19(9.5%). It was clear that majority 141(70.5%) of the staff nurse were females and remaining 59(29.5%) were males. It is revealed that 79(39.5%) of the staff nurse were BSc (N) graduates, 62(31.0%) were studied GNM, 44(22.0%) studied PBBSc (N), and remaining 15(7.5%) were studied MSc (N). Regarding speciality area of 56(28%) were selected from medical ward, 54(27%) were surgical ward, 49 (24.5%) from Intensive care unit, 28(14%) were from post operative ward and very few 13 (6.5%) were from Emergency ward. Majority of the staff nurses had 73 (36.5%) had more than 10 years of work experience, 67(33.5%) had 7-9 years of experience and 60(30%) had 0-3 years of experiences. Most of the 149(74.5%) had previous knowledge or exposure to CNE Program on CAUTIs 51(25.5%) had no exposure to any kind of CNE program on CAUTI.

Table 2: Comparison of pre and post test level of knowledge and practice of staff nurses regarding CAUTIs

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Level of Knowledge</th>
<th>Pre-test</th>
<th>Post-Test</th>
<th>Level of Practice</th>
<th>Pre-test</th>
<th>Post-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F P (%)</td>
<td>F P (%)</td>
<td></td>
<td>F P (%)</td>
<td>F P (%)</td>
</tr>
<tr>
<td>1</td>
<td>Poor Knowledge</td>
<td>46 23.0</td>
<td>02 1.00</td>
<td>Good</td>
<td>198 99.0</td>
<td>150 75.0</td>
</tr>
<tr>
<td>2</td>
<td>Medium Knowledge</td>
<td>131 65.5</td>
<td>64 32.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Good Knowledge</td>
<td>23 11.5</td>
<td>122 61.0</td>
<td>Excellent</td>
<td>02 1.0</td>
<td>50 25.0</td>
</tr>
<tr>
<td>4</td>
<td>Excellent Knowledge</td>
<td>00 00</td>
<td>12 6.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>200 100.0</td>
<td>200 100.0</td>
<td>Total</td>
<td>200 100.0</td>
<td>200 100.0</td>
</tr>
</tbody>
</table>

And 198(99.0%) of the staff nurse had good practice before intervention and it was reduced 150(75.0%) after intervention. Only 2(1.0%) staff nurse had excellent practice regarding CAUTIs before intervention and as increased to 50(25.0%) after intervention.

The mean pre-test practice of staff nurse regarding CAUTIs was 72.17 whereas post test mean practice score was 83.04 and was statistically significant with p-value < 0.000.
Table 3: Comparison of Incidence of among the patients before and after evidence based Practice protocol (EBPP)

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Ward</th>
<th>Before EBPP</th>
<th>After EBPP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>CAUTI</td>
<td>N</td>
</tr>
<tr>
<td>1</td>
<td>Medical Ward</td>
<td>45</td>
<td>06</td>
</tr>
<tr>
<td>2</td>
<td>Surgical Ward</td>
<td>60</td>
<td>05</td>
</tr>
<tr>
<td>3</td>
<td>Intensive care Unit</td>
<td>15</td>
<td>02</td>
</tr>
<tr>
<td>4</td>
<td>Post-Operative Ward</td>
<td>20</td>
<td>03</td>
</tr>
<tr>
<td>5</td>
<td>Emergency Ward</td>
<td>40</td>
<td>04</td>
</tr>
<tr>
<td>Total</td>
<td>180</td>
<td>20(11.1%)</td>
<td>135</td>
</tr>
</tbody>
</table>

Table 3 revealed that out of 60 patients in surgical ward, 5 had CAUTI before protocol and it was reduced to 2 patients out of 50 after EBPP, in medical ward, out of 45 patients 6 had CAUTI before EBPP which was fall down to 3 patients out of 40. Regarding Intensive care unit and Post operative ward before EBPP 2 patient had CAUTI out of 15 in ICU and 3 had CAUTI out 20 in Postoperative ward, after Protocol administration out of 5 patients in ICU and 10 patients in postoperative ward none of the patient had CAUTI. Regarding emergency ward out of 40 patients 4 had CAUTI before EBPP and it was reduced to 2 patients out of 30 after EBPP. It reveals that out of 180 patients, 20 patients had catheter associated urinary tract infection (CAUTIs) before administration of EBPP. The overall incidence rate of CAUTI was 20(11.1%). After EBPP out of 135 patients incidence rate of CAUTI was 07(5.3%).

From the above Table 4, it was clear that pre test knowledge of the staff nurse regarding CAUTIs was not associated with socio demographic variables such as age, gender, work experience and CNE program but it was highly associated with educational qualification and area of specialty. And pre test practice of the staff nurse’s regarding CAUTIs was not associated with any of the socio demographic variables.

**Discussion**

CAUTI is a very challenging risk factor among hospital acquired infection. Enhancing knowledge and level of practice among health professionals would helps to prevent this problem. This quasi experimental one group pretest posttest study was conducted to prevent catheter associated urinary tract infections (CAUTIs) in adult critical care patients, by using evidence based practice protocol. Total 200 staff nurses were included for this study. The study findings reveals that the overall incidence rate of CAUTI in adult critical care patients was 11.1% before administration of evidence based practice protocol. The results of the study shows that in pretest, 46(23%) had poor, 131(65.5%) had moderate level, 23(11.5%) had good knowledge and 198(99%) shown good level and only 02(1%) excellent level of practice. The present study was supported by another study conducted by Anns M. Benny, Anju S. Indiculla, and Annamary Kanjumon to assess the nurses’ knowledge on prevention of Catheter Associated Urinary Tract Infection in a selected hospital of Mangaluru. Total 235 staff nurses working in medical college hospital with National Accreditation Board of Hospitals were involved. Structured knowledge questionnaire was used to assess nurses’ knowledge on CAUTI. The result of this study shows that majority of nurses (80.85%) had average knowledge and few (7.23%) nurses had high knowledge regarding CAUTI. Study concludes that the knowledge of Nurses on prevention of CAUTI and indicated that nurses had an average knowledge. In the post test of present study, 2(1%) had poor, 64(32%) had moderate level, 122(61%) had good knowledge, 12(6%) had Excellent knowledge and 150(75%) shown good level and only 50(25%) excellent level of practice. Pre test mean knowledge of staff nurse regarding CAUTIs was 13.68, whereas post test knowledge score was 16.5 and was statistically significant with p-value <0.0001. The findings of post test confirmed that the effectiveness of evidence based practice protocol in terms of significantly improve the knowledge and practice of staff nurses and also reduce the incidence of CAUTI.
Table 4: Association between level of knowledge and practice of study participants regarding CAUTIs with their Socio-demographic Profile

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pre-Test Knowledge Level</th>
<th>Chi-square</th>
<th>df</th>
<th>P-value</th>
<th>Variables</th>
<th>Pre-test Practice Level</th>
<th>Chi-square</th>
<th>df</th>
<th>P-value</th>
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</thead>
<tbody>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22-35</td>
<td>Good 18</td>
<td>7.79</td>
<td>6</td>
<td>0.253</td>
<td>22-35</td>
<td>Good 59</td>
<td>1.17</td>
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<td></td>
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<td>36-45</td>
<td>Poor 19</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td>55 &amp; above</td>
<td>Good 59</td>
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<td></td>
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<td>55 &amp; above</td>
<td>Medium 54</td>
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<td>(NS)</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td>55 &amp; above</td>
<td>Poor 19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>Good 59</td>
<td></td>
<td></td>
<td></td>
<td>Gender</td>
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<td></td>
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<td>Male</td>
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<td></td>
<td>Male</td>
<td>Poor 19</td>
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<tr>
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<td>2</td>
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<tr>
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<td></td>
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<tr>
<td>Specialty Area</td>
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<td>13.97</td>
<td>6</td>
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<tr>
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<td>Good 18</td>
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(*S*): Significant at p < 0.05
among critical care patients. Mariam Sabry Shaheb conducted a quasi-experimental study to evaluate the impact of protocol of care of catheterization on nurses. 50 staff nurses working with patients undergoing for urinary catheterization were assessed for knowledge. The results show that the knowledge of staff nurses was (28%) in pre test, (100%) immediately after the administration of protocol and (98%) 3 months after and (96%) 6 months after the protocol intervention. The study suggest that providing ongoing in-service education for nurses to update knowledge related to care of catheterized patients.11

Recommendations

- Several experts and organizations have published guidelines or recommendations on the identification, management and prevention of catheter associated urinary tract infections (CAUTIs).
- A similar study can be conducted on a large sample may help to draw more definite conclusions and make generalization.
- Our study highlighted the importance of the use of the urethral catheters only for appropriate indications, sterile techniques for insertion and necessary for continuous assessment, and maintenance of a sterile, continuously closed drainage system that allows unobstructed urine flow.

Conclusion

Prevention is the main step against CAUTI. Priorities with proven value are the appropriate use and early removal of catheters, aseptic insertion, and the maintenance of a closed urinary drainage system. Infection control programs must develop, implement, and monitor policies and practice to minimize infections associated with use of these devices. A major focus of these programs should be to limit the use of indwelling urethral catheters, and to remove catheters promptly when no longer required.

Conflict of Interest: We declare that there is no conflict of interest

Source of Funding: Rajiv Gandhi University of Health Sciences, Bangalore, Karnataka

References

Green Synthesis of Copper Nanoparticles using Green Tea and Neem Formulation and Assessment if its Antimicrobial Effects

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Abstract

Aims: Nanotechnology is the science which is about manipulating matter, atom by atom and is associated with particles smaller than 100 nm in size. Copper nanoparticles are used mainly due to its surplus amount, low cost, easy availability and biocompatible property. Green synthesis of copper nanoparticles is very simple, economical and eco-friendly method that does not involve any toxic chemicals. The aim of our study is green synthesis of copper nanoparticles using green tea and neem formulation and assessment of its antimicrobial effects.

Place and Duration of Study: Sample: Department of Nanotechnology, Saveetha dental college and hospitals, between December 2020 and January 2021

Methodology: 20 mM of copper sulphate solution is mixed with 40mL of plant extract and 60 mL of distilled water was added and made it into 100 ml solution. Once the copper nanoparticles are synthesized the solution is characterized using UV- vis-spectroscopy and was scanned in double beam UV-vis- spectrophotometer from 300 nm to 700nm wavelength. The antimicrobial property of copper nanoparticle is evaluated by agar well diffusion method.

Results: The colour change from green to brown and peak observed in UV-vis-spectrophotometer was associated with the synthesis of copper nanoparticles. Copper nanoparticle from green tea and tea extract has good antimicrobial activity against S.mutans, C.albicans, E.faecalis, S. aureus.

Conclusion: Copper nanoparticles can be efficiently synthesised from green and neem formulation. These copper nanoparticles showed good antibacterial properties and are effective against oral pathogens.

Keywords: Copper, nanoparticles, neem, green tea, plant extract, antimicrobial activity
Introduction

Nanotechnology is the science which is about manipulating matter, atom by atom\(^1\). Nanotechnology is associated with particles smaller than 100 nm in size\(^2\). Due to its small size and high surface area, nanoparticles increase the state of activity. The size of nanoparticles depends on the method of reduction and its surrounding environment. Nanodentistry is the science and technology of maintaining good oral health through the use of nanomaterials including tissue engineering and nanorobotics\(^3\). Currently nanoparticles are of use in various fields of biomedical and pharmaceutical like diagnostics, biomarkers, bio-imaging, cosmetics, antibacterial, anticancer, immunology, cardiology, genetic engineering, drug delivery for treating cancer and other infectious diseases.\(^4\)

Due to their small size, physical and chemical properties, high surface area for interaction and wide area of application of metal nanoparticles are increasing\(^5\). Copper nanoparticles are used in the field of nanotechnology and nanomedicine due to their good optical, electrical and anti-fungal/bacterial properties\(^6\). Copper nanomaterials have been used mainly due its abundant amount, low cost and availability when compared to other metals such as silver and gold. Copper is the basic and biocompatible element that has good therapeutic and antibacterial property known since a very long time\(^7\). The copper nanoparticle was synthesised by various methods such as metal vapours synthesis\(^8\), laser irradiation\(^9\), exploding wire method\(^10\), vacuum vapour deposition, and microemulsion\(^11\). But these techniques are of high cost and involve the use of toxic chemicals.

The herbal preparation plays an immense role in synthesising the nanoparticles naturally. Nanoparticles synthesised from plant extract is able to overcome many toxic effects of conventional methods\(^12\). Green synthesis of nanoparticle is a very simple, economical, eco-friendly and repeatable method which does not require intense energy, pressure, temperature, or toxic chemicals\(^13\). The previous studies showed that the antimicrobial and anticancer activities of nanoparticles synthesised from plant extract is better\(^14\). The green synthesis techniques usually uses water, biological extracts, biological systems, and microwave-assisted synthesis which is non toxic and eco-friendly.

There are many methods of green synthesis of nanoparticles. The commonly used methods for green synthesis are using biological routes using microorganisms such as bacteria, yeast, plant and animal extracts and enzymes or their byproducts\(^15\). These are most commonly used because they are eco-friendly, non-toxic, cost efficient, and mild conditions.

The objective of study was green synthesis of copper nanoparticles using green tea (Camellia Sinensis) and neem formulation and assessment of its antimicrobial effects.

Materials and Methods

Preparation of Plant Extract

Green tea and neem were purchased from the herbal care center. 2 grams of green tea and 2 grams of neem were measured and added to a conical flask and were dissolved in 100 ml of distilled water. Then the extract was heated at 60°C for 7-8 minutes in a heating mantle. Using a blotting paper the extract was then filtered into another conical flask using a Whatmann No. 1 filter paper. The filtrate which is in the conical flask is needed plant extract.

Synthesis of Silver Nanoparticles

20 millimoles of copper sulphate is mixed with 40 mL of plant extract and 60 mL of distilled water in a conical flask. The flask was then placed in the orbital shaker at 65 rpm and then in a magnetic stirrer at 450 rpm for uniform distribution. The color change was noted regularly with an interval of one hour for two days. With the help of UV spectroscopy the prepared copper containing green tea and neem extract was recorded to check for the synthesis of nanoparticle. It was then subjected to centrifugation at 10,000 rpm for 10 mins in a centrifuge. The copper nanoparticle pellets were then collected to perform antimicrobial activity tests.

Characterisation of nanoparticles

Once the nanoparticles are synthesised the solution is characterised by using UV- vis-spectroscopy.
the solution is taken in cuvette and scanned in double beam a UV-visible spectrophotometer (ELICO SL 210 UVVis spectrophotometer) from 300 nm to 700 nm wavelengths and the results were recorded graphically.

**Antimicrobial activity of copper nanoparticle**

The antimicrobial efficiency of copper nanoparticles were assessed using agar well diffusion method. The antibacterial activity of copper nanoparticles tested against four different bacterial isolates like *E. Faialis, Streptococcus mutans, Streptococcus aureus and Candida albicans*. Fresh bacterial cultures were prepared on the surface of Muller-Hinton agar plates in a broth medium. Different concentrations of copper nanoparticles (25, 50, and 100 μL) were incorporated into the wells and the plates were incubated at 37°C for 24 hours. The antibiotic discs (ampicillin) were used as control. After incubation, the zone of incubation formed around the discs were measured and notes down.

**Results**

**Visual observation**

Nanoparticles have a great interest due their unique optical properties. During their process of synthesis they exhibit a different range of colours. The plant extract contains various phytochemicals that converts the copper sulphate into copper nanoparticles identified by the colour change. This colour change from green to brown indicating the formation of copper nanoparticles (Fig 1).

**UV-visible Spectroscopy**

The copper nanoparticles were synthesized using copper sulphate and green and neem extract displays an absorption peak at 340 nm (Fig 2). This peak was associated with the synthesis of copper nanoparticles. The broadened SPR peak that was observed in the UV-visible spectrum confirmed that polydispersed nanosized particles are synthesized.

![Figure 2. Graphical representation of synthesis of copper nanoparticles.](image)

**Antibacterial activity of copper nanoparticles against oral pathogens**

Copper has an excellent antimicrobial activity against a wide range of oral pathogens and this property is enhanced with its nanoparticle form. The zone of growth inhibition of cells is because of the distraction of cell membrane by copper nanoparticles, which leads to the break down of cell enzymes. The results of our study reveals that the green tea and neem mediated copper nanoparticles showed effective antibacterial activity (Fig 3, 4).
Discussion

Copper as a metal is well known for its antimicrobial and anti-inflammatory properties since several years\(^\text{18}\). The copper nanoparticles can be easily synthesized and is cost-effective material so it can be an alternative for gold and silver nanoparticles\(^\text{19}\). Currently, environmental friendly synthesis of copper nanoparticles has gained much interest\(^\text{20}\). Green synthesis of nanoparticles is more trending as the plant itself acts as both reducing and capping agent also because of its eco-friendly\(^\text{21}\). Green tea (Camellia sinensis) contains many phytoconstituents like epigallocatechin-3-gallate (EGCG), theanins, catechins and polyphenols and is reported to have neuroprotective activity\(^\text{22}\). It is reported that they even prevent proliferation of carcinoma cells\(^\text{23}\). Green tea is considered as a reducing agent for the synthesis of the different morphology of copper nanoparticles because it contains a high amount of polyphenols and other organic groups in it. The reduction mechanism happens in two steps initially when the precursor is added a complex is formed by breaking the \(-\text{OH}\) bond and a partial bond with a metal ion is formed. Then there is transfer of electrons wherein the metal ions gets reduced to nanoparticles by the breakage of the partial bond, and then it get oxidized to ortho-quinone\(^\text{24}\). Green synthesis of copper nanoparticles using green tea and neem formulation is a simple, economical, eco-friendly process. In our study the colour change indicates the synthesis of copper nanoparticles which is in accordance with the previous studies\(^\text{25}\). In general UV-vis spectra can be used for assessing the size and shape-controlled nanoparticles in the aqueous solution with 200-800nm wavelength range\(^\text{26}\). In our study it was observed that the copper nanoparticles have good

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Figure 3: Antimicrobial activity of copper nanoparticle synthesized from green and neem formulation against S.mutans, C.albicans, E.faecalis, S. aureus.

Figure 4: Graphical representation of antimicrobial activity of copper nanoparticle synthesized from green and neem formulation against S.mutans, C.albicans, E.faecalis, S.aureus. S. aureus is having almost comparable antimicrobial activity as that of the control antibiotic disc.
antimicrobial properties against oral pathogens. The zone of inhibition is increased with the increase in concentration of copper nanoparticles. These results were homogeneous when CuNPs synthesized using glycerol-polyvinyl alcohol, polyurethane with silver and copper nanoparticles, copper based additives and copper-resistant Bacillus cereus.

### Conclusion

Copper nanoparticles were efficiently synthesised from green and neem formulation. The use of toxic chemicals is avoided since the nanoparticles are synthesised using green synthesis method, which is non toxic, economical and eco friendly. These copper nanoparticles showed good antibacterial properties also. Since it is effective against the oral pathogens and they can be used in toothpaste and oral medicines. Hence the copper nanoparticles could be expected to be used in future for the effective drug systems and immunity against diseases.

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### Conflict of Interest: Authors declare no conflict of interest

### Ethical Clearance: Ethical clearance taken from Saveetha University, ethical approval number is IHEC/SDC/ORTHO-1902/21/286.

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12. Rajeshkumar S, Bharath LV. Mechanism of plant-mediated synthesis of silver nanoparticles – A review on biomolecules involved, characterisation and antibacterial activity [Internet]. Vol. 273, Chemico-


Determinant Factors Related to the Unmet-Need of Family Planning Program among Married Women in Indonesia

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Abstract

Unmet family planning is one of the common causes of low contraceptive prevalence rates in developing countries, including Indonesia. Contraceptive in family planning is used to monitor family planning. Globally, the unmet for family planning remains high. The population growth rate is due to birth (fertility), death (mortality), and population. The high population growth is one of the problems in Indonesia and other developing countries. One of the population growth efforts is carrying out a family planning program to control fertility because it is critical for managing population growth, especially in Indonesia [1]. Program from the Indonesian government contains population growth, including the Family Planning Program, Reproductive Health, Prosperous family, and family empowerment. Based on the Indonesian Demographic Health Survey, it is evident that the proportion of unmet needs for family planning in 2012 was 11.4%, and in 2017 10.6%. This study aims to factors associated with the family planning program used by married women. Thus, this study is designed to assess the prevalence and associated factors of unmet needs of family planning programs.

This study uses secondary data from 2017 IDHS carried out in 34 provinces in Indonesia. The total sample was 34,086 of 49,627 mother’s aged 15-49 years. The dependent variable was the family planning program. The independent variables consisted of socio-demographic and socioeconomic factors, knowledge, discussion with husband, and access to health services. Data analysis was performed through a binary logistic regression test to obtain the adjusted odds ratio of each factor. Data analysis using binary logistic regression STATA.

The results showed that determinant factors related to the unmet-need of family planning program were the use of contraception (p=0.000), maternal age (p=0.000), mother’s education (p=0.000), number of children’s ownership (p=0.000), history of child death (p=0.000) 0.000), wealth index (p=0.011), province of residence (p=0.000), knowledge about contraceptive use (p=0.000) and ever used anything to delay getting pregnant (p=0.000). The proportion of unmet needs in this analysis was 61% with p-value= 0.000 OR= 2.49 (95% CI= 2.43- 2.56).

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The proportion of unmet needs in Indonesia is still high. Programs intervention should be targeted at women to decrease associated factors, especially in the productive period.

**Keyword:** unmet-need, family planning

**Introduction**

Indonesia was a country with a population in the fourth position globally with growth relatively high. The point of the task in family planning, in this case, was clear, lower fertility to reduce the burden of development to achieve happiness and prosperity and the nation of Indonesia. Family planning is a key objective and the main feature for sustainable development goals (SDGs) launched in 2015 by the United Nations. Reproductive health is explicitly mentioned in goal three on good health and wellbeing but may also be considered as part of goal 5, which aims at gender equality and women’s empowerment[1].

Family planning has proven benefits for individuals and society as it prevents unintended pregnancies, limits the number of children, and controls birth intervals and timing[2]. Generally, it has been observed that the unmet need for family planning is inversely related to contraceptive prevalence 8.9, with a few exceptions. As contraceptive uptake increases, the level of unmet need reduces. Globally, the contraceptive prevalence has increased 5.10, and the unmet need for contraceptive use has decreased. The large population in Indonesia can cause various problems such as stunted economic growth, increasing unemployment, crime, and abuse [3]. The government continues its efforts to overcome or suppress the population rate. One of the government’s efforts to reduce population growth in Indonesia is the family Planning Program. The population problem in Indonesia is characterized by an increasing population, unequal population distribution, and an unfavorable situation for the population’s age structure where there are many productive ages[4].

Good information from health providers in helping a client choose and determine the type of contraceptive used. Good communication will increase client satisfaction impact. Contraceptive use longer, thus assisting the success of family planning. In addition, the husband also supports affect the use of contraception. The client is provided with support by the husband will be using contraceptives continuously while the husband would not have the support.[4]

A study in Indonesia using the 2017 IDHS secondary data analysis found that the associated factor related to unmet needs family planning program is communication with husbands. There is no publication of determinants of unmet needs using IDHS 2017 data. In the past five years, there have been considerable changes in Indonesia, including infrastructure, national health insurance program, and the significant increase budget allocated to all villages throughout, with a possible impact in decreasing the unmet need for family planning. This study aims to determine the factors associated with unmet needs for family planning in Indonesia by analyzing the 2017 IDHS data. Thus, this study is designed to assess the prevalence and associated factors of unmet needs of family planning programs.

**Methods**

This study refers to secondary data from the 2017 IDHS. The IDHS data sets were download online after registration and notification to Indonesia’s National Population and Family Planning Board. The 2017 IDHS survey was conducted on 35,681 women of childbearing age (15-49 years) using a two-stage stratified sampling and thorough study at the national level. Detailed information about study design, sample size calculations, survey instruments, data collection, and other survey procedures are available in the 2017 IDHS report.

In our analysis, eligible subjects are women of productive age and married marital status. Aged 15-49 years who married/living together with a partner. The total number of analyzed subjects or samples was 34.086 from 49.627 all women. Excluded subjects were menopausal or infertile women and women with marital status banned married. The dependent variable is the unmet need for a family planning program. And the independent variable is the
mother’s age, education, number of living children, total children ever born, birth in last year, birth in the month of interview, wealth index, province, place of residence, knowledge of any method, ever used anything to delay getting pregnant, current contraceptive method. Mother’s age is < 20 years old, 20-29 years old, 30-39 years old and more than 40 years old. Education are ranked as a university, graduated from high school, not graduated from high school, graduated from elementary school, not graduated from elementary school, and never go to school. Number of living children and total children ever born is categorized not having 1-2 son and more than 2 sons. Birth in last children and birth in the month of interview is categorized as not having 1-2 son and more than 2 sons.

Wealth index is categorized poorest, poorer, middle, richer, richest. Regions are categorized as “Sumatera,” “Java and Bali,” “Kalimantan,” “Sulawesi.” The place of residence is classified as “rural” and “urban.” Bivariate analysis was performed with a chi-square test to display data on the characteristics of women of childbearing age with unmet needs. The bivariate analysis results are used for developing a model of the multivariate analysis. Multivariate analysis was performed with a binary logistic regression using the backward method to obtain the adjusted odds ratio of each variable.

**Result**

**Table 1: Analysis Factor Family Planning in Indonesia Demographic Health Survey 2017**

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<td>p value = 0.003 OR =0.95 (0.92- 0.98)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Number of living children</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>382 (1.12%)</td>
<td>2.176 (6.38%)</td>
</tr>
<tr>
<td>≤2</td>
<td>17.701 (51.93%)</td>
<td>1.980 (5.80%)</td>
</tr>
<tr>
<td>&gt;2</td>
<td>11.033 (33.16%)</td>
<td>814 (2.39%)</td>
</tr>
<tr>
<td>p value = 0.000 OR =2.37 (2.28- 2.47)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Variable Family Planning Program

<table>
<thead>
<tr>
<th></th>
<th>Family planning</th>
<th>Do not family planning program</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of children died</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>12.278 (36.02%)</td>
<td>1.019 (2.99%)</td>
</tr>
<tr>
<td>Yes</td>
<td>19.473 (57.13%)</td>
<td>1.316 (3.86%)</td>
</tr>
<tr>
<td><strong>p value</strong></td>
<td>0.000 OR =0.81</td>
<td>(0.748 – 0.886)</td>
</tr>
<tr>
<td><strong>Wealth Index</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poorest</td>
<td>4.441 (13.03%)</td>
<td>3.252 (9.54%)</td>
</tr>
<tr>
<td>Poorer</td>
<td>4.199 (12.32%)</td>
<td>2.472 (7.25%)</td>
</tr>
<tr>
<td>Middle</td>
<td>4.169 (12.23%)</td>
<td>2.454 (7.2%)</td>
</tr>
<tr>
<td>Aricher</td>
<td>4.062 (11.92%)</td>
<td>2.559 (7.5%)</td>
</tr>
<tr>
<td>Richest</td>
<td>3.918 (11.49%)</td>
<td>2.560 (7.51%)</td>
</tr>
<tr>
<td><strong>p value</strong></td>
<td>0.011 OR =1.02</td>
<td>(1.00 – 1.035)</td>
</tr>
<tr>
<td><strong>Place of residence</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>10.479 (30.74%)</td>
<td>10.310 (30.25%)</td>
</tr>
<tr>
<td>Rural</td>
<td>6.742 (19.78%)</td>
<td>6.555 (19.23%)</td>
</tr>
<tr>
<td><strong>p value</strong></td>
<td>0.593 OR =1.01</td>
<td>(0.96 – 1.05)</td>
</tr>
<tr>
<td><strong>Province</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sumatera</td>
<td>7.385 (21.67%)</td>
<td>1.383 (4.06%)</td>
</tr>
<tr>
<td>Jawa</td>
<td>10.407 (30.53%)</td>
<td>1.314 (3.86%)</td>
</tr>
<tr>
<td>Bali- Nusa Tenggara</td>
<td>2.286 (6.7%)</td>
<td>468 (1.37%)</td>
</tr>
<tr>
<td>Kalimantan</td>
<td>2.815 (8.26%)</td>
<td>322 (0.95%)</td>
</tr>
<tr>
<td>Maluku- Papua</td>
<td>6.223 (18.26%)</td>
<td>1.483 (4.35%)</td>
</tr>
<tr>
<td><strong>p value</strong></td>
<td>0.000 OR =0.92</td>
<td>(0.91- 0.94)</td>
</tr>
<tr>
<td><strong>Knowledge of any method</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No method</td>
<td>0</td>
<td>152 (0.44)</td>
</tr>
<tr>
<td>Traditional method</td>
<td>10 (0.03)</td>
<td>12 (0.035)</td>
</tr>
<tr>
<td>Modern method</td>
<td>20.779 (60.96%)</td>
<td>13.133 (38.53)</td>
</tr>
<tr>
<td><strong>p value</strong></td>
<td>0.000 OR =8.48</td>
<td>(5.17 – 13.91)</td>
</tr>
<tr>
<td><strong>Ever used anything to delay getting pregnant</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>0 (0)</td>
<td>4.970 (14.58)</td>
</tr>
<tr>
<td>Yes</td>
<td>20.789 (60.99%)</td>
<td>8.327 (24.43)</td>
</tr>
<tr>
<td><strong>p value</strong></td>
<td>0.000 OR =2.49</td>
<td>(2.43 – 2.56)</td>
</tr>
</tbody>
</table>

**Mother’s age**

Maternal age factor is one factor that influences family planning; this is indicated by = 0.000 were associated AOR 0.71 (95% CI [0.71-0.76]). The majority of mothers aged 30-39 years support the family planning program as many as 8,883 people (26.06%).

**Education**

The mother’s education factor is a factor that affects family planning; this is indicated by =0.003 were associated AOR 0.95 (95%CI [0.92-0.98]) with most of the mothers who participated in the family planning program mostly secondary education or high school level as many as 11,105 people (32.5%).
Number of living children and children died

The factor of the number of children living and children died is a factor that affects family planning, this is indicated by $= 0.000$ were associated AOR 2.37 (95% CI [2.28-2.47]) and in children who die are also factors that affect family planning, this is indicated by $= 0.000$ were associated AOR 0.81 (95% CI [0.748-0.886]) with most having children less than equal two.

Wealth Index

The wealth index is a factor that affects family planning; this is indicated by $= 0.011$ were associated AOR 1.02 (95% CI [1.00-1.035]) with some who follow the family planning program having a low wealth index or poorest of 4.441 people (13.03%).

Place of residence

Place of residence and region/province are factors that affect family planning; this is indicated by $=0.593$ were associated AOR 1.01 (95% CI [0.96-1.05]) while province also affected family planning as indicated by $=0.000$ were associated AOR 0.92 (95% CI [0.91-0.94]) with 10,479 people mostly living in urban areas and 10,407 people (30.53%) living in Java.

Knowledge of any method of contraception

Knowledge of any method of contraception is a factor that affects family planning; this is indicated by $= 0.000$ were associated AOR 8.48 (95% CI [5.17-13.91]) with most of the users knowing modern contraceptive methods as many as 20,779 people (60.96%).

Using contraception and ever used anything to delay getting pregnant

Contraceptive use factors and slowing pregnancy affect family planning; this is indicated by $= 0.000$ were associated AOR 2.49 (95% CI [2.43-2.56]) with the majority of delaying pregnancy as many as 20,789 people (60.99%).

Discussion

This study assesses the determinants of contraceptive use among married women in Indonesia. The benefits of using contraception are to delay or space getting pregnant and to limit the number of children

Mother’s age

Maternal age factor is one factor that influences family planning; this is indicated by $= 0.000$ were associated AOR 0.71 (95% CI [0.71-0.76]). The majority of mothers aged 30-39 years support the family planning program as many as 8,883 people. We first found that mother’s age was significantly associated with contraceptive use among married women, especially older age. This result is consistent with the previous studies conducted in Ghana and Nigeria, documenting that older women have a lower concern with modern contraceptive use 7,9. This finding is related to their less active sexual desires.

At this age, it is a period of achieving work success, stability in lifestyle, attitudes, values of life, and a good and healthy diet to maintain health. At that age, it is a mature age to have a baby and have more experience than at a younger generation so that an older mother will be more accepting of her pregnancy[5].

Based on the table, it is known that according to the age of the mother, most of the respondents who need family planning are at risk, namely $<20$ years or $>35$ years. The results can obtain the results of these statistical test calculation using the chi-square as presented in the table can be obtained p-value $0.001<\alpha$ (0.05) so that there is a significant relationship between maternal age and statistically unwanted pregnancy ($p=0.001$, CI 95%). The unmet need based on this study can occur at various ages, both at the young and old reproductive ages. In this study, the most incidence of unmet need were respondents aged $>35$ years. They were no longer reproductive and considered themselves old at that age, so the possibility of pregnancy was very slight. Based on the results of the 2007 IDHS shows that there is a significant relationship between the respondent’s age and the status of unmet need or unmet need for family planning. The older age of the woman causes it. The more experience she will have in using family planning to choose a suitable family planning tool or method and minimize the experience of unmet family planning needs. A woman’s age will affect the psychological and physiological aspects of experience in family planning and not only affect a woman’s motivation to control her fertility.
Education

The mother’s education factor is a factor that affects family planning, and this is indicated by $= 0.003$ were associated AOR 0.95 (95%CI [0.92-0.98]) with most of the mothers who participated in the family planning program mostly secondary education or high school level as many as 11,105 people.

The average education of the respondents is moderate education or equivalent to high school; education is one of the factors that affect knowledge.

Following research conducted in India, which showed that the higher the mother’s education level, the lower the number of want to pregnancy A woman’s level of education is related to her ability to capture available information such as awareness, the value of small family benefits and knowledge about contraception and family planning. A high level of education allows a person to have a higher level of knowledge. It is easier to accept something new, especially if it is beneficial for himself and his family. Including in terms of preventing pregnancy with various kinds of contraception. Education also increases a person’s awareness of the benefits of having fewer children so that can encourage someone to take part in family planning.[6][7][8].

Number of living children and children died

The factor of the number of children living and children dying is a factor that affects family planning, this is indicated by $= 0.000$ were associated AOR 2.37 (95% CI [2.28-2.47]) and in children who die are also factors that affect family planning, this is indicated by $= 0.000$ were associated AOR 0.81 (95% CI [0.748-0.886]) with most having children less than equal to two or more than two as many as 17,701 people.

Parity is the number of children born to the mother, both alive and dead. mother with primipara is defined as a woman who has given birth for the first time where the fetus has reached the age of 28 weeks or more. Parity has a significant relationship with the incidence of unwanted pregnancy. The more children ever born, the higher the chances of unwanted pregnancies. The 2012 IDHS shows that the proportion of unwanted pregnancies also increases with the order in which children are born[1]. Unmet needs for family planning can also occur in low parity or high parity. Some couples of childbearing age want to delay pregnancy, space, or end pregnancies but do not use contraception for reasons of fear of side effects when using contraception and, if using contraception, are afraid that fertility will not return immediately. The pattern of contraceptive use is different between women with high parity and low parity. Contraceptive use increased in women with high parity. The number and sex of living children significantly influence accepting the family planning method. The more the number of children still alive, the more contraception will increase. Women with one living child have lower contraceptive use than those with two or more than three children. Women with a small number of children desire to have children of a different gender.

Wealth Index

The wealth index factor is a factor that affects family planning; this is indicated by $= 0.011$ were associated AOR 1.02 (95% CI [1.00-1.035]) with some who follow the family planning program having a very poor wealth index of 4.441 people. This is relevant where someone with more children has more living costs in the family. Low use among contraception in poor women, its related to sociodemographic status[9,10].

Place of residence

Place of residence and region/province factors are factors that affect family planning; this is indicated by $=0.593$ were associated AOR 1.01 (95% CI [0.96-1.05]) while province also affected family planning as indicated by $=0.000$ were associated AOR 0.92 (95% CI [0.91-0.94]) with 10,479 people mostly living in urban areas and 10,407 people living in Java.

This is relevant where the distribution of the majority of Indonesia’s population is on the island of Java.

Knowledge method of contraception

Knowledge of any method of contraception is a factor that affects family planning; this is indicated by $= 0.000$ were associated AOR 8.48 (95% CI [5.17-13.91]) with most of the users knowing modern contraceptive methods as many as 20,779 people.

According to Green’s theory (1980), predisposing factors are one of the factors that motivate to behave. Knowledge of contraceptives is one of the predisposing factors for family planning. This is following previous research, which showed a relationship between contraceptive methods and
family planning knowledge. In addition, individuals who have good knowledge will be better able to accept reasons to practice because they have good knowledge and attitudes about family planning as a basis for behavior and support for family planning programs[10–12].

Using contraception and ever used anything to delay getting pregnant

Contraceptive use factors and slowing pregnancy affect family planning; this is indicated by \(= 0.000\) were associated AOR 2.49 (95% CI [2.43-2.56]) with the majority of delaying pregnancy as many as 20,789 people.

However, family planning decisions can be heavily influenced by men’s socioeconomic characteristics, views, and perceptions about family planning services [13, 14].

Conclusions

Determinant factor related to unmet family planning programs are the use of contraception where most use modern contraception (e.g., IUDs, injections, implants, etc.) to delay pregnancy with \(p\) value\(= 0.000\), mother’s age where the majority are aged 30-39 years, education factor, wealth index, province and knowledge. While the place of resident is not a determinant factor related to unmet family planning.

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Reference


Dental Prosthetic Status and Prosthetic Needs of Geriatric Population: A Cross Sectional Study

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1D-Care Dental Clinic, Consultant Prosthodontist, Kerala, 2Professor and Head, Department of Prosthodontics, AJ Institute of Dental Sciences, Mangalore, India, 3Assistant Professor, Department of Prosthodontics, Manipal College of Dental Sciences, Manipal, Manipal Academy of Higher education, Manipal, India, 4-6Assistant Professor, Department of Prosthodontics, AJ Institute of Dental Sciences, Mangalore, India.


Abstract

Objective: Oral health is considered as an indicator for health and quality of life in geriatric patients. It becomes essential to assess the prosthetic status and need in order to promote oral health. Hence, a survey was conducted on geriatric patients visiting a dental teaching institute in India.

Materials and Method: A cross-sectional survey was conducted among geriatric population aged above 60. The sample size was estimated to be 570. The information related to prosthetic status, prosthetic need was obtained using the World Health Organisation Oral Health Assessment Form 1997. Chi-square test was used to analyse the data.

Results: Out of 570 elderly people, 308 were males and 262 were females. The study results showed that 77% and 78% of the total subjects had no prostheses in maxillary and mandibular arch, respectively. Prosthetic status was better in males than in females. The level of prosthetic need was higher in females (82%) in mandibular arch compared to males (75%). In the age group of 80-85 years need for complete denture was more. Requirement for single and multi unit prosthesis was higher in age groups of 60-65 years. Less percentage of subjects in the upper socio-economic categories needed prosthesis compared to those in the lower socio-economic categories.

Conclusion: Unmet prosthetic needs were more in geriatric population attending the institutional outpatient department. There was no significant association of gender with prosthetic status and need and significant association was observed between age and socioeconomic status with prosthetic status and need.

Keywords: Prosthetic status, prosthetic need, complete denture, geriatric population.

Introduction

The lifespan of an individual has improved with the advancements in the field of medical science and improvement in social conditions.1 Oral health is an important part of general health and plays a crucial role in improving the quality of life.2 An improved
oral health allows geriatric patients to gain self-confidence, increase social networking, physical and mental well being. Untreated dental caries and periodontal diseases can lead to tooth loss which is quite common in developing countries such as India.

Often oral health is ignored because of the poor awareness and financial constraints. It is usually sought only for emergencies such as pain relief. Hence, extraction of painful tooth is an only option, which makes the person partially or completely edentulous. Usually, the prosthodontic treatment is denied by most of the patients due to the expense prevailing it. Systematic assessment of prosthetic treatment needs is essential to determine unmet oral health care needs and to bring about beneficial change to the oral health of the population.

Individuals with poor dentition consume soft, easily chewable foods that are low in dietary fiber and have low nutrient density which may affect the general health. Therefore, for optimum oral health in geriatric population, there is a need to find out prosthetic status and prosthetic need. The baseline information extracted may help health agencies to formulate policy, plan and monitor oral health services. Hence, the objective of this study was to evaluate the prosthetic status and treatment needs of geriatric population attending a dental institution in India.

Materials and Methods

A cross sectional study was conducted among the geriatric patients who visited the outpatient department of Prosthodontics, of a dental institution in India. Sample size was estimated to be 570. The inclusion criteria considered were participants aged 60 years and above who were willing to provide signed informed consent.

Data on general information of the participants was collected as per Annexure II. Modified Kuppuswamy socio-economic scale was used to classify subjects according to socio-economic status.

Prosthetic status and needs was clinically assessed according to the criteria described by the World Health Organization (WHO) and Oral Health Assessment form 1997. Data was analysed using the statistical package SPSS 22.0 (SPSS Inc., Chicago, IL) and level of significance was set at p<0.05. Descriptive statistics was performed to assess the proportion of each category of the respective groups. Normality of the data was assessed using Shapiro Wilkenson test. Inferential statistics to find out the difference between the groups was done using chi square test to find out the association between the groups. The level of significance was set at <0.05. Analysis was done using chi-square test.

The study was conducted after obtaining permission from the institutional ethics committee.

Results

A total of 570 subjects comprising 262 females (46%) and 308 males (54%) aged 60 years and above (mean age 71.2±7.7 years) formed the study population.

77% and 78% of the subjects had no prosthesis in their maxillary arch and in mandibular arch respectively. Evaluation of prosthetic status showed no significant differences between the gender in the maxillary (X² = 0.509; P = .99) and mandibular arches (X² = 6.075; P = .29) respectively. No significant association between prosthetic status and gender (p<0.05) (Table 1). The need was highest for complete prosthesis (37% and 38% in the maxillary and mandibular arch) followed by the need for a multiunit prosthesis (28% and 24% in maxillary arch mandibular arch); 23% and 22% subjects did not require any prosthesis in maxillary arch and mandibular arches respectively. Evaluation of prosthetic needs showed no significant differences between the gender in the maxillary (X² = 0.509; P = .99) and mandibular arches (X² = 6.075; P = .29) respectively. No significant association was found between prosthetic needs and gender (p<0.05) (Table 2).
Table 1: Distribution of study subjects according to gender & prosthetic status of their lower and upper arch.

<table>
<thead>
<tr>
<th>Prosthetic status</th>
<th>Male (Lower)</th>
<th>Female (Lower)</th>
<th>Total</th>
<th>X2 value</th>
<th>P value</th>
<th>Male (Upper)</th>
<th>Female (Upper)</th>
<th>Total</th>
<th>X2 value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No prosthesis</td>
<td>230(75%)</td>
<td>215(82%)</td>
<td>445(78%)</td>
<td>6.075</td>
<td>0.29</td>
<td>230(75%)</td>
<td>215(82%)</td>
<td>445(78%)</td>
<td>0.509</td>
<td>0.99</td>
</tr>
<tr>
<td>Bridge</td>
<td>13(4%)</td>
<td>6(2%)</td>
<td>19(3%)</td>
<td></td>
<td></td>
<td>13(4%)</td>
<td>6(2%)</td>
<td>19(3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>More than one bridge</td>
<td>2(0.6%)</td>
<td>1(0.3%)</td>
<td>3(0.5%)</td>
<td></td>
<td></td>
<td>2(0.6%)</td>
<td>1(0.3%)</td>
<td>3(0.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partial denture</td>
<td>37(12%)</td>
<td>19(7%)</td>
<td>56(10%)</td>
<td></td>
<td></td>
<td>37(12%)</td>
<td>19(7%)</td>
<td>56(10%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Both bridge &amp; partial bridge</td>
<td>1(0.3%)</td>
<td>0</td>
<td>1(0.1%)</td>
<td></td>
<td></td>
<td>1(0.3%)</td>
<td>0</td>
<td>1(0.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full removable denture</td>
<td>25(8%)</td>
<td>21(8%)</td>
<td>46(8%)</td>
<td></td>
<td></td>
<td>25(8%)</td>
<td>21(8%)</td>
<td>46(8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>308(100%)</td>
<td>262(100%)</td>
<td>570(100%)</td>
<td></td>
<td></td>
<td>308(100%)</td>
<td>262(100%)</td>
<td>570(100%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05 is statistically significant

Table 2: Distribution of study subjects according to gender & the prosthetic need of their lower and upper arch.

<table>
<thead>
<tr>
<th>Prosthetic needs</th>
<th>Male (Lower)</th>
<th>Female (Lower)</th>
<th>Total</th>
<th>X2 value</th>
<th>P value</th>
<th>Male (Upper)</th>
<th>Female (Upper)</th>
<th>Total</th>
<th>X2 value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No prosthesis required</td>
<td>78(25%)</td>
<td>47(18%)</td>
<td>125(22%)</td>
<td>2.579</td>
<td>0.63</td>
<td>72(23%)</td>
<td>57(23%)</td>
<td>129(23%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Need for single unit prosthesis</td>
<td>30(10%)</td>
<td>35(13%)</td>
<td>65(11%)</td>
<td></td>
<td></td>
<td>25(8%)</td>
<td>20(8%)</td>
<td>45(8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Need for multi-unit prosthesis</td>
<td>75(24%)</td>
<td>63(24%)</td>
<td>138(24%)</td>
<td></td>
<td></td>
<td>84(27%)</td>
<td>76(30%)</td>
<td>160(28%)</td>
<td>0.535</td>
<td>0.97</td>
</tr>
<tr>
<td>Needs for combination of single or multi-unit prosthesis</td>
<td>10(3%)</td>
<td>15(6%)</td>
<td>25(4%)</td>
<td></td>
<td></td>
<td>10(3%)</td>
<td>13(5%)</td>
<td>23(4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Need for full mouth prosthesis</td>
<td>115(37%)</td>
<td>102(39%)</td>
<td>217(38%)</td>
<td></td>
<td></td>
<td>117(38%)</td>
<td>96(38%)</td>
<td>213(38%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>308(100%)</td>
<td>262(100%)</td>
<td>570(100%)</td>
<td></td>
<td></td>
<td>308(100%)</td>
<td>262(100%)</td>
<td>570(100%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05 is statistically significant

78% of the subjects had no prostheses in their mandibular arch. Among the age group of 60-65 years 83% of them did not have any prosthesis. 81% of the population in the age group of 65-70 years needed prosthesis in maxillary arch. Evaluation of subjects showed significant differences between the age and the prosthetic status of their maxillary arches (X2 =64.579; P = 0.0001) and mandibular arches (X2 =54.413; P = 0.0005).

Among the age group of 80-85, 72% needed complete prosthesis in the maxillary and mandibular arch. Need for multi unit prosthesis was highest in the age group of 60-65 years. Evaluation of subjects according to age and the prosthetic need of their maxillary arches showed significant differences between the age (X2 = 137.28; P = .0001) whereas in the mandibular arch, the difference was significant (X2 = 150.811; P = 0.0001).

86% of upper middle and lower middle class subjects had not opted for prosthetic rehabilitation in the upper arch. In mandibular arch 87% of lower middle-class subjects did not replace the missing teeth. Evaluation of subjects showed significant differences between the socioeconomic status and the prosthetic status of their maxillary arches (X2 =119.232; P = 0.0001) and mandibular arches (X2 =103.259; P = 0.0001). Significant association was found between prosthetic status of mandibular arch, maxillary arch and socioeconomic status (p<0.05)

The need of complete prosthesis was highest in lower middle-class subjects. Prosthetic need, according to socio-economic status was statistically significant in the maxillary (χ2 = 146.375, P = 0.0001)
and mandibular arch ($\chi^2 = 146.613, P = 0.0001$). Significant association was found between prosthetic need and socioeconomic status ($p<0.05$)

**Discussion**

Prosthetic status among the geriatric patient was unsatisfactory which may be attributed to older people not using the available dental facilities, lack of awareness, financial constraints, reduced mobility, misconceptions about dentures and lack of interest. The result was similar to the studies done by Choudhury GK et. al. and Soh et. al.

Prosthetic status was better in males than in females which could be attributed to a lower level of education, lack of access, dependency on male members and unemployment. The difference was not significant.

The clinical possibilities to prosthetic replacement for each patient according to the missing teeth were significantly different from patient desire. The level of prosthetic need was higher in females in mandibular arch compared to males.

Patients aged 70 years and above had better prosthetic status compared to other age groups. This may be due to geriatric patients having lower expectations and frequently retaining them beyond their useful life which may be either due to economic circumstances or a learned pattern of functioning. Prosthetic status was better with increasing age. Similar findings were found in study conducted by Pavan T P et. al. This could be an indication of a lower subjective demand for prosthetic care among partially edentulous subjects when compared to edentulous subjects.

Requirement for one-unit prosthesis was higher in age groups of 60-65 years (17% in mandibular arch, 8% in maxillary arch), the need for multi-unit prosthesis was higher in age group of 60-65 years (35% in mandibular arch and 39% in maxillary arch). The need for complete denture was more in age group of 80-85 years (72% both in maxillary and mandibular arch). Requirement of prosthesis increases with advancement of age with complete edentulism and the findings are in agreement with studies done by George et. al., and Hamasha et. al.

Tooth loss without prosthetic intervention in elderly results in psychological feeling of inferiority, mental stress due to general appearance and malnutrition. Rehabilitation of the physical, psychological and social state of elderly, along with restoration of masticatory function plays a significant in improving the quality of life.

Prosthetic status was better in lower socioeconomic group compared to the upper socioeconomic groups which is in agreement with the studies by Hanson et. al. Eklund et. al. The social pressure of maintaining aesthetics and functions in upper class may influence people to replace their missing teeth.

An inverse relationship was observed between the socioeconomic status and prosthetic need. Unmet prosthetic needs were approximately three-fold greater than the prosthetic status, which is a cause of concern. The probable reasons could be passivity of enthusiasm for aesthetics, scarcity of information, lack of availability of dental clinics, monetary limitations.

**Conclusion**

Within the limitations of the study, the results showed that unmet prosthetic needs were more in geriatric population attending the institutional outpatient department. There was no significant association of gender with prosthetic status and need and a significant association of age and socioeconomic status with prosthetic status and need.

**Source of support:** Nil

**Conflict of interest:** None

**References**


Abuse of Chemical Substances Cause Poisoning in Dogs and Cats: A Review

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Abstract

Chemical substances are used worldwide as rodenticides, insecticides and herbicides, and also to protect animals against ectoparasites. Poisoning from chemical substances can occur in dogs and cats from both non-accidental and accidental causes. Non-accidental causes include the intentional abuse of chemical substances. This is regarded as animal cruelty and is illegal in many countries. By contrast, accidental causes result from animals ingesting poisonous substances unintentionally such as baits containing rodenticides or eating dead or alive poisoned rodents. Knowledgeable use of poisonous chemicals is vital for the safety of pets. Clinical signs of poisoning in dogs and cats depend on the type of chemical substances, species, lethal dose (LD50) and duration after exposure. This review summarises the common chemical substances that can endanger dogs and cats including rodenticides, insecticides, molluscicides, herbicides, paracetamol and antifreeze chemicals (ethylene glycol).

Key words: Chemical substance, Poison, LD50, Clinical signs, Dogs, Cat

Introduction

Poisoning agents can exist in solid, liquid or gaseous forms.1 When toxic substances enter the body they cause disturbances in the various processes of organ functions.2 Classification of toxic substances is based on several criteria such as chemical structure, physical characteristics, the severity of the poison, the target organ causing toxicity and source of the toxic substances (natural or synthetic).3 Toxicity can be divided into three types as (1) acute toxicity, animals show clinical signs after poisoning once or multiple times within 24 hours, (2) subchronic toxicity, animals show toxicity after consecutive exposure to a small amount of a toxic substance for about 1-3 months and (3) chronic toxicity, animals show toxicity in several organ systems after receiving a small amount of toxic substances for more than three months until toxicity develops.4

Dogs and cats are often reported with toxic chemical exposure.5 Types of toxic chemical substances in pets vary depending on each area. In some areas anticoagulant rodenticides are common, while organophosphates may be found in others.2,5 This review addresses toxic chemical substances often found in dogs and cats including rodenticides, insecticides, molluscicides, herbicides, paracetamol and antifreeze chemicals (ethylene glycol).

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Anticoagulant rodenticides

Anticoagulant rodenticide classification follows two groups of chemical structure: shydroxycoumarine and indandione. Hydroxycoumarine subdivides into first-generation (warfarin, coumachlor and coumafuryl) and second-generation (bromadiolone, brodifacoum and flocoumafen), while the indandione group comprises pindone, chlorophacinone and diphacinone. The mechanism of anticoagulant rodenticides involves impeding the recycling of active vitamin K1 by inhibiting vitamin K epoxide reductase, which discourages the activation of blood clotting factors (II, VII, IX and X), resulting in blood coagulopathy. Dogs and cats can be poisoned by ingestion of baits containing anticoagulant rodenticide substances or ingestion of dead or alive poisoned rodents. Substance quantity that causes toxicity depends on the anticoagulant rodenticide type. Warfarin shows oral toxicity (LD50) in dogs at 11-323 mg/kg, with 20-50 mg/kg in cats. Clinical signs of toxicity in dogs and cats depend on the amount and duration after ingestion and include anorexia, weakness, pale mucous membrane, urination, polydipsia, hematochezia, melena, hemorrhage on the skin and mucous membrane, nasal bleeding, pulmonary edema, intrapulmonary and pleural hemorrhage. Recommended treatment for dogs and cats poisoned during the first 4 hours is ingestion of activated charcoal and emetics, while an antidote (vitamin K1) should be administered immediately.

Bromethalin rodenticide

Bromethalin is a highly potent rodenticide that occurs as an odourless crystalline solid that was developed for anticoagulant rodenticide resistance. The bromethalin rodenticide mechanism involves neurotoxicity by decreasing oxidative phosphorylation within the mitochondria of the central nervous system. This impacts ATP production, resulting in reducing the amount of energy to maintain the function of the Na/K-ATPase pump. As a result, the cells lose the ability to regulate osmotic pressure, causing intracellular sodium accumulation and subsequent cell enlargement from the accumulation of water within the myelin sheath. This promotes vacuolation in the nervous system and reduces the efficiency of nerve impulse, transport. Dogs and cats are intoxicated by ingestion of bait directly or ingestion of carcasses of poisoned rodents. The quantity of the chemical substance that causes oral toxicity (LD50) in dogs is 2.5 mg/kg and 0.45 mg/kg in cats. Clinical signs in dogs and cats depend on the amount ingested and duration after ingestion and include weakness, anorexia, vomiting, paralysis, severe muscle tremors, hyperesthesia, hyperthermia, convulsions, coma and death. There is no specific antidote for bromethalin. Supportive care and preventing gastrointestinal absorption are considered.

Cholecalciferol rodenticide

Cholecalciferol rodenticide or vitamin D3 can be ingested by dogs or cats as baits or as food additions. The mechanism increases renal tubular reabsorption of calcium and decreases parathyroid hormone synthesis. This results in the inability to control calcium and phosphorus levels in the plasma. Oral LD50 in dogs is reported as 88 mg/kg, while some dogs show clinical signs when given 0.5 mg/kg. The toxic dose reported in cats is doubtful but reports suggest that cats are more resistant than dogs. Clinical signs depend on the amount of cholecalciferol ingested and the hypercalcemia situation and present as polyuria, polydipsia, lethargy, abdominal pain, hematemesis, melena, vomiting, diarrhea and seizures. Treatment involves decontamination of the chemical substances and adjusting the calcium-phosphorus level balance in the bloodstream. This chemical substance has no known antidote.

Strychnine

Strychnine is an alkaloid substance extracted as a white powder from the seeds of Strychnosnux-vomica and S. ignatii and used as a rodenticide. The mechanism involves a selective competitive antagonist by inhibiting glycine activity at the glycine receptor, with increased glutamic acid and amino acid levels that act as neurotransmitters involved in stimulating the contraction of muscles in the central nervous system. This results in the skeletal muscles becoming more sensitive to stimulation. Oral toxicities reported in both dogs and cats are 0.5 mg/kg. Clinical signs depend on the amount and duration after ingestion and include ataxia,
muscle stiffness, tachypnea, hypersalivation and seizures. There is no specific antidote for strychnine poisoning. Treatment involves controlling the seizures and maintaining oxygen levels within the body to prevent hypoxia and dehydration.

Zinc/aluminium phosphide

Zinc/aluminium phosphide is a powdered rodenticide that smells like acetylene and occurs in many colors such as grey-black, grey-yellow and brown. The toxicity mechanism of zinc phosphide occurs when the chemical substance hydrolyses in humid or acidic conditions to form phosphine, while aluminium phosphide is hydrolysed at a neutral pH. Phosphine gas irritates the gastrointestinal tract and the respiratory system, inducing free radical and oxidative stress that directly affect cell destruction. The toxic dose in dogs and cats is 20-40 mg/kg. Many factors are related to poisoning including the amount of food in the stomach. Dogs and cats with empty stomachs are more resistant to toxic effects. Toxicity increases when ingested with food because the gastric juices catalyse the hydrolysis reaction and convert phosphide to phosphine. Clinical signs in dogs and cats after ingestion include anorexia, vomiting, hematemesis, melena, cyanosis, ataxia and seizures. Supportive care and preventing gastrointestinal absorption are treatment considerations.

Organophosphate and Carbamate

Organophosphate and carbamate are organic phosphorus compounds that are used as ingredients in various insecticides and pesticides. Contained substances include malathion, parathion, diazinon, fenitrothion, dichlorvos, chlorpyrifos and ethion. Organophosphate and carbamate have similar structural formulae, properties and mechanisms of action. The mechanism works by inhibiting the enzyme acetylcholine esterase, resulting in overstimulation of the muscarinic and nicotinic receptors. LD$_{50}$ in dogs and cats depends on chemical substance types. For marathon, oral toxicity is 500 mg/kg. Clinical signs in dogs and cats of organophosphate and carbamate poisoning depend on the quantity of the insecticide and include hypersalivation, vomiting, depression, dyspnea, ataxia, weakness of hind limbs, bradycardia and seizures. Pralidoxime and atropine are used as antidotes in poisoned pets following supportive care to prevent gastrointestinal absorption.

Organochlorine

Organochlorine insecticides are a group of synthetic chemicals comprising hydrogen, carbon and chlorine. They can accumulate in the environment for a long time with high risk of contamination in the food chain. Organochlorines can be divided according to structural formulations into three groups. The first group contains diphenyl aliphatic compounds such as DDT, methoxychlor, perthane and dicofox. The second group comprises hexachlorocyclohexane and its derivatives such as benzene hexachloride, lindane, gamma hexachlorocyclohexane and paradichlorobenzene, while the third group consists of cyclodienes such as aldrin, dieldrin, endrin, chlordane, heptachlor, endosulfan and isobenzan. The mechanism of toxicity involves stimulation of the central nervous system by disturbing the sodium channel in the neuron membrane. This increases sodium content in the cells and reduces or inhibits potassium exit from the cells, resulting in increased intracellular positive values. Nerves become depolarised, causing pets to have seizures. Dogs and cats are intoxicated through ingestion, inhalation or direct contact. The toxins are well absorbed by ingestion. LD$_{50}$ in dogs and cats depends on the type of chemical substance. Aldrin has oral toxicity of 65-95 mg/kg for dogs and 10-15 mg/kg for cats. Clinical signs in dogs and cats depend on quantity and duration after poisoning and include ataxia, hypersalivation, vomiting and seizures. Supportive care and preventing gastrointestinal absorption are treatment considerations.

Metaldehyde

Metaldehyde is a molluscicide that is used to control snails and scallops in agriculture. Some products such as carbaryl are mixed with insecticides. Metaldehyde has the action mechanism of metaldehyde is unclear but it has been reported to impact γ-aminobutyric acid (GABA) that is involved in CNS transmission. The reduction of GABA decreases other metabolites such as serotonin and norepinephrine, which are associated with a decrease in the seizure threshold. The oral toxic dose in dogs and cats is 100 mg/kg and 207 mg/kg respectively. After ingestion, clinical
signs in dogs and cats may appear as hypersalivation, vomiting, diarrhea, ataxia, anxiety, tachycardia, tremors and seizures. No specific antidote for metaldehyde toxicosis is available and treatment focuses on preventing substance absorption, with supportive care to control the clinical signs.

**Paraquat**

Paraquat is a quaternary nitrogen compound that has been used as a herbicide since 1962 in England and later spread worldwide. The toxicity mechanism occurs when the substance is reduced by nicotinamide adenine dinucleotide phosphate (NADPH) in cells to a substance capable of converting free oxygen molecules into superoxide radicals. Superoxide is the primary oxygen free radical produced in mitochondria that leads to cell death. The oral toxic dose in cats is 35-50 mg/kg. Oral LD$_{50}$ in dogs is unknown but higher than for cats. After intoxication, clinical signs in dogs and cats may appear as vomiting, hematochezia, melena, tachypnea, hyperthermia, and hemorrhage at the mucous membrane. Preventive gastrointestinal absorption and supportive care are essential treatments because there is no specific antidote for this substance.

**Acetaminophen**

Acetaminophen (paracetamol) is a non-steroidal anti-inflammatory drug (NSAID) and most commonly used in humans for mild pain and fever. Cats are susceptible to this drug because they lack methemoglobin reductase enzyme and their red blood cell structure contains up to eight sulfhydryl groups bound to this toxin. Free radicals are produced after the drug’s metabolites produce glutathione and appear as oxidative injury. The free radicals cause hemolysis and result in methemoglobinemia. The oral toxicity dose in cats is less than 60 mg/kg, while for dogs it is over 300 mg/kg. Clinical signs of intoxication in dogs and cats appear as facial swelling, hypersalivation, cyanosis and vomiting. Treatment is by administering N-acetylcysteine (NAC) to prevent hepatotoxicity and supportive care follows clinical signs.

**Ethylene glycol**

Ethylene glycol is found in commercial antifreeze for vehicle cooling systems at approximately 95%. This substance is inexpensive and easy to find. After ingestion, oxalic acid from the metabolic process leads to acidosis in the body. Oxalic acid binds with calcium in the serum resulting in the formation of calcium oxalate crystals. These crystals damage the ureter and induce renal failure. Cats are more susceptible to this toxin than dogs; The lethal dose of undiluted ethylene glycol is 1.5 ml/kg in cats and 6.6 ml/kg in dogs. After ingestion, clinical signs in dogs and cats appear as vomiting, polydipsia and polyuria, abdominal pain, ataxia, oliguria and seizures. Treatment in dogs and cats aims to decrease the absorption of ingested ethylene glycol and supportive care follows clinical signs.

**Pyrethrin-Pyrethroid**

The pyrethrin group is extracted from chrysanthemum flowers, while the pyrethroid group comprises synthetic compounds. Both groups have similar properties but the pyrethroid group has higher stability and is not easily decomposed. The pyrethrin and pyrethroid groups comprise many substances used as drugs including cinerin, jasmolin, permethrin, cyfluthrin, cyhalothrin and cypermethrin. The mechanisms of the two groups interfere with neuronal voltage-sensitive sodium channels by inducing slow sodium channel closure. This decreases the intracellular voltage potential, resulting in cellular hyperexcitability. These toxins are used in external forms and absorption of toxin through the skin rarely occurs. The substance mainly enters the body through ingestion and inhalation. The toxic dose in dogs and cats depends on pyrethrin-pyrethroid type. Commercial flea and tick spray products containing 0.09% fenvalerate and 9.0% diethyl-toluamide (DEET) have oral toxic dose at 4 mg/kg in dogs and 2 mg/kg in cats. Clinical signs in dogs and cats from intoxication are mild (anorexia, vomiting), moderate (hypersalivation, blurred vision) and severe (seizures, coma) depending on the pyrethrin-pyrethroid type, quantity and duration after intoxication. Supportive care follows clinical...
signs, and decontamination of toxins is the current treatment in dogs and cats.48

**Macrocyclic lactone**

Macrocyclic lactone is a chemical substance widely used to control and eliminate internal and external parasites in pets and livestock.51 It contains several drugs including doramectin, eprinomectin, ivermectin, milbemycin, moxidectin and selamectin.52 Macrocyclic lactone exerts its toxic actions through high affinity for ligand-gated chloride channels, particularly those mediated through the neurotransmitters GABA and glutamate. The binding of macrocyclic lactone to glutamate-gated chloride channels causes increased chloride conductance through the cell membrane, resulting in hyperpolarization.53 LD$_{50}$ in dogs and cats depends on macrocyclic lactone type and status of the $p$-glycoprotein transport system (P-GP) individual. The P-GP is a protein coded by the ABCB1(formerly MDR1), while normal P-GP is tolerant to the toxin. Members in macrocyclic lactone such as ivermectin gave oral toxicity in cats at 1 mg/kg, dogs 80 µg/kg (ABCB1 defective) and 0.2-1 mg/kg (ABCB1 normal).53 Clinical signs of poisoning with these substances are related to the nervous system. In cases of acute toxicity from high doses, exposure can be induced within hours and pets show signs of depression, ataxia and hypersalivation. In severe cases, dogs and cats may fall asleep and become unconscious.54 No antidote for this substance exists. Supportive care is an important treatment.

**Fipronil**

Fipronil is a broad use chemical insecticide. This substance is used in veterinary medicine to prevent and eliminate external parasites such as ticks, fleas or mites.55 The mechanism of toxicity inhibition at the GABA receptor-chloride complex causes sustained activation of the central nervous system, resulting in convulsions, loss of consciousness and death. Fipronil has a selective binding that only catches glutamate-gated GABA chloride channel, which is found only in invertebrates. Thus, invertebrates are the group most at risk of being affected.55 Based on toxicity in dogs and cats, fipronil is classified as a moderately toxic substance. Skin contact alone produces little or no toxicity. Oral LD$_{50}$ in dogs and cats is necessary receipt accumulate several months will show clinical signs (0.2-0.5 mg/kg/day).56 Clinical signs are often neurological-related symptoms including loss of appetite, muscle tremors, and aggressive behavior.57 There is no specific antidote for the toxicity of fipronil. Supportive care follows clinical signs and decontamination is a treatment consideration.55

**Amitraz**

Amitraz is an insecticide used to prevent and eliminate insects such as ticks, fleas, and mites in pets and livestock.58 The product can be a powder, collar, spray or bath. The mechanism of action interferes with the function of the nervous system This results in paralysis of the insect’s mouth, with inability to suck blood from the host. High doses of amitraz can cause toxicity to mammals through action of the alpha 2-adrenergic agonist and a monoamine oxidase inhibitor (MAOI) that influence the nervous system and cause cardiovascular system disorder.58, 59 Oral LD$_{50}$ in dogs is 100 mg/kg but less in cats.30 The toxicity in dogs and cats is divided into two main categories as acute toxicity and chronic toxicity. Acute toxicity usually appears within 30 minutes to 2 hours after intoxication. Clinical signs in acute intoxication include nausea, vomiting, diarrhea, arrhythmia, bradycardia, decreased blood pressure and hypothermia. Chronic intoxication affects the endocrine and reproductive systems.58, 60 There is no specific antidote for amitraz poisoning. Treatment should prevent substance absorption, with supportive care to control the clinical signs.

**Conclusions**

Toxic poisoning in dogs and cats can occur as a result of non-accidental and accidental causes. Dogs or cats can accidentally ingest baits containing rodenticide substances, or dead or alive poisoned rodents if the owner lacks knowledge of the correct use of the chemicals. Non-accidental causes include intentional abuse, which is considered animal cruelty and is illegal in many countries. Clinical signs in dogs and cats after intoxication depend on the type of substances, quantity ingested and duration after exposure. Defining the type of substance requires variation assessment including information from the owner, physical evidence, clinical examination on
pets and detection of substances in the laboratory. Treatment should be managed immediately when known pets are exposed to toxic substances to avoid progress of the clinical signs.

**Ethical clearance:** There was no involvement of subjects hence ethical permission is not required.

**Financial support and sponsorship:** Nil.

**Conflicts of interest:** There are no conflicts of interest.

**References**


Correlation Study Between Age and Level of Education with the Act of Consuming Iron Tablets in Teenage Girls to Prevent Stunting in Gresik Regency

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Abstract

Introduction: Indonesia is ranked 5th in the world with a stunting prevalence of 37%. Pregnant women who are malnourished and anemia are one of the causes of the birth of stunting children. Anemia can be prevented by regularly taking iron tablets since adolescence. This study aimed to analyze the correlation of age and level of education with adolescent girls in consuming iron tablets to prevent stunting in Gresik Regency.

Methods: This type of study is an observational-analytic with a cross-sectional design. The sample of this study was 310 teenage girls who went to school in the Gresik Regency area with proportionate stratified random sampling technique. Data were collected using online questionnaire which refers to the theory of planned behavior. The independent variables in this study were age and level of education the teenage girls. The dependent variable was the act of consuming iron tablets in teenage girls. Pearson correlation coefficient was used to measure the correlation between independent and dependent variables.

Results: The results of the pearson’s r analysis showed that there is an influence between age and the act of consuming iron tablets (p = 0.009). The influence between level of education and the act of consuming iron tablets was also significant (p = 0.013).

Discussion: The results of this study indicate that age and level of education are related to the act of consuming iron tablets in teenage girls. This means that support from a positive surrounding environment is needed so that the consumption of iron tablets in teenage girls can be better and the incidence of stunting in the future can be reduced.

Keywords: stunting, iron tablets, anemia, teenage girls

Introduction

Stunting is a health problem throughout the world where this health problem occurs in children. Indonesia is ranked as the 5th highest in the world with the highest prevalence of stunting under five. This health problem must be handled properly because children with stunting conditions will affect
their long-term health condition like cognitive and motor development. This circumstance is measured by using a length or height that is much less than – 2 SD (minus two standard deviation) from the WHO’s median infant growth standard. Stunting is associated with other nutritional health problems such as anemia in women of reproductive age\(^1\).

During pregnancy, a woman with anemia has the potential to give birth to a stunted child. This can happen because when a person is anemic, the hemoglobin in red blood cells will decrease. This can happen because when someone is anemic, someone will lack iron. Iron is what plays an important role in the formation of hemoglobin. This causes a lack of oxygen and nutrition for the child in the womb. Therefore, it is important to meet the needs of sufficient iron for our bodies, especially in women of reproductive age.

Anemia is one of the most frequent international malnutrition troubles and influences 23% female over the age of 15 and 37% pregnant women. There are 37.1% teenage girls in Indonesia aged 15-19 years with a risk of anemia\(^2\). Therefore, improving the nutritional status of adolescent girls can break the chain of stunting, prevent anemia, increase iron reserves in the body, and prepare for a healthy, high-quality, and productive generation. Iron intake can be obtained from foods derived from animal protein, such as liver, fish, and meat. However, not all people can eat these foods. Thus, it is necessary to take additional iron intake obtained from iron tablets.

Age is one of the determining factors for a person to take an action. The level of maturity of a person’s age can affect the pattern of thinking that will lead to a certain behavior. Variables related to compliance taking Fe tablets is age (<20 years)\(^7\). A person’s level of education can also influence an individual to take action. Someone with higher education tends to consider taking an action by seeing the benefits of the action. Meanwhile, someone with lower education tends to ignore or not know the benefits that may exist in an action. So that it makes someone with lower education choose to avoid a certain action. A study states that there are relationship between education and adherence to consuming Fe tablets\(^4\).

**Material and Methods**

This type of study is an observational-analytic with a cross-sectional design. The study was conducted in June - August 2021 by distributing questionnaires to respondents through Google form. The population of this research is 1,544 teenage girls consisting of 10th, 11th, and 12th grade in Gresik Regency. The calculation of the sample size for the cross-sectional study design used binomunal proportions which resulted in as many as 310 respondents for this study. The independent variables in this study were age and level of education teenage girls in Gresik Regency. The dependent variable was the act of consuming iron tablets in teenage girls in Gresik Regency.

The research instrument used in this study adapted from theory of planned behavior by Icek Ajzen\(^6\) which has been modified and adapted and has gone through validity and reliability tests. The data that has been obtained were tested using SPSS to find the relationship between the independent variable and the dependent variable. The statistical test used is the Pearson correlation coefficient. Pearson correlation coefficient is the statistics test that measures the statistical relationship, or association, between two variables.

**Results**

Respondents in this study were differentiated by level, that is their age and level of education. A study claim that variables related to compliance taking Fe tablets is age (<20 years)\(^7\) and some study states that there are relationship between education and adherence to consuming Fe tablets\(^5\). The results of that research was later also proven in this study which resulted in a relationship between age and education level with consumption of iron tablets.
Table 1: Distribution Frequency Age of Teenage Girls in Gresik Regency (n = 310)

<table>
<thead>
<tr>
<th>Age</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
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<tbody>
<tr>
<td>Valid</td>
<td></td>
<td></td>
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<tr>
<td>15 Years Old</td>
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<td>16 Years Old</td>
<td>94</td>
<td>30.3</td>
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<td>17 Years Old</td>
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<tr>
<td>Total</td>
<td>310</td>
<td>100.0</td>
<td></td>
<td>100.0</td>
</tr>
</tbody>
</table>

Based on the frequency distribution table above, it can be seen that most of the respondents in this study were 17 years old that is 120 people (38.7%). There are also 96 people aged 15 years old (31%), and 94 people aged 16 years old (30,3%). Most respondents are 12th grade which is 120 people (38,7), then 116 people are 10th grade (37,4%), and also 74 people are 11th grade (23,9%). From the distribution frequency table of action of consuming iron tablets it can conclude that mostly teenage girls has an average category of action of consuming iron tablets which is 179 people (57,7). Then, there are also 95 people (30,6%) categorized good. But, there was still 36 people (11,6%) who categorized poor of action of consuming iron tablets.

Table 2: Distribution Frequency Level of Education of Teenage Girls in Gresik Regency (n=310)

<table>
<thead>
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<th>Level of Education</th>
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<tr>
<td>Valid</td>
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</tr>
<tr>
<td>10th Grade</td>
<td>116</td>
<td>37.4</td>
<td>37.4</td>
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</tr>
<tr>
<td>11th Grade</td>
<td>74</td>
<td>23.9</td>
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<td>61.3</td>
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<td>12th Grade</td>
<td>120</td>
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<tr>
<td>Total</td>
<td>310</td>
<td>100.0</td>
<td></td>
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</table>

Table 3: Distribution Frequency Action of Consuming Iron Tablets of Teenage Girls in Gresik Regency (n=310)

<table>
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<tr>
<th>Action</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
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<tr>
<td>Valid</td>
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<tr>
<td>Poor</td>
<td>36</td>
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<tr>
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<tr>
<td>Good</td>
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<tr>
<td>Total</td>
<td>310</td>
<td>100,0</td>
<td></td>
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</tr>
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Table 4: Correlation Between Age and Action of Consuming Iron Tablets of Teenage Girls in Gresik Regency (n=310)

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<th>Age</th>
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<td>Sig. (2-tailed)</td>
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<tr>
<td>N</td>
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</tr>
</tbody>
</table>

Based on the Pearson correlation coefficient table above show the correlation between age and action of consuming iron tablets of teenage girls in Gresik Regency is significant that obtain \( p \) - value = 0.009 which is less than \( \alpha = 0.05 \). The correlation between level of education and action of consuming iron tablets of teenage girls in Gresik Regency is significant that obtain \( p \) – value = 0,013 which is less than \( \alpha = 0.05 \). It can conclude that there are correlation between those variables.

Table 5: Correlation Between Level of Education and Action of Consuming Iron Tablets of Teenage Girls in Gresik Regency (n = 310)

<table>
<thead>
<tr>
<th>Action</th>
<th>Level of Education</th>
</tr>
</thead>
<tbody>
<tr>
<td>Action</td>
<td>Pearson Correlation</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.013</td>
</tr>
<tr>
<td>N</td>
<td>310</td>
</tr>
</tbody>
</table>

Based on the Pearson correlation coefficient table above show the correlation between level of education and action of consuming iron tablets of teenage girls in Gresik Regency is significant that obtain \( p \) – value = 0,013 which is less than \( \alpha = 0.05 \). It can conclude that there are correlation between those variables.
**Discussion**

Age is the size of the time a man or woman has lived considering birth. The older a man or woman is, the maturity degree and power of that person will be higher; mature in wondering and working. In phrases of trust, people who are more adults will be extra dependent on than people who are not excessive adequate level their maturity. This is as an end result of the journey and maturity of their soul. The greater mature an individual is, the greater mature and orderly the way of wondering becomes perform health behavior.

Education is the technique of altering the attitudes and behavior of an individual or group of human beings in efforts to mature people through educating and training efforts. Education is intently associated with knowledge. The greater stage of a person’s education, it is anticipated that the greater the expertise the man or woman has about diet and health. Higher education can make anybody pay more interest to food to meet the consumption of vitamins that balanced. Action is a structure of implementation of the intention on the person as a behavioral disposition. This can occur when there is the proper time and possibility to manifest intentions in the structure of action.

Pearson correlation coefficient test between age and the act of consuming iron tablets in teenage girls resulted in a p-value = 0.009. Because p-value < α, where α = 0.05 it means those variable above is significant, which means that there is a relationship between age with the act of consuming iron tablets in teenage girls in Gresik Regency. This result is accordance with research that implies variables related to compliance taking Fe tablets is age (<20 years).

Pearson correlation coefficient test between level of education and the act of consuming iron tablets in teenage girls resulted in a p-value = 0.013. Because p-value < α, where α = 0.05 it conclude that those variables is significant, which means there are correlation between level of education with the act of consuming iron tablets in teenage girls in Gresik Regency. This end result is accordance with research that the results of the analysis show that there are relationship between education and adherence to consuming Fe tablets.

**Conclusion**

The results of this study indicated that age and level of education are related to the act of consuming iron tablets in teenage girls in Gresik Regency. The implications of the outcomes of this study are imperative information for the government to make a wise and right regulation for preventing the incidents of stunting and anemia of teenage girls in Gresik Regency so that teenage girls are more prepared and healthy when they become pregnant women in the future and also give birth to healthy children. Further research is needed to explore various other factors that cause stunting.

**Conflict of interest:** Nil

**Ethical Clereance:** Taken from the Faculty of Dental Medicine Health Research Ethical Clearance Commission of Airlangga University number 354/HRECC.FODM/VII/2021.

**Source of funding:** Self funding

**References**

Stevens-Johnson Syndrome induced by Piroxicam: A Case Report

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Abstract

Stevens Johnson Syndrome (SJS) is acute life-threatening mucocutaneous reactions characterized by extensive necrosis and detachment of the epidermis and mucosal epithelium less than 10% of the total body surface area. The pathophysiology of SJS is still unclear; however, drugs are identified as the main cause of SJS in most cases. Drugs are the most important etiologic factor, >20% idiopathic or caused by infection. Many nonsteroidal anti-inflammatory drugs (NSAID) were suspected. Oxicam derivates showing the highest risk. The accurate diagnosis, avoidance of the use culprit of the drugs and rapid treatment with symptomatic and supportive care may improve the prognosis.

Keywords: Stevens-Johnson syndrome, adverse drug reaction, piroxicam

Introduction

Stevens-Johnson syndrome (SJS) is a rare life-threatening reaction, acute, and potentially fatal skin reactions involving loss of skin and, in some cases, mucosal membranes accompanied by systemic symptoms.¹ SJS is characterized by sudden apoptosis of keratinocytes leading to mucous membrane erosions and epidermal detachment. Detachment of less than 10% of the total body surface area defines SJS.² Some drugs that often cause of SJS are NSAIDs (45%), carbamazepine (20%), herbal medicine (13.3%), and the other drugs such as amoxicillin, cotrimoxazole, dilantin, chloroquine, and ceftriaxone.³ Drugs are identified as the main cause of SJS in most case. NSAIDs oxicam derivatives showing the highest risk.⁴ Piroxicam are medicines widely used for the relief of pain and inflammation and are commonly purchased over-the-counter in addition to being prescribed. Piroxicam is an enolic derivative of the oxicams class of NSAIDs with antiinflammatory, analgesic, antipyretic, and antiplatelet properties.⁴ The pathophysiological mechanism of SJS is not fully understood. It is believed to be a delayed hypersensitivity reaction mediated by Th1 cells. SJS is traditionally thought to be a T-cell-mediated disorder. T cells are activated by binding of drugs...
to T cell receptors (TCRs) from antigen-presenting cells (APCs). Widespread apoptosis of keratinocytes is provoked by the activation of a cell-mediated cytotoxic reaction and amplified by cytokines, mainly granulysin. Management of patients with SJS requires three measures: Early discontinuation of all medicines, particularly drugs known to be high-risk; supportive measures and active interventions. 

Case

A woman, 43 years old, 65 kg, came to emergency unit with a chief complaint of blisters in almost over her body since 2 days before hospitalized, accompanied by swollen eyes, sore on the lips and genital. Initially appeared itchy red spots on the chest and then spread throughout the body, then patches are partly form blister which containing clear fluid then burst and left a wound. Before appeared sore and blisters, she got prodromal symptoms like fever, malaise, sore throat, myalgia, and arthralgia, then she buys and consumed allopurinol and mefenamic acid because of sore throat, myalgia, and arthralgia. The patient had history of pain on her both legs and hyperthyroid, she had been go to the doctor, she got the therapy piroxicam, bisoprolol, and propranolol two weeks before. After receiving piroxicam for 2 weeks, the prodromal symptoms and skin lesion were appeared.

The medical history like this before was denied. There was history of hyperthyroid. No history of asthma, drug and food allergy to the patient or the family. History of applying other topical medication was denied. No history of hypertension and diabetes mellitus in patients or the family.

On physical examination, the patient’s general state obtained weak, awareness compost mentis. Presented blood pressure of 110/80 mmHg, pulse rate of 90 beats per minute, respiratory rate of 18 breaths per minute, and temperature of 38.0 degrees Celsius. Based on dermatological examination on the generalize region showed erythematous macules, irregularly shaped which progressively coalescence with multiple vesicle, containing clear fluid, erosion, and there was epidermal detachment less than 10% BSA (figure 1) with a positive Nikolsky’s sign. There was conjunctival hyperemia, lacrimation and discharge in both eyes. On the area of lips and oral mucous membrane were found erosions and some hemorrhagic crust, and also there was erosions on the genital area.

Initial laboratory test showed the hemoglobin 12.1 g/dL (N: 11.0-14.7), total count of WBC 7.98 x10³/µL (N: 3.37-10.00) with normal differential count of leukocyte. From clinical chemistry examination results were abnormal: BUN 12 mg/dL (N: 10-20), albumin level 3.8 g/dL (N: 3.40-5.00), serum creatinine 0.8 mg/dL (N: 0.50-1.20), SGOT 24 U/L (N: < 41), SGPT 41 U/L (N: < 38), uric acid 5.4 mg/dL (N: < 5.7). There was slightly alteration of potassium electrolyte level 3.5 mmol/L (N: 3.8-5.0). Routine and microscopic examination of urine revealed all within normal limits. Other laboratory results from blood gas analysis HCO3 22.8 mEq/L, glucose level was 92 mg/dl, FT4 1.310 mg/dl and TSH 0.221 ulu/mL. From patient’s laboratory result, has been collected several data to complete the SCORTEN scale, consist of the patient was 43 years of age, heart rate 90 beats per minute, BSA involved <10%. The level of BUN 12

![Figure 1: SJS lesions day-1.](image-url)
mg/dL and random blood glucose were 92 mg/dL. Serum bicarbonate was 22.8 mEq/L. The SCORTEN scale of this patient was 1 with mortality rate 3.2%.

Based on the history taking, physical and laboratory examination, the diagnosis of SJS was established. At first the five of suspected drugs was discontinued immediately. Stopping consumption of the suspected drugs were our first treatment to prevent worsen condition. Then we treat the patient with appropriate supportive treatment with IVFD triofuchsin:RD 5%:aminofluid = 1:2:1 500 cc/24 hours, gentamycin injection 2x80mg iv, wound dressing with normal saline for the erosion and crusted lesion, high calori and high protein diet, and compress if fever. Initial dose of dexamethasone 0.12 mg/kg/day was given intravenously with tapered dose as the improvement of the skin lesions. After 10 days hospitalization, the skin recovery and re-epithelialization were established, temperature decreased, and mucosal complications stabilized (figure 2). There was leaving residual pigmentation on the site of skin lesions before (hyperpigmented macules). The patient was discharged after 10 days of hospitalization.

![Figure 2: SJS lesions day-10.](image)

To identify the suspected causal agent, DPT was performed 6 weeks after all the lesions completely healed. DPT using As-Is piroxicam, allopurinol, mefenamic acid, bisoprolol and allopurinol. The DPT As-Is were applied on the back and read on 48, 72, and 96 hours after applying as suggested in International Contact Dermatitis Research Group guideline (ICDRG). On 48-hour and 72-hour reading, the piroxicam patch test was positive (++), and on 96-hour reading, the piroxicam patch test was positive (+++). The patch test result of piroxicam was give crescendo tendency on 72- and 96-hour readings.

![Figure 3: Drug Patch Test.](image)
Discussion

SJS is acute life-threatening mucocutaneous reactions characterized by extensive necrosis and detachment of the epidermis and mucosal epithelium.2 The diseases can start with prodromal symptoms lasting up to 1 week, such as fever, malaise, sore throat, coughing, eye burning, myalgia, and arthralgia. After the prodromal symptoms occurred may precede the mucocutaneous lesions by 1 to 3 days. Detachment of the skin, as well as mucous involvement in this case are on the eyes, oral, and genital mucosa was also observed. The eruption is initially symmetrically distributed on the face, upper trunk and proximal part of the limbs.1,2 The initial skin lesions are characterized by erythematous, dusky red, purpuric macules, irregularly shaped, which progressively coalescence. The epidermis is detached or “detachable” (Nikolsky’s sign is positive) in erythematous zones was less than 10%. SJS is characterized by involvement of <10% body surface area; SJS-TEN overlap signifies 10%–30% involvement and the most severe form of the spectrum, TEN is characterized by involvement of >30% body surface area. Mucosal inflammation (oral, ocular, and genital) is nearly universal. Histopathology is usually not required for the diagnosis of SJS-TEN.2,6

The risk seems confined to the first 8 weeks of treatment and most inducing medications revealed the first continuous exposure between 4 and 28 days before reaction onset. The epidermal detachment progresses for 5 to 7 days. Then patients enter a plateau phase, which corresponds to progressive reepithelialization. This can take a few days to a few weeks, depending on the severity of the disease and the prior general condition of the patient. During this period, life-threatening complications such as sepsis or systemic organ failure may occur. The prognosis of the disease is determined using the score of TEN (SCORTEN). It consists of 7 parameters: Age ≥40 years, heart rate ≥120/min, presence of cancer/hematologic malignancy, >10% body surface area involvement, raised blood urea nitrogen (>28 mg/dL), serum bicarbonate <20 mmol/L, serum glucose level >14 mmol/L, calculated within the first 24 h of admission of the patient.2,6

The immunologic pattern of early lesions suggests a cell-mediated cytotoxic reaction against keratinocytes leading to massive apoptosis. Drugs are the most important etiologic factors. The pharmacologic interaction of drugs with the immune system could result in binding of the responsible drug to MHC-1 and the T cell receptor. Most of CADR are type IV hypersensitivity reactions by Gell and Coombs, and it can be sub classified into type IVa, IVb, IVc and IVd according to the effector phenotype of the involved T cells. The pathomechanism of SJS is type IVc hypersensitivity reaction, induced by CD4+ and CD8+ T cells producing cytotoxic mediators (perforin and granzyme B). The epidermal damage in the skin lesions of SJS/TEN patients is considered to be of apoptotic origin. Apoptosis is induced by cytotoxic CD8+ T cells through the Fas-Fas ligand (FasL) pathway or the perforin/granzyme pathway.2,5

Many nonsteroidal anti-inflammatory drugs (NSAIDs) were suspected to be associated with SJS. NSAIDs oxicam derivatives showing the highest risk. Piroxicam is an enolic derivative of the oxicams class of NSAIDs, inhibitor of the cyclooxygenase 1 and 2 pathway, with anti-inflammatory, analgesic, antipyretic, and antiplatelet properties. Retrospective studies implicate piroxicam in 1.6% to 12% of cutaneous adverse drug reaction cases.2,4,7

SJS is a life-threatening disease that requires optimal management, early recognition, and withdrawal of the offending drugs in drug-induced cases and supportive care in an appropriate hospital setting. Immediate withdrawal of all the suspected drugs is the key to the management of SJS, earlier withdrawal of the drug is associated with better prognosis.6 Management of SJS consists of symptomatic treatment, specific treatment in acute stage, and sequelae treatment. The mainstay of treatment in SJS is symptomatic and supportive care which includes fluids and electrolyte replacement, early nutritional support, control of infection, topical skin care, and eye care. Whereas specific treatment consists of corticosteroids, intravenous immunoglobulin, cyclosporine, plasmapheresis or hemodialysis, antitumor necrosis factor agents, and treatment of sequele.2,6

Currently, clinical diagnosis is still considered the gold standard for delayed immunologically mediated ADRs but there is consensus that in vivo testing, such as drug patch test (DPT). DPT is usually performed to the implicated drug(s) at least 4–6 weeks after delayed hypersensitivity reaction at the recommended non-irritating concentrations.8 The basic principles and methodology for drug patch test remains same as that in patch testing for contact dermatitis. The mechanism of delayed hypersensitivity reaction in the
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Stevens-Johnson Syndrome induced by Piroxicam: A Case Report

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The DPT should be used as the first line of investigation or defining relevant drug in severe type of CADR including SJS. The sensitivity of DPT can vary depending on the vehicle used and the drug tested. The DPT sensitivity also varies with the type of eruption (appears most useful for generalized eczematous, maculopapular eruption, AGEP and FDE, on the other hand DPT is least useful in the investigation of SJS. Whereas the specificity of DPT have not ben yet determined. The DPT is safer than other in vivo diagnostic tests to establish the diagnosis of non immediate drug hypersensitivity reactions. DPT is not invasive procedure and the lowest possibility to re-induce severe DHR, if it is compared to prick test, intradermal skin test or even drug provocation test. Therefore, we consider performing DPT with commercially available drug (As-Is) to confirm the causative drug inducing SJS in this case, in order to prevent the second or more attack of severe CADR in this patient.

Conclusions

Stevens-Johnson Syndrome (SJS) is acute life-threatening mucocutaneous reactions characterized by extensive necrosis and detachment of the epidermis and mucosal epithelium. It can cause by non-steroidal anti-inflammatory drugs (NSAIDs). Oxicam derivatives showing the highest risk. The mainstay of treatment in SJS is symptomatic and supportive care which includes fluids and electrolyte replacement, early nutritional support, control of infection, topical skin care and eye care. Avoidance of use causative drug and rapid prompt treatment may improve the prognosis. Drug patch testing with positive reactions have high predictive value to avoid using causative drug in the future.

Patient Informed Consent: Proper patient consent was taken from patient for writing this case report.

Funding: This research received no external funding.

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

References


Surgical follow up after Focused Assessment with Sonography for Trauma Exam in Blunt Abdominal Trauma

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Abstract

Introduction: Blunt injury sustained from a blunt force may be related to motor vehicle accidents, falls, blows or crush injuries from animals, blunt objects or assailants. FAST is a low cost and portable method for evaluating BAT. It provides reasonable accuracy and has a high negative and positive predictive value for diagnosis of intraperitoneal injuries. Hence the aim of the study was to determine a relationship between the findings in focussed assessment with sonography in trauma and clinical outcome.

Materials and Methods: A total of 80 patients who underwent FAST were included in the study. A prospective analytical study was carried over 2 year’s duration. The FAST findings were recorded and clinical follow up for seven days.

Result: There were more male patients with blunt injury abdomen than female patients. Most common age group was between 20 to 39 years. The most common cause of BAT was RTA followed by falls. Out of 80 patients with BAT in our study, 29.2% patients were FAST positive and 70.8% patients were FAST negative.

Discussion and Conclusion: On follow up patient with negative FAST had fewer falls in hemoglobin and hematocrit compared to the patient with positive FAST. It concluded that FAST scan is a predictor of clinical outcome in BAT. It was concluded that FAST is a better diagnostic modality in ruling in than ruling out a patient with blunt abdominal trauma.

Keywords: Blunt injury, Focused assessment, Sonography, Trauma

Introduction

Blunt injury sustained from a blunt force may be related to motor vehicle accidents, falls, blows or crush injuries from animals, blunt objects or assailants. The World Health Organization estimates that, by 2020, trauma will be the first or second leading cause of years of productive life lost for the entire world population.¹
About 1.25 million people die each year as a result of road traffic crashes. Road traffic injuries are the leading cause of death among young people, aged 15–29 years. 90% of the world’s fatalities on the roads occur in low- and middle-income countries, even though these countries have approximately half of the world’s vehicles. Half of those dying on the world’s roads are vulnerable road users: pedestrians, cyclists and motorcyclists.2

The importance of the FAST examination is emphasized by the fact that it has been included as a part of the Advanced Trauma life support (ATLS) course. FAST is a quick and effective tool to evaluate BAT in emergency situations. It should be considered the 5th modality in the assessment of trauma situations. Currently FAST with its ability to rapidly determine any life-threatening injuries has become the method of choice in evaluating hemodynamically unstable patients. There is evidence that patient care is improved when the FAST exam is included in the initial work up of the patients.3,4

FAST is a low cost and portable method for evaluating BAT. It provides reasonable accuracy and has a high negative and positive predictive value for diagnosis of intraperitoneal injuries. However for spinal fractures, pulmonary contusions, diaphragmatic rupture, vascular injuries, bowel and mesenteric injuries and in the evaluation of injuries related to retroperitoneal structures like the pancreas its accuracy has been disputed.5,6

Over the past several years, major changes in the management of blunt abdominal injury have occurred. Because of the progress that has been made in the quality and wide availability of the multidetector computed tomography (MDCT) scan combined with minimally invasive intervention options like angioembolization7, Nonoperative management (NOM) has evolved to be the treatment of choice for hemodynamically stable patients. NOM is a safe treatment for stable patients with traumatic liver, splenic or kidney injuries and success rates of up to 95% are described in the literature. Hence the aim of the study was to determine a relationship between the findings in focussed assessment with sonography in trauma and clinical outcome.

Materials and Methods

A prospective observational study carried out on patients with blunt abdominal trauma presenting to the Emergency Department of medical college and associated hospital. The duration of study was of 2 years. A total of 80 patients were included in the study. The required written consent was obtained from the institutional review board.

Inclusion criteria:

All patients with blunt abdominal injury undergoing FAST within 24 hours of the trauma event.

Exclusion criteria:

1. Patients with penetrating abdominal injuries.
2. Patients lost to follow up within 7 days of admission with history of blunt abdominal trauma.

Study methods:

All patients with a history of BAT presenting to the emergency medicine department were evaluated with FAST performed by an emergency physician. The parameters such as age, sex, mechanism of injury (RTA, fall) were noted. Haemodynamically stable patients without free fluid identified on FAST (FAST negative) were observed for a period of seven days. Those haemodynamically stable patients detected to have free fluid (FAST positive) underwent a CECT of the lower thorax, abdomen and pelvis to identify injuries which would require operative management. Any patient with haemodynamic instability will undergo exploratory laparotomy. Ultrasonography by a radiologist, echocardiography by a cardiologist, plain radiography of the chest or abdomen and CECT of the thorax and abdomen only if required for the patients’ routine clinical management.

Patients were taken to exploratory laparotomy based on one or more of the following findings

1. Clinical deterioration with increasing abdominal distention.
2. Unexplained sustained hypotension (systolic BP < 90 mm of mercury) not responding to intravenous fluid infusion in the setting of BAT.
3. Signs of continuing intra-abdominal haemorrhage with decline in haematocrit.
4. Pneumoperitoneum detected on erect chest or abdominal radiography.
5. Presence of free fluid in the peritoneum detected by FAST or radiologist performed ultrasound scan with hemodynamic instability.

The clinical outcome of the patients in our study was categorized based on FAST scan findings into FAST positive and FAST negative cases. FAST positive cases with stable hemodynamics were NOM and were investigated further with CECT where as FAST positive cases with unstable hemodynamics underwent OM. FAST negative patients with stable hemodynamics were discharged from the Emergency Department with outpatient follow-up and FAST negative patients with associated other systems injuries were admitted for observation. All FAST positive cases were serially monitored for drop in Hemoglobin (Hb) and Hematocrit (PCV). The findings of FAST in these patients were compared with CECT and / or intraoperative surgical findings.

**Result**

The purpose of the present research was to determine a relationship between the findings in focussed assessment with sonography in trauma and clinical outcome. Total of 80 patients were included in study. In the present study, the youngest patient was 1 years old, and the oldest was aged 75 years. Maximum patients were in the age range of 20 to 39 years. Out of 80 patients from this study, 55 patients were male and 25 patients were female.

In this study out of the 80 patients included; 68 of the patients sustained blunt trauma abdomen by road traffic accident (RTA) and 12 patients had a history of fall. Among the patients studied 77 patients had stable vitals on arrival and 3 patients had unstable vitals on arrival. Among the patients who were included in the study 42 patients did not have any other injury on secondary survey. 18 patients had abrasions, 7 patients had long bone fractures and 7 patients had pelvic fractures. Head injury was present in 5 of the patients, and chest injury was present in 1 of the patients.

Patients with the presence of free fluid on FAST study and with stable vitals underwent CECT of the abdomen and pelvis after renal function test. Hemoperitoneum was seen in 22 of the patients, 3 patients had hemothorax. Solid organs like Liver was injured in 7 patients, Spleen was injured in 10 patients, Kidneys in 1 patients, 1 patient sustained pancreatic injury, 1 patients had Bowel/Mesentery injury.

In this study, 43 cases were negative for intra-abdominal injury with hemodynamic stability was discharged based on the FAST findings within 24 hours of admission. All these patients had an uneventful follow-up on out - patient basis for 7 days.

Among the 22 FAST positive patients in this study 12 patients were operated and 10 FAST positive patients were conservative managed. Among the 58 FAST negative patients, 15 patients stayed more than 24 hours has this patients had significant other injuries like head injury, long bone fractures or pelvic fractures.

The sensitivity in this study was calculated with respect to how accurate FAST findings were in guiding patient management and to predict clinical outcome. Based on FAST findings, operative management was advised for 11 patients out of the 22 positive FAST scans.

**Table 1: Clinical outcome of FAST management guidelines**

<table>
<thead>
<tr>
<th>Follow up for one week</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAST positive (NOM)</td>
<td>10</td>
</tr>
<tr>
<td>FAST negative (Discharge)</td>
<td>43</td>
</tr>
<tr>
<td>FAST (OM)</td>
<td>12</td>
</tr>
<tr>
<td>FAST negative (Other injuries)</td>
<td>15</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
</tr>
</tbody>
</table>

**Discussion**

FAST is a rapid, repeatable noninvasive bedside method that was designed to answer one single question: Whether free fluid is present in the peritoneal, pleural and pericardial cavity. It has been a valuable investigation for the initial assessment of blunt abdominal trauma as shown in large series from several North America trauma centers.8,9
FAST in patients with abdominal blunt trauma is used as first method at admission because it can be done quickly and can show posttraumatic intraperitoneal, pleural or pericardial fluid. Sometimes, the presence of free air or meteorism can make very difficult or impossible to see the lesions. CT exam brings additional information regarding fluid density, laceration’s extension, active bleeding or especially pancreatic trauma. The presence of pelvic fractures implies to perform delayed scan sequences for the excretory phase which can show extravasation.10,11

In this study, the youngest patient was 1 years old, and the oldest was aged 75 years with mean age 34. Maximum patients were in the age range of 20 to 39 years. Among 80 patients in this study 55 patients were male and 25 patients were female. The predominant population involved was young males. Sanjeev Bhoi et al12 showed that 86.6% were male and 13.4% were female with mean age of patient was 28 years, which shows that chest and abdominal traumas are more common in young people. In other words, trauma affects the quality of life and life itself, in a group that consists of the most efficient in society, and this brings about huge economic losses.

In the present study, RTA accounted for 84.2% of cases followed by 15.8% had fall. This was equivocal with other studies conducted by Perry and Morton et. al.13 were RTA was most common cause of BAT. Thus prevention of accidents can decrease fatality in BAT. Among the patients studied 77 patients had stable vitals and 3 patients had unstable vitals on arrival, all the 4 unstable eventually underwent emergency laparotomy this was in consistent to studies conducted by D. Dammers et. al.14 were among 637 patients with BAT 607 (95.29%) patients were stable and 30 (4.7%) were unstable.

In our study during secondary survey 7 patients had long bone fracture, 7 had pelvic fractures, 5 had Head injury, and 1 had chest injuries. This was similar to studies by Nikil Mehta et al15 were 40% had chest injuries, 14 had head injuries, 10% had long bone fractures and 10% had pelvic fractures.

In our study, FAST is 92.10% Sensitivity and 93.4 % specificity compared to CT in detecting haemoperitoneum with Positive predictive value of 93.10% and Negative predictive value of 97.40% in detecting blunt abdominal trauma. Alexander Y. et. al. found that FAST had a sensitivity of 20.0%, specificity of 98.3%, positive predictive value of 73% and negative predictive value of 84% compared to CT.

In our study, follow up patient with negative FAST had fewer falls in hemoglobin and hematocrit compared to the patient with positive FAST. D. Dammers et. al. reported that patients with a positive FAST had a lower Haemoglobin (Hb) level and often received blood transfusions than patients with a true negative FAST.

In our study, 44 cases were negative for intra-abdominal injury with hemodynamic stability were discharged based on the FAST findings within 24 hours. Alexander Y et. al. found that 47.31% patients who underwent FAST never required a CT at all. This practice of ruling out intra-abdominal injury with FAST, which may be occurring with increasing frequency as ultrasound use became more prevalent. Thereby our findings stress that even in stable patients, one should not rely on a single negative FAST-exam to exclude serious abdominal injuries: either careful observation, or a repeated FAST-exam should be performed.

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

**Conflict of Interest:** None

**Source of Support:** Nil

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Surgical Venous Ablation in Varicose Veins Lower Extremity under Sonographic Guidance

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Abstract

Introduction: The EVLA method can be applied in approximately 70% of the patients with complaints of varices. The aim of this study is to assess the efficacy of the EVLA procedure in treatment of saphenous venous insufficiency and the resultant varicose veins.

Materials & Method: Total of 100 patients were included in the study. The EVLA procedure was done using a 1470 nm laser diode by the Interventional Radiologist. The patients were evaluated clinically and by using color Doppler before the procedure, two months and five months after the procedure.

Results: The decrease in Venous clinical severity score and Venous disability score from the pre-procedural score to the second and fifth month scores (post procedure) was found to be statistically significant (p<0.05). Decrease in saphenous vein diameters from the pre-procedure measurement to the second and fifth month diameters (post procedure) at various levels as described was found to be statistically significant (p<0.05). Significant decrease in the number of patients with venous reflux was noted on fifth month follow-up after the procedure.

Discussion & Conclusion: EVLA is a minimally invasive technique. EVLA results in irreversible damage to the vein which is ablated. The venous occlusion is mainly due to progressive fibrosis which causes obliteration of the vein rather than thrombotic occlusion. As this is a new technique, data regarding the long-term outcome of EVLA is required to confirm the durability of the procedure.

Keywords: EVLA, Saphenous vein, Venous insufficiency; varicose veins

Introduction

Venous disease is a common health problem encountered by many people. The effects of this in terms of disability and health care costs are considerable. Etiology is multifactorial ranging from thrombotic obstruction, loss of vein wall elasticity, to valve incompetence. The most common cause of chronic venous insufficiency and varicose veins is saphenous vein insufficiency.¹ ² Refluxing venous
blood leads to venous hypertension. This results in venous reflux due to poorly functioning or incompetent venous valves. Distension of the vein as well as endothelial activation can be seen which promotes leukocyte extravasation. The resulting inflammatory state in the superficial dermis and in the vein wall itself causes a range of symptoms and physiologic changes including pain, edema, varicosities, stasis dermatitis, and ulceration.3

Symptoms of superficial venous disease include telangiectasia, varicose veins, edema or skin changes. Skin or venous ulceration can also be present in severe cases. The prevalence of chronic venous disease in adults is usually indicated by the presence of varicose veins. This has been noted to vary between 5% and 65% of the population.4 The prevalence of chronic venous disease is noted to be higher in the western countries than in the developing countries. However, an international, observational, prospective study called the vein consult program which consisted of about 90,000 patients across different regions of the globe showed that the prevalence of chronic venous disease was mostly the same across the globe.5

Though some studies have shown that varicose veins and chronic venous disease are more commonly reported in females than in males, the established risk factors for development of chronic venous disease are increasing age and previous pregnancy.

Some of the lesser risk factors are noted to be female sex alone (without previous pregnancy), high BMI (Body mass index), work which involves prolonged standing throughout the day or prolonged sitting throughout the day. Symptomatic chronic venous disease patients usually face a measurable decrease in the quality of their lives. QOL is used to denote quality of life. The symptoms can cause hinderance to the social life of the patient and can limit the physical or occupational activities of the patient. This can even lead to financial consequences due to disability resulting from the disease causing loss of work time or loss of the ability to continue the present occupation.6

Treatment options for varicose veins include conservative methods, minimal invasive procedures and surgery. Conservative treatment options include the avoidance of prolonged standing and straining, elevation of the affected leg, exercise, external compression, medical therapies, and weight loss.7 External compression therapies with bandages or support stockings have been recommended as the initial therapy for varicose veins. Conservative therapies may relieve the patient’s discomfort and slow the progression of the disease but cannot cure it.8

In surgical treatment, the most common operation is ligation and stripping. However, disadvantages include requirement of general anaesthesia, postoperative pain and cut scar. In addition, recurrence is observed in approximately half of the patients in the first 5 years after treatment.9

Endovenous Laser Ablation (EVLA) is one of the best minimally invasive techniques for the treatment of varicose veins. Laser therapy for varicose veins was described in 1985 but the current technique of EVLA was first used by Dr Bone in 1999, and subsequently reported in large series by Dr Luis Navarro and Dr Robert J. Min.10 The most important advantages of this method include performance under local anesthesia, absence of pain, absence of wound or scar and mobilization of the patient immediately after the procedure. The EVLA method can be applied in approximately 70% of the patients with complaints of varices. The aim of this study is to assess the efficacy of the EVLA procedure in treatment of saphenous venous insufficiency and the resultant varicose veins.11

Materials and Method

The source of data for this study will be patients referred to the Department of Radio Diagnosis, in the Medical College and Hospital, for the treatment of saphenous vein insufficiency. The study was conducted for the period of two years. The ethical committee of the institute was informed about the study and the ethical clearance certificate was obtained. The inclusion and exclusion criteria of the study were as below:

Inclusion Criteria

1. Patients diagnosed with symptomatic saphenous vein insufficiency.

Exclusion Criteria

1. Patients diagnosed with deep vein thrombosis (DVT).
2. Patients diagnosed with severe arterial insufficiency.

3. Pregnant or breast feeding patients.

4. Patients with past history of allergy against local anaesthetics.

All patients referred to the Department of Radio Diagnosis, in Medical College and Hospital, for the treatment of saphenous vein insufficiency were the source of the data. Total of 100 patients were included in the study. All the patients included were informed about the procedure and purpose of the study. Written informed consent was obtained from all the included patients. Those who did not provide the consent were excluded from the study. The detail history was recorded.

Before the EVLA procedure is done, the condition of patients would be evaluated by the following measures:

Presence of venous reflux would be assessed using colour Doppler ultrasonography, in case of venous insufficiency of the long saphenous vein; the diameter of the vein would be measured along the course at the following sites using ultrasonography:

a. 3 cm distal to Saphenofemoral junction.

b. 1 cm above knee.

c. 1 cm below knee.

d. 1 cm above medial malleolus.

In case of venous insufficiency of the short saphenous vein, the diameter of the vein would be measured along the course at the following sites using ultrasonography:

a. 1 cm below knee.

b. 1 cm above lateral malleolus.

The EVLA procedure is done by the interventional radiologist in the department using a 1470 nm laser diode. The outcome of the EVLA procedure would be assessed by follow-up of the patient and evaluation by:

- Measurement of the diameter of saphenous veins at the above mentioned sites.

All statistical analyses were performed via Statistical Package for the Social Sciences (SPSS).

Results

The present study was performed with an aim of evaluating the early efficacy of Endovenous Laser Ablation treatment in Saphenous vein insufficiency. Our findings were tabulated using Microsoft Excel and have been provided within the Annexure.

A total of 100 patients were included in the study. All the patients were diagnosed with great saphenous vein insufficiency. The data was recorded, compiled and analyzed. Of the 100 included patients, there were 46 males and 54 females.

Sonography assessment of saphenous vein diameters pre-procedure and during followup:

Pre-procedure:

The mean diameter of GSV 3 cm below SFJ was noted to be 6.23 ± 0.67 mm.

The mean diameter of GSV 1 cm above knee was noted to be 6.02 ± 0.43 mm.

The mean diameter of GSV 1 cm below knee was noted to be 6.01 ± 0.94 mm.

The mean diameter of GSV 1 cm above ankle was noted to be 5.84 ± 0.82 mm.

Two months after the procedure:

The mean diameter of GSV 3 cm below SFJ was noted to be 3.21 ± 0.56 mm.

The mean diameter of GSV 1 cm above knee was noted to be 3.02 ± 0.86 mm.

The mean diameter of GSV 1 cm below knee was noted to be 2.86 ± 0.39 mm.

The mean diameter of GSV 1 cm above ankle was noted to be 2.11 ± 0.69 mm.

Five months after the procedure:

The mean diameter of GSV 3 cm below SFJ was noted to be 1.11 ± 0.02 mm.
The mean diameter of GSV 1 cm above knee was noted to be 1.21 ± 0.16 mm.

The mean diameter of GSV 1 cm below knee was noted to be 1.12 ± 0.12 mm.

The mean diameter of GSV 1 cm above ankle was noted to be 0.64 ± 0.01 mm.

The decrease in saphenous vein diameters from the pre-procedure measurement to the second and fifth month diameters (post procedure) at various levels as described were found to be statistically significant (p<0.05).

**Venous clinical severity score pre-procedure and during followup**

The mean Venous Clinical Severity Score pre-procedure was 19.2 ± 4.2.

The mean Venous Clinical Severity Score two month after procedure was 11.25 ± 3.1.

The mean Venous Clinical Severity Score five month after procedure was 1.86 ± 0.9.

The decrease in Venous clinical severity score from the pre-procedural score to the second and fifth month scores (post procedure) was found to be statistically significant (p<0.05).

**Venous Disability Score Pre-Procedure and During Follow-Up**

The mean venous disability score pre procedure was 1.4 ± 0.23. The mean venous disability score one month after procedure was 1.1 ± 0.32. The mean venous disability score three months after procedure was 0.82 ± 0. All patients were noted to have a venous disability score of 0 six months after the procedure. The decrease in Venous disability Score from the pre-procedural score to the first, third and sixth month scores (post procedure) was found to be statistically significant (p<0.05).

**Superficial Venous Reflux Pre-Procedure and During Follow-Up:**

Reflex was noted to be present in 72 out of the total 100 Great Saphenous veins. Theses veins did not show reflux in the USG examinations done at 2nd month and 5th months post-procedure. All 100 patients of the Great saphenous veins who were ablated showed occlusion at end of 2nd months and 5th months post procedure. Recanalization of GSV was not found in any of the 100 Great saphenous veins 5th months after the procedure.

**Discussion**

Endovenous laser ablation provides a minimally invasive, very effective and safe treatment option for varicose veins. Laser diodes of varying wavelengths (810 - 1470 nm) can be used to perform the venous ablation. In our study, the 1470 nm laser diode along with radial fibre is used. This diode produces light energy which is primarily absorbed by the water molecules in the vein. The light energy produced by the laser diodes of lower wavelength are preferentially absorbed by the haemoglobin in the vein. Use of a radial fibre along with a laser diode of 1470 nm wavelength results in significant reduction in the power required to achieve the venous ablation. This method also reduces the discomfort of the patient after the treatment.

In a study performed by Desmyttere et al. in 500 patients (436 women, 64 men, treatment with EVLA was performed and occlusion with a rate of 98% was obtained immediately after the procedure. In the one-year follow-up visit, the LSV was mostly not visualized or was seen as a fibrous cord. Even when the patients were followed up after 4 years, the venous occlusion rate was found to be 97.1%. In our study, all 100 of the ablated Great saphenous veins were noted to be occluded at 2nd month and 5th months.

EVLA treatment was found to be successful in 85.5% of patients in a study done by Sharif et al. The procedure was performed in 145 limbs of 136 patients who had Great saphenous vein insufficiency. A complete occlusion rate of 89.7% and a partial occlusion rate of 7.7% were observed at a 2 month follow-up visit. At the 12-month follow-up visit, complete occlusion was observed with a rate of 76% and partial occlusion was found with a rate of 18%. In our study, all 100 of the ablated Great saphenous veins were noted to be occluded at 2nd month and 5th months.

In a study performed by M Beyazal et al.
a 980 nm diode laser, the mean diameter of the saphenous veins pre-procedure was 4.9 ± 0.8 mm. After months post-procedure, the mean diameter of the saphenous veins was 1.8 ± 0.2 mm. The pre-procedure VCSS score in this study was 5.91 ± 1.2 mm. The VCSS score 5th month post-procedure in this study was 0.8 ± 0.6 mm.

In our study, 1470 nm diode laser has been used. The mean diameter of saphenous vein pre-procedure and 5th month post-procedure measures 3 cm below SFJ were found to be 6.23 ± 0.67 mm and 1.11 ± 0.02 mm respectively. The mean Venous Clinical Severity Score pre-procedure was 19.2 ± 4.2. The mean Venous Clinical Severity Score six month after procedure was 1.86 ± 0.9. There is a larger mean difference in Saphenous vein diameter and VCSS score in our study and can be due to the usage of 1470 nm laser diode with radial tip fibre.

In a study performed by Rathod et al. EVLA was performed on 108 veins on 76 limbs using 1470 nm diode laser and were followed up on day 2, 1 month, 6 months, and 12 months post-procedurally. The venous occlusion rate at the end of 1 year follow-up was 98.61%. The pre-procedure clinical grade and the venous disability score also showed significant improvement.

In a study performed by Mundy et al., rate of closure of GSV has been reported as 88-100%. Relief in symptoms along with reduction in superficial varicosities has been observed. Separate treatment of individual varicosities is not needed binitially. Any varicosities which are remaining 6 weeks after the EVLA procedure can be treated using delayed sclerotherapy.

In our study, we found that there was a significant progressive reduction in the Saphenous vein diameter at various levels.

When the pre-procedure value and the postprocedure values after 2nd month and 5th month were compared (p<0.05). We also found that there was a significant reduction in the VCSS and VDS scores when the pre-procedure values and the post-procedure values after 2nd month and 5th months were compared (p<0.05). Greenhouse-Geisser correction was used to calculate the p value and thus evaluate the significance of the progressive reduction in saphenous vein diameters along with the VCSS and VDS scores. No evidence of venous reflux nor recanalization was noted in any of the 50 Great saphenous veins which were ablated.

**Conclusion**

EVLA is a minimally invasive technique. Cannulation of the vein is done using a needle and the further procedure is performed through this site. Few injections are given surrounding the superficial vein while injecting the local anaesthetic. This also provides excellent cosmesis. EVLA results in irreversible damage to the vein which is ablated. The venous occlusion is mainly due to progressive fibrosis which causes obliteration of the vein rather than thrombotic occlusion. As this is a new technique, data regarding the long-term outcome of EVLA is required to confirm the durability of the procedure.

**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

**References**


Aerobic Weight and Non-Weight Bearing Exercise Effects on Myocardium: An in vivo Study on Elderly Mus Musculus

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Abstract

Background: Due to the process of aging, the function of human organs and muscles decreases. Exercise is a solution to improve organ and muscle function. But it all must be in proportion. So far, aerobic exercise is an exercise solution for the elderly, but which aerobic exercise has not been widely studied.

Purpose: To test aerobic weight bearing exercise and aerobic non weight bearing exercise as a sports solution for old age.

Methods: The experimental laboratory used a completely randomized post-test design using 26 elderly females' mice samples with three actions, namely 9 mice that were not exercised, 9 mice with aerobic weight bearing exercise and 8 mice with aerobic non weight bearing exercise. this process is carried out for 6 weeks with a frequency of exercise 5 times a week, and processed statistically using the statistical product and service solution (SPSS) program.

Results: Aerobic weight-bearing exercise and aerobic non-weight-bearing exercise resulted in an enlargement of the myocardium but there was significant difference between groups due to the One Way ANOVA test, with p-value < 0.05.

Conclusion: weight bearing exercise induce hipertrophic effects and significant differences between aerobic weight bearing exercise and aerobic non weight bearing exercise between the two groups.

Keywords: aerobic weight bearing exercise, aerobic non weight bearing exercise, elderly, myocardium

Introduction

The heart is one of the most vital organs in the human body systems. On the other hand, as humans age, they are going to advance the final stage of the human cycle, the elderly. Elderly is a period in which humans have reached maturity in size and organ function, whose functions have also shown a decline over time, as well as a process of slowly disappearing the ability of tissues to repair in order to maintain their normal functional structure.1 Accordingly, the heart as a part of the cardiovascular system is also going to show a decline in function as they are aging.

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The aging process will cause anatomical, physiological, and biochemical changes which might significantly affect the function and ability of the body as a whole, including muscle mass decrease, changes in muscle blood distribution, muscle cell pH alteration, muscle stiffening, and muscle strength weakening.\(^1\)

In addition, the female physical strength equal to two-thirds of that male possessed. Poitrast reported that at the age of twenty, women had 65% lifting strength compared to men while her pushing and pulling strength was approximately 75% of the man.\(^2\) Moreover, optimal muscle strength is at the age of 20-39 years and will decrease by 20% at the age of 60 years. Hence, it could be understood that women’s physical endurance tends to be relatively lower than men’s and might be considered as a more vulnerable group.

Exercise is one of the solutions to improve organ and muscle functions. So far, aerobic exercise is an exercise solution for the elderly. Aerobic exercise might improve body composition, such as body fat, bone health, muscle mass, muscle endurance, muscle strength, and muscle flexibility so that the elderly are expected to be healthier and fitter and lower the risk of falling. The benefits of aerobic exercise for the elderly include prolonging life expectancy, nourishing the cardiorespiratory and musculoskeletal organs, promoting being more independent, preventing obesity, reducing anxiety and depression, and gaining higher self-confidence. Aerobic exercise for health maintenance is suggested to be done in the morning, without exceeding the 60 - 70% maximum heart rate.\(^1\)

Aerobic exercise comprises activities that make an individual bear his body weight (weight-bearing), such as walking, or activities that do not directly support his body weight (non-weight bearing), such as cycling and swimming. The usefulness of an exercise program for the elderly also relies on the program being conducted. Therefore, it is recommended that the exercise program should fulfill principles of Frequency, Intensity, Time, Type (FITT).\(^3\)

According to Aziz et. al. (2021) study, the heart rate of athletes was significantly higher when they predominantly performed anaerobic compared to aerobic exercise.\(^5\) This might be explained as the oxygen demand of the aerobic exercise-performing population is considerably high and constant. An increase in preload and volume becomes the major stimulus for ventricular adaptation in the form of hypertrophy and dilatation of the ventricular wall, thereby increasing the effectiveness of heart-pumping function, which in turn would reduce the heart rate in predominantly aerobic athletes. This finding might support the idea that aerobic is better than anaerobic exercise, especially for elderly population. Furthermore, Rahima et. al. (2015) added that there might also be changes in the levels of Brain Natriuretic Peptide (BNP) in the heart muscle which affected changes in the histological size of the heart muscle.\(^5\) However, the study concerning which type of aerobic exercise is better for elderly still becomes a research topic that should be explored. Therefore, this study aimed to investigate the effect of weight-bearing and non-weight bearing aerobic exercise on myocardiac histological properties in animal model of elderly Mus musculus.

**Materials and Methods**

A laboratory experimental study employing a randomized posttest design was conducted in Animal Study Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia, from January to February 2021. All experimental procedure has been reviewed and approved by Medical Research Ethics Committee, Faculty of Medicine, Universitas Airlangga (No.77/EC/KEPK/FKUA/2021).

A total of twenty-seven female mice (Mus Musculus) aged 7–8-month-old with good physical fitness were obtained from Animal Study Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga. Mice were randomly assigned to three study groups evenly, generating three observed groups (control [K0], aerobic weight bearing [K1], and aerobic non weight bearing group [K2]). Mice were placed at cages measuring 60x20x40 cm made from plastic covered with gauze equipped with a place to eat and drink bottles. Each cage was occupied by each group consisting of nine mice. Mice were placed at room temperature and the lighting was regulated by a 12-hours light-dark cycle. The feeding was done at 09.00 - 10.00 a.m. ad libitum. Formerly, all
mice were acclimatized in their cages for two weeks and were habituated with treadmill and swimming activity during their second week’s acclimatization, 5 minutes/day.

K1 group were treated with zero-degree-inclination treadmill exercise, conveyed at 21 cm/s in velocity. Each exercise session took 30 minutes and was given 5 times/week for six weeks (Souza et. al., 2007). K2 group received 30 minutes of swimming exercise for each session, given 5 times/week for six weeks (Darsana et al, 2019).

On the last day of sample observation, the myocardiac tissue of each animal model was obtained by surgical process done under ketamine HCl anesthesia. However, one of the studied mus musculus from the K2 group should be excluded since there was significant technical damage in its myocardiac specimen. Therefore, the final myocardium specimens that met eligibility criteria were twenty-six specimens. All specimens were prepared for histopathological examination on Hematoxylin Eosin Staining and were analyzed microscopically through 400x magnifications. The myocardiac thickening was also examined by using the ImageJ v1.33 software downloaded from the NIH website (http://rsb.info.nih.gov/ij).

Obtained the data were then processed and analyzed statistically using SPSS v16 for Windows. The normality test was carried out using the Kolmogorov-Smirnov test following by the homogeneity test to determine the similarity between the treatment groups. The test used to compare the average comparison values of the research studied was carried out using the One-Way ANOVA test. This finding was considered significant at p<0.05.

**Results and Discussion**

Out of twenty-six eligible specimens from three studied group, the baseline characteristics of myocardium thickness were summarized in the Table 1. Additionally, the representative of histopathological finding of each group were depicted in Figure 1.

**Table 1: Baseline characteristics of myocardium thickness of each investigated group**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Myocardium thickness (µm)</th>
<th>Minimum Thickness (µm)</th>
<th>Maximum Thickness (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K₀</td>
<td>9</td>
<td>15.952,72 ± 9571,67</td>
<td>1.643,25</td>
<td>35.734,75</td>
</tr>
<tr>
<td>K₁</td>
<td>9</td>
<td>10.049, 59 ± 7,255,05</td>
<td>839,23</td>
<td>28.571,05</td>
</tr>
<tr>
<td>K₂</td>
<td>8</td>
<td>8.227,61 ± 7,988,26</td>
<td>213,81</td>
<td>31.301,56</td>
</tr>
</tbody>
</table>

**Figure 1: Histopathological appearances of K0 (a); K1 (b); K2 (c)**

After analyzing the comparative studies, this study found that the thickness of the myocardium of K1 was significantly lower than that of K0, as was K2. However, the thickness of the K1 myocardium was significantly higher than that of K2. Comparative studies of the thickness of the myocardium in each group can be seen in Table 2 below.
Tabel 2: Comparative study of myocardium thickness of each group (ANOVA-one way test)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>ANOVA-one way test</th>
</tr>
</thead>
<tbody>
<tr>
<td>K₀</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>K₁</td>
<td>9</td>
<td>0.004*</td>
</tr>
<tr>
<td>K₂</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

* significant at p<0.050

Evidence of significant thickness of the myocardium seen in the aerobic weight bearing exercise group may result in mild enlargement of the ventricular myocardium. This is in line with research by Soya et. al. (2007) who reported that treadmill aerobic exercise causes myocardial hypertrophy. This is also supported by another study which showed mild ventricular hypertrophy after being given treatment in the form of running for 6 weeks, compared to the group without intervention.

Similarly, the findings of a significant thickness of myocardium were seen in the aerobic non weight bearing exercise group of the study reported by Medeiros et. al. (2004) who observed that aerobic non weight bearing exercise can cause myocardial adaptation in the form of various changes, including enlargement and renewal of the myocardium. In addition, these findings are in line with another study in adult mice treated with swimming for 4 weeks which reported that the treated mice only experienced an increase in ventricular mass, ventricular mass-weight ratio, and an increase in heart wall thickness compared to adult mice. those who do not exercise.

This study also showed that both aerobic weight-bearing exercise and aerobic non weight bearing exercise groups caused a hypertrophic effect. However, when viewed from the descriptive mean test, the results without exercise have the highest value compared to the average of aerobic weight bearing exercise and aerobic non weight bearing exercise. Possibly this was due to the low number of mice in the aerobic non weight bearing exercise treatment, as well as the high oxygen consumption in the mice that rested too much. However, there has been no further research that discusses the difference between aerobic weight bearing exercise and aerobic non weight bearing exercise but based on previous research that discusses the effects of exercise can cause hypertrophy.

**Conclusion**

This animal model study suggests that the effect of aerobic weight bearing exercise and aerobic non weight bearing exercise on elderly mice myocardium differ significantly. However, more research concerning this topic still needed to add more information in this field.

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

**Source of Funding:** No financial support was provided for this project.

**References**

Study of Lopinavir/Ritonavir in Severe COVID-19 Patients with Comorbid Hypertension and Diabetes Mellitus

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Abstract

\textbf{Background:} Lopinavir/ritonavir is an antiviral studied for its effectiveness in healing severe COVID-19 infection; however, it remains debated. This study determined the efficacy and evaluated the side effects of lopinavir/ritonavir on severe COVID-19 patients with comorbid hypertension and diabetes mellitus.

\textbf{Methods:} Retrospective data collection was conducted from June to December 2020. The data were then analyzed descriptively. Severe COVID-19 patients with hypertension and diabetes mellitus who received lopinavir/ritonavir were grouped into groups A, B, C, D and observed based on other pharmaceutical therapies (antibiotics, corticosteroids, anticoagulants, multivitamins and supplements, comorbid therapy, and symptomatic therapy) obtained. The efficacy of lopinavir/ritonavir was identifiable through RT-PCR, oxygenation saturation, and chest X-ray. The side effects of lopinavir/ritonavir were also be evaluated.

\textbf{Results:} Only 21 total sample met the inclusion criteria. The oral administration of lopinavir/ritonavir (2 x 400/100 mg) was effective in groups A, B, C, and D whose negative RT-PCR results were respectively 10%, 48%, 5%, and 14%; normal oxygen saturation with the administration of oxygen therapy was 10%, 33%, 5%, and 5%, respectively; and chest X-ray improvement was 10%, 29%, 5%, and 14%, respectively. Lopinavir/ritonavir could increase SGOT (46%), SGPT (27%), and as a QT interval prolongation (5%).

\textbf{Conclusion:} The administration of lopinavir/ritonavir is effective to rule out negative RT-PCR results, improve chest X-ray imaging and oxygen saturation. Monitoring side effects merits more attention to avoid those potential side effects of the therapies.

\textbf{Keywords:} COVID-19, efficacy, lopinavir, ritonavir, side effects
Introduction

COVID-19 infection is happening due to Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). On March 11, 2020, WHO declared the COVID-19 outbreak a pandemic.\(^1\) Southeast Asia was ranked in third place after Europe and America for the cumulative increase in new confirmed cases (12%) and deaths (8%).\(^2\) The clinical manifestations of COVID-19 are divided into mild, moderate, severe, and critical symptoms.\(^3\)

Antivirals are one of the pharmacological therapy strategies administered to all stages of COVID-19. However, the effectiveness of the antivirals against SARS-CoV-2 remains unclear. In vitro, lopinavir has antiviral activity against SARS-CoV-2.\(^4\) A cohort study comparing the effects of hydroxychloroquine and lopinavir/ritonavir on severe COVID-19 patients with comorbidities showed that the lopinavir/ritonavir group had a lower hospital mortality rate and a lower percentage of patients transferred to the ICU than the hydroxychloroquine group. Besides, the percentage of negative PCR test results in the lopinavir/ritonavir group was higher than in the hydroxychloroquine group. Meanwhile, the discontinued use of lopinavir/ritonavir in patients occurred because of side effects (nausea and diarrhea) and the size of the drug tablets, as well as increased liver enzymes in some patients.\(^5\)

An RCT study on severe COVID-19 patients in China demonstrated that the administration of lopinavir/ritonavir did not significantly accelerate clinical improvement, as well as reduce mortality and viral RNA levels. Recovery in the lopinavir/ritonavir group was only one day earlier than in standard care. Lopinavir/ritonavir was discontinued early in 13 patients because of gastrointestinal side effects, and a QT prolongation effect was also found in the lopinavir/ritonavir group.\(^6\)

The Indonesian Task Force for COVID-19 (Gugus Tugas Percepatan Penanganan COVID-19) reported the highest variations of comorbidities found in COVID-19 patients were hypertension (50.5%), diabetes mellitus (34.5%), and heart disease (19.6%).\(^7\) An observational study stated that the high mortality rate (87.1%) occurred to severe COVID-19 patients with comorbid diabetes mellitus and hypertension.\(^8\) Given those facts, this study took advanced look to describe the effectiveness and evaluate the side effects of lopinavir/ritonavir in severe COVID-19 patients with comorbid hypertension and diabetes mellitus at Bhayangkara H.S. Samsoeri Mertojoso Hospital, Surabaya.

Material and Methods

This study was observational research collecting data retrospectively from the medical records of patients at Bhayangkara Hospital, Surabaya. It involved severe COVID-19 patients with comorbid hypertension and diabetes mellitus who received lopinavir/ritonavir (2 x 400/100 mg) orally. The administration of lopinavir/ritonavir was categorized as 4 groups: Group A (antibiotics, corticosteroids, anticoagulants, multivitamins and supplements, comorbid therapy, and symptomatic therapy), B (therapy in group A without corticosteroids), C (therapy in group A without anticoagulants), and D (therapy in group A without corticosteroids and anticoagulants).

Patients who received an antiviral therapy other than lopinavir/ritonavir and patients who were discharged against medical advice were excluded from the criteria. The time-limited sampling of data collection was conducted from June 2020 to December 2020. Descriptive analysis was done to the data including patient profiles (age and gender), past medical history, Lopinavir/Ritonavir therapy (duration of administration and side effects), length of hospital stays, diagnosis, clinical and laboratory data.

Results and Discussion

Demographics

In this study, 21 medical records of patients were observed, and they consisted of 19 discharged patients and 2 patients who died. Table 1 shows that 15 patients under investigation were mostly male (71%), while six patients were female (29%). Li, et. al.\(^9\) found that severe COVID-19 occurred more often in men (28.2%) than women (18.8%). Thus, the risk of severity and mortality were higher 1.6 times in men than in women. Genetic factors affect ACE-2 receptor expression which commonly was more expressed in man’s lungs than woman’s.\(^10\)
Table 1: Characteristics of COVID-19 patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number of patients</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender (n=21)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>15</td>
<td>71</td>
</tr>
<tr>
<td>Female</td>
<td>6</td>
<td>29</td>
</tr>
<tr>
<td><strong>Age Range (n=21)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 – 25 years old</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>26 – 45 years old</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>46 – 65 years old</td>
<td>16</td>
<td>76</td>
</tr>
<tr>
<td>&gt;65 years old</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td><strong>Comorbidities (n=21)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>9</td>
<td>43</td>
</tr>
<tr>
<td>Hypertension</td>
<td>7</td>
<td>33</td>
</tr>
<tr>
<td>Hypertension and Diabetes Mellitus</td>
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<td>24</td>
</tr>
<tr>
<td><strong>Blood Pressure (mmHg) (n=19)</strong></td>
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<tr>
<td>Normotension on admission</td>
<td>12</td>
<td>63</td>
</tr>
<tr>
<td>Normotension on discharge</td>
<td>16</td>
<td>84</td>
</tr>
<tr>
<td>Hypertension on admission</td>
<td>7</td>
<td>37</td>
</tr>
<tr>
<td>Hypertension on discharge</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td><strong>Blood Glucose Level (mg/dL) (n=19)</strong></td>
<td></td>
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<tr>
<td>Normoglycemia on admission</td>
<td>11</td>
<td>42</td>
</tr>
<tr>
<td>Normoglycemia on discharge</td>
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<td>89</td>
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<tr>
<td>Hyperglycemia on admission</td>
<td>8</td>
<td>58</td>
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<tr>
<td>Hyperglycemia on discharge</td>
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<td>11</td>
</tr>
<tr>
<td><strong>Clinical Symptoms</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>16</td>
<td>76</td>
</tr>
<tr>
<td>Cough</td>
<td>14</td>
<td>67</td>
</tr>
<tr>
<td>Shorness of breath</td>
<td>12</td>
<td>57</td>
</tr>
<tr>
<td>Nausea</td>
<td>11</td>
<td>52</td>
</tr>
<tr>
<td>Loss of Appetite</td>
<td>7</td>
<td>33</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>6</td>
<td>29</td>
</tr>
<tr>
<td>Vomiting</td>
<td>5</td>
<td>24</td>
</tr>
<tr>
<td>Weakness</td>
<td>4</td>
<td>19</td>
</tr>
<tr>
<td>Headache</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Myalgia</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Cold</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Dry throat</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Flatus</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

Following that 16 patients aged 46-65 years (elderly) were the most dominant among the groups (76%). Aging influences physiological changes in the immune system which are categorized into immunosenescence (the immune system changes associated with age) and inflame-aging (chronic low-grade inflammation that develops with aging). These factors may lead to a progressive decrease in the ability of the immune system against new and latent infections.

**Comorbid**

Diabetes mellitus is the most common comorbid among the patients (43%). Unlike this finding, previous research discovered that COVID-19 patients mostly suffered form hypertension (22.6%), followed by diabetes mellitus (11.5%). Their might be due to different number of patients, years, and places of research.

**Clinical Symptoms**

In Table 1, the common clinical symptoms experienced by patients include fever (76%), cough (67%), shortness of breath (57%), and nausea (52%). Fever and cough are common symptoms in COVID-19 patients. Setting, and Participants: Case series of patients with COVID-19 admitted to 12 hospitals in New York City, Long Island, and Westchester County, New York, within the Northwell Health system. The study included all sequentially hospitalized patients between March 1, 2020, and April 4, 2020, inclusive of these dates. Exposures: Confirmed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Further meta-analysis studies explained that the incidence of shortness of breath occurred in severe cases of COVID-19. The digestive symptoms among COVID-19 patients likely occurs due to the ACE-2 and TMPRSS as the SARS-CoV-2 receptors also on enteroendocrine cells (EEC) can act directly on brain stem structures although variable, is comparable with diarrhea. Poor definition of N, confusion with appetite loss, and reporting of N and/or V as a single entity may contribute to reporting variability and likely underestimation. We propose that emetic mechanisms are activated by mediators released from the intestinal epithelium by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

**Treatment of Comorbid Hypertension and Diabetes Mellitus**

Table 1 also shows patients admitted to the
hospital with hypertension (37%) and hyperglycemia (58%). Previous retrospective observational study where COVID-19 patients with high blood pressure were twice as likely to die as patients without high blood pressure.\textsuperscript{15} A retrospective cohort study in China found that increase in blood glucose levels is an independent risk factor for critical/death conditions among non-critical COVID-19 patients.\textsuperscript{16} Therefore, an additional therapy is necessary for COVID-19 patients with comorbidities.

### Tables 2: Hypertension and diabetes mellitus therapies of COVID-19 patients

<table>
<thead>
<tr>
<th>Comorbidities</th>
<th>Therapy</th>
<th>Total</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug Class</td>
<td>Medications</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>Insulin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glargine</td>
<td>6</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Aspart</td>
<td>4</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Glulisine</td>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Insulin sensitizer</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Metformin</td>
<td>9</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>Pioglitazone</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Insulin secretagogue</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glimepiride</td>
<td>6</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Glibenclamide</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Diuretic</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hydrochlorothiazid</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>CCB</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Amlodipine</td>
<td>8</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>Nifedipine</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>ACE inhibitor</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lisinopril</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>ARB</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Candesartan</td>
<td>7</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>Beta-blocker</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bisoprolol</td>
<td>5</td>
<td>24</td>
</tr>
</tbody>
</table>

Note: one patient can have more than one medication; ACE inhibitor, Angiotensin-converting enzyme inhibitors; ARB, Angiotensin Receptor Blocker; CCB, Calcium Channel Blocker.

Patients with diabetes and hypertension also received treatment (see table 2). Amlodipine was a calcium channel blocker (CCB) antihypertensive. All CCB drugs were metabolized by CYP450, especially CYP3A4 (except nisoldipine).\textsuperscript{17} The use of CCBs with ritonavir simultaneity could cause drug interactions, causing the risk of bradycardia and hypotension, as well as amlodipine levels to increase.\textsuperscript{18} The interactions occur as ritonavir is a strong inhibitor of CYP3A4.\textsuperscript{19} Therefore, regular blood pressure monitoring and dose adjustment based on blood pressure must follow. Metformin is an oral antidiabetic drug that lowering blood sugar by increasing insulin sensitivity. Another advantage of metformin in COVID-19 patients is its therapeutic potential for cardiovascular disease.\textsuperscript{20} A retrospective study showed metformin on COVID-19 patients contributed to reducing the incidence of hospital mortality.\textsuperscript{21}

### Median Duration of Lopinavir/Ritonavir Administration and Length of Hospital Stay

The median length of oral lopinavir/ritonavir (2 x 400/100 mg) administration was 10 days (see Table 3). In several other studies, the length of oral lopinavir/ritonavir (2x400/100 mg) administration is 14 days as the incubation period for COVID-19 is about one to 14 days.\textsuperscript{22} Besides, the median length of hospital stay was 15 days. This finding was almost similar to previous research which demonstrated the median length of hospital stays for severe COVID-19 patients without comorbid hypertension and diabetes mellitus receiving lopinavir/ritonavir therapy was 17 days.\textsuperscript{21} The length of hospital stay for COVID-19 patients is influenced by several factors, for example gender and age.\textsuperscript{23} Further, serious health conditions also influence the length of hospital stay.\textsuperscript{24}
### Tables 3: Duration of Lopinavir/Ritonavir administration and length of stay Profile

<table>
<thead>
<tr>
<th>Profile</th>
<th>Therapy</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A (n = 3)</td>
<td>B (n = 12)</td>
</tr>
<tr>
<td>Median duration of administration (Day)</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>Median length of stay (Day)</td>
<td>19</td>
<td>18</td>
</tr>
</tbody>
</table>

### Tables 4: Effectivity parameters of Lopinavir/Ritonavir therapy Profile

<table>
<thead>
<tr>
<th>Effectivity Parameters</th>
<th>Therapy</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A (n = 3)</td>
<td>B (n = 12)</td>
</tr>
<tr>
<td>RT-PCR Negative</td>
<td>10%</td>
<td>48%</td>
</tr>
<tr>
<td>RT-PCR Positive</td>
<td>5%</td>
<td>10%</td>
</tr>
<tr>
<td>Oxygen Saturation Normal With oxygen therapy</td>
<td>10%</td>
<td>33%</td>
</tr>
<tr>
<td></td>
<td>5%</td>
<td>19%</td>
</tr>
<tr>
<td></td>
<td>0%</td>
<td>5%</td>
</tr>
<tr>
<td>Chest X-Ray Improve</td>
<td>10%</td>
<td>29%</td>
</tr>
<tr>
<td></td>
<td>5%</td>
<td>19%</td>
</tr>
<tr>
<td>Death</td>
<td>0%</td>
<td>5%</td>
</tr>
</tbody>
</table>

Note: *Two patients with incomplete chest X-ray data; RT-PCR, reverse transcription-Polymerase Chain Reaction.

### Tables 5: Liver enzyme levels and QT interval data of COVID-19 patients

<table>
<thead>
<tr>
<th>No</th>
<th>Patients Initials</th>
<th>SGOT (µ/L) Admission</th>
<th>Discharge</th>
<th>SGPT (µ/L) Admission</th>
<th>Discharge</th>
<th>QT Interval (ms) Admission</th>
<th>Lopinavir/Ritonavir (day to)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Mr JM*</td>
<td>58</td>
<td>37</td>
<td>50</td>
<td>25</td>
<td>Mr JM*</td>
<td>492</td>
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<tr>
<td>2</td>
<td>Mr GI*</td>
<td>55</td>
<td>33</td>
<td>46</td>
<td>39</td>
<td>Mr GI**</td>
<td>441</td>
</tr>
<tr>
<td>3</td>
<td>Mr Su*</td>
<td>45</td>
<td>43</td>
<td>16</td>
<td>11</td>
<td>Mr Su**</td>
<td>***</td>
</tr>
<tr>
<td>4</td>
<td>Mr Sj*</td>
<td>41</td>
<td>19</td>
<td>25</td>
<td>20</td>
<td>Mr Sj**</td>
<td>455</td>
</tr>
<tr>
<td>5</td>
<td>Mrs AS*</td>
<td>33</td>
<td>31</td>
<td>26</td>
<td>30</td>
<td>Mrs AS*</td>
<td>482</td>
</tr>
<tr>
<td>6</td>
<td>Mr PW*</td>
<td>31</td>
<td>43</td>
<td>28</td>
<td>39</td>
<td>Mr PW**</td>
<td>***</td>
</tr>
<tr>
<td>7</td>
<td>Mr DP*</td>
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<td>26</td>
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<td>Mr DP**</td>
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<td>Mr TH*</td>
<td>30</td>
<td>25</td>
<td>20</td>
<td>20</td>
<td>Mr TH**</td>
<td>***</td>
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<tr>
<td>9</td>
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<td>57</td>
<td>82</td>
<td>41</td>
<td>Mrs S**</td>
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</tr>
<tr>
<td>11</td>
<td>Mr Ts**</td>
<td>62</td>
<td>39</td>
<td>26</td>
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<td>Mr Ts**</td>
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<td>40</td>
<td>36</td>
<td>25</td>
<td>Mr I**</td>
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<tr>
<td>13</td>
<td>Mr SE**</td>
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<td>17</td>
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<td>42</td>
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<td>14</td>
<td>Mr K**</td>
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<td>31</td>
<td>58</td>
<td>22</td>
<td>Mr K**</td>
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</tr>
<tr>
<td>15</td>
<td>Mr O**</td>
<td>41</td>
<td>32</td>
<td>30</td>
<td>29</td>
<td>Mr O**</td>
<td>409</td>
</tr>
</tbody>
</table>
Effectiveness of Lopinavir/Ritonavir Therapy

Table 4 shows the effectiveness of lopinavir/ritonavir therapy in patients based on RT-PCR results, improvement in oxygen saturation, and improvement in chest X-ray results. The patients mostly had RT-PCR results negative (76%) after the therapies. Supporting this finding, a cohort study in Austria showed the use of lopinavir/ritonavir could make severe COVID-19 patients (64.5%) recovered and having negative RT-PCR swab results in 9 to 17 days.\(^5\) Lopinavir/ritonavir has contributed to the effectiveness of COVID-19 convalescence due to its mechanism of action. It could inhibit the translation process, an important step in the viral maturation process. In vitro studies showed that lopinavir has an antiviral activity against SARS-CoV-2.\(^{25}\) It is likely able to inhibit the enzyme 3-chymotrypsin-like protease (3CLpro) which is important in the viral life cycle; thus, it could interfere with the process of replication and transcription.\(^{26}\)

Improvements in the chest X-ray (radiological) results and oxygen saturation (clinical) are determinants of patient discharge.\(^3\) The administration of lopinavir/ritonavir to severe COVID-19 patients with comorbid hypertension and diabetes mellitus overall led to better chest X-ray results (57%; n=12). Lopinavir/ritonavir is likely effective in improving lungs condition (chest X-ray) of COVID-19 patients.\(^{27}\) Besides, supporting with oxygen therapy, 53% (n=11) of the patients receiving lopinavir/ritonavir had better oxygen saturation. The results of this study are in line with the latest research, where severe COVID-19 patients were more in need for ventilators (oxygen therapy) along with the lopinavir/ritonavir administration.\(^{28}\) Severe COVID-19 patients without comorbid hypertension and diabetes mellitus who received lopinavir/ritonavir therapy also required a ventilator during their treatment.\(^{21}\) In short, both types of patients need a ventilator for treatment.

Side Effects of Lopinavir/Ritonavir

The side effects upon the administration of lopinavir/ritonavir included increased liver enzyme levels (SGOT of 46% and SGPT of 27%) and QT interval prolongation (5%) (see Tables 5). Such increased liver enzymes from lopinavir might be due to extensive metabolic processes in the liver, mostly by cytochrome P450 (CYP3A4), which could result in the production of harmful intermediates.\(^{29}\) The use of low-dosage ritonavir in combination with other drugs seems not to increase the frequency or severity of elevated liver enzyme levels. However, due to its enzymatic properties, it could increase the plasma concentration of the co-administered drug (lopinavir); hence, it could increase the risk of hepatotoxicity.\(^{30}\)

A case study conducted by Zhu, et al\(^{31}\) no studies have reported an association between corrected QT (QTc found a side effect of QT prolongation during the administration of lopinavir/ritonavir in COVID-19 patients. Because of the side effects caused by therapy, the complications must be given attention as they can affect the clinical condition of the patients. One of ways to anticipate the complications is monitoring and evaluating therapy in patients by pharmacists and other health workers. To sum up the findings, this retrospective study is not enough to see
the side effects of lopinavir/ritonavir therapy. None of the patients had to discontinue lopinavir/ritonavir therapy due to the side effects. Besides, supportive therapy that patients receive might vary and affect the effectiveness and side effects of lopinavir/ritonavir therapy in different ways.

Conclusion

The oral administration of lopinavir/ritonavir (2 x 400/100 mg) in this study was effective in providing negative RT-PCR results, improving chest X-ray results, and improving clinical conditions of oxygen saturation. The side effects were detected to increase SGOT of 46%, SGPT of 27%, and QT interval prolongation of 5%.

Acknowledgements: The author would like to express a gratitude to the Bhayangkara H.S. Samsoeri Mertojoso Hospital Surabaya which has assisted researchers in providing facilities for data collection.

Ethical Clearance: This study was approved by the Ethics Committee of Bhayangkara Hospital (Ethics Number: 06/IV/2021/KEPK/RUMKIT).

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References


The Effectiveness and Side Effects of Remdesivir in COVID-19 Patients with Pneumonia

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Abstract

Background: Remdesivir is one of the antiviral agents to treat COVID-19 infection. It has an antiviral activity directly related to activation of pro-inflammatory responses, causing cytokine storm, multiorgan damage and mortality. With these potential activities, this study investigated the effectiveness and evaluated side effects of remdesivir used for COVID-19 patients with pneumonia.

Methods: This cohort observational study collected data retrospectively from medical records of COVID-19 patients at Bhayangkara H.S Samsoeri Mertojoso Hospital, Surabaya. Patients who received remdesivir with loading dose (1 x 200 mg) IV had to follow maintenance dose (1 x 100 mg) IV. They were analyzed in three groups from October 2020 to February 2021. The data conveyed PCR/rapid Ag Swab, oxygen saturation, chest X-rays imaging, BUN, SCr, SGOT and SGPT.

Result: A total of 60 patients were evaluated in three therapeutic groups, and the administration of remdesivir within 5-9 days was effective. Mostly, the patients (77%) had negative PCR/rapid Ag Swab; most of them (52%) had better oxygen saturation by supplementary oxygen therapy; more than a half had better chest X-rays imaging parameters (68%), Common side effects included an increase in SGOT (85.30%), SGPT (79.88%), BUN (74.23%), and serum creatinine (43.33%).

Conclusion: The antiviral effect of remdesivir with loading dose (1 x 200 mg) IV needs to follow maintenance dose (1 x 100 mg) IV for 5-9 days. Hence, COVID-19 patients with pneumonia could have clinical improvement with increased level of liver and kidney function.

Key words: antiviral, COVID-19, pneumonia, remdesivir

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E-mail: didikhasmono@ff.unair.ac.id
Introduction

Since March 2021, coronavirus disease 2019 (COVID-19) caused by Severe Acute Respiratory Coronavirus-2 (SARS-CoV-2) has globally reached more than 116 million confirmed cases, including 2.5 million deaths. Suggested from the COVID-19 prevalence in Indonesia, the number of COVID-19 cases continuesly increase, and out of more than 1.3 million cases, the death rate was thirty-seven thousand deaths. Currently, COVID-19 patients receive antiviral therapy as primary pharmacological management. It can inhibit viral replication of RNA transcription which can cause worse inflammation and epithelial injury of the lung and cytokine storm. Cytokine storm is excessive inflammatory reactions mediated by the rapid production of cytokines leading to acute respiratory distress syndrome (ARDS), multi-organ failure, and even death.

Remdesivir is one of the antiviral drugs approved by the Food and Drug Administration (FDA) for the treatment of COVID-19. Beigel et al’s study conducted a study where remdesivir was given to 514 patients with lower respiratory tract infections for ten days. The initial dose of remdesivir (1 x 200 mg) IV was given on the first day, followed by next doses (1 x 100 mg) IV on 9 to 10 days. Results showed that patients who received remdesivir had an average recovery time of 10 days which were five days earlier than in patients who received a placebo. The overall report of mortality over 29 days showed 11.4% deaths in the remdesivir group and 15.2% in the placebo group. The administration of remdesivir for COVID-19 infection treatment was also given to severe COVID-19 patients for 5 or 10 days. Remdesivir therapy was given to 397 patients divided into two groups: 200 patients for 5 day administration and 197 patients for 10 day administration. The dose of remdesivir (1 x 200 mg) IV was given on the first day and (1 x 100 mg) IV on the next day. The results showed no significant difference between 5 day and 10 day remdesivir therapy in severe COVID-19 patients who did not require mechanical ventilation.

Material and Methods

This current study was a cohort observational-retrospective study using data on medical records of patients with pneumonia who received remdesivir therapy at Bhayangkara H.S Samsoeri Mertojoso Hospital, Surabaya. Samples were selected through time-limited sampling on October 2020 to February
Patients who received remdesivir with loading dose (1 x 200 mg) IV received maintenance dose (1 x 100 mg) IV. They were observed into three groups: Group 1 (remdesivir, antibiotics, dexamethasone, multivitamins, anticoagulant, N-Acetylcysteine, symptomatic and comorbid therapy); Group 2 (therapies in group 1 without anticoagulant); and Group 3 (therapies in group 1 without dexamethasone).

The data consisted of PCR/rapid Ag Swab, oxygen saturation, and chest X-rays imaging that bear the effectiveness of therapy. Then, BUN (blood urea nitrogen), SCr (serum creatinine), SGOT (serum glutamic oxaloacetic transaminase), and SGPT (serum glutamic pyruvic transaminase) after remdesivir administration were evaluated to find the side effects. All data were analyzed descriptively, except data on patients who were diagnosed with tuberculosis and incomplete medical records.

**Result and Discussion**

Sixty medical records of COVID-19 patients with pneumonia were collected. Patients were grouped based on the medical records of patients with or without comorbidities. Therapeutic Group 1 was patients who received remdesivir, antibiotics, dexamethasone, multivitamins, anticoagulants, N-Acetylcysteine, symptomatic and comorbid therapy. Therapeutic Group 2 was patients who did not have anticoagulants; and therapeutic Group 3 was patients who did not receive dexamethasone. Table 1 shows patient characteristics including gender, age, and comorbidities. Most of the patients in Group 1 were males (72%) at the age range mostly between 50-59 years (42%). Therapeutic groups commonly suffered from hypertension followed by diabetes. Other comorbidities (asthma, coronary heart disease) occurred the least (5%). Other drugs besides remdesivir were administered to COVID-19 patients with pneumonia (Table 2). To determine the effectiveness of the therapy and their side effects, the PCR/Rapid Ag Swab, oxygen saturation, chest X-rays imaging, liver and kidney function values were evaluated after remdesivir administration. Overall, the patients with or without comorbid showed negative PCR/rapid Ag Swab (77%), improved oxygen saturation by supplementary oxygen therapy (52%), and better chest X-rays imaging (68%) (Table 3). The administration of remdesivir yield an increase in liver function: SGOT (17 people; 85.30%) and SGPT (28 people; 79.88%). The effect on kidney function could cause BUN values in nine patients (74.23%) and serum creatinine in 8 patients (43.33%) to increase (Table 4).

**Patient Characteristics**

Out of sixty patients under investigation, most of them were males (Table 1). It was assumed that usually biological factors triggers infection by sexes. The expression of the angiotensin converting enzyme 2 (ACE2) receptor helps the virus to enter host cells differently between sexes,7caused by the coronavirus SARS-CoV-2, exhibits differences in morbidity and mortality between sexes. Here, we present a meta-analysis of 3,111,714 reported global cases to demonstrate that, whilst there is no difference in the proportion of males and females with confirmed COVID-19, male patients have almost three times the odds of requiring intensive treatment unit (ITU With its activity, men have higher plasma ACE2 levels than women which allows higher expression for SARS-CoV-2 infection. It explains why men are more susceptible to COVID-19 infection. In addition to biological factors, lifestyle is likely associated with higher COVID-19 infection rate in men than women. Men are less likely to take preventive measures against COVID-19 such as washing hands, social distancing, and looking for medical assistance.8 By the fact, higher smoking behaviour in men could lead to respiratory diseases compared to women.9,10much emphasis was initially placed on the elderly or those who have preexisting health conditions such as obesity, hypertension, and diabetes as being at high risk of contracting and/or dying of Covid-19. But it is now becoming clear that being male is also a factor. The epidemiological findings reported across different parts of the world indicated higher morbidity and mortality in males than females. While it is still too early to determine why the gender gap is emerging, this article point to several possible factors such as higher expression of angiotensin-converting enzyme-2 (ACE 2; receptors for coronavirus COVID-19 patients with pneumonia mostly were aged 50-59 years which are of productive age. People at productive age are more susceptible to COVID-19
infection as they do activities more often outside the home. As many as 37% of COVID-19 patients with pneumonia had one or more comorbidities, most likely hypertension. This is in line with a retrospective study on 654 COVID-19 cases where the highest comorbidities were hypertension (43.1%), diabetes (33.2%), and coronary heart disease (26.0%).

Table 1: Characteristic COVID-19 patient with pneumonia

<table>
<thead>
<tr>
<th>Category</th>
<th>Therapeutic 1 (n = 36)</th>
<th>Therapeutic 2 (n = 14)</th>
<th>Therapeutic 3 (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>26 (72)</td>
<td>6 (43)</td>
<td>5 (50)</td>
</tr>
<tr>
<td>Female</td>
<td>10 (28)</td>
<td>8 (57)</td>
<td>5 (50)</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-29</td>
<td>2 (6)</td>
<td>0 (0)</td>
<td>2 (20)</td>
</tr>
<tr>
<td>30-39</td>
<td>3 (8)</td>
<td>1 (7)</td>
<td>1 (10)</td>
</tr>
<tr>
<td>40-49</td>
<td>8 (22)</td>
<td>6 (43)</td>
<td>2 (20)</td>
</tr>
<tr>
<td>50-59</td>
<td>15 (42)</td>
<td>7 (50)</td>
<td>3 (30)</td>
</tr>
<tr>
<td>60-69</td>
<td>5 (14)</td>
<td>0 (0)</td>
<td>1 (10)</td>
</tr>
<tr>
<td>≥ 70</td>
<td>3 (8)</td>
<td>0 (0)</td>
<td>1 (10)</td>
</tr>
<tr>
<td><strong>Comorbid</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>6 (17)</td>
<td>1 (7)</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>4 (11)</td>
<td>0 (0)</td>
<td>2 (20)</td>
</tr>
<tr>
<td>Hypertension + diabetes</td>
<td>5 (14)</td>
<td>0 (0)</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Other</td>
<td>2 (5)</td>
<td>1 (7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Non comorbid</td>
<td>19 (53)</td>
<td>12 (86)</td>
<td>6 (60)</td>
</tr>
</tbody>
</table>

Table 2: Use of other drugs in COVID-19 patient with pneumonia

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Total Samples (n=60)</th>
<th>Total (Patients)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotic</td>
<td>60</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Anticoagulant</td>
<td>46</td>
<td>76.6</td>
<td></td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>50</td>
<td>83.3</td>
<td></td>
</tr>
<tr>
<td>N-acetylcysteine</td>
<td>60</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Multivitamin and supplement</td>
<td>60</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Effectiveness

The COVID-19 patients with pneumonia were grouped into three therapeutic groups (Table 1). Remdesivir is the antiviral administered to COVID-19 patients with pneumonia and serves as a prodrug that will be converted into a pharmacologically active form of triphosphate. Remdesivir works by binding to RdRp (RNA-dependent RNA polymerase) which inhibits viral replication by premature termination of RNA transcription. Remdesivir is given intravenously 200 mg IV loading dose then followed by 100 mg maintenance dose until Day 5. Its administration can be extended up to 10 days if the patients have not shown any clinical improvement.

The effectiveness parameters can be observed from the results of PCR/rapid Ag Swabs, oxygen saturation, and chest X-rays imaging. Effective therapies are observable from these aspects. The negative PCR/rapid Ag Swab occurred to Groups 1, 2, and 3 with a percentage of 48%, 17%, and 12%, respectively. Oxygen saturation was better in the groups in the same order with a percentage of 34%, 13%, and 5%, respectively. Oxygen saturation could improve since the use of lower supplementary
oxygen therapy was in place after the remdesivir administration. In the same order, chest X-rays imaging also showed better images with a percentage of 40%, 19%, and 9%, respectively (Table 3).

In vitro studies shows remdesivir is known to be effective in inhibiting Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and Severe Acute Respiratory Coronavirus-1 (SARS-CoV-1) replication. Remdesivir also potentially inhibits the replication of SARS-CoV-2 in vitro in primary human airway epithelial (HAE). An in vivo study using chimeric mice infected with SARS-CoV-2 targeting the SARS-CoV-2 RdRp showed a decrease in viral load in the lungs and an increase in lung function. In a placebo-controlled study, double-blind RCTs were performed to 1,062 patients which were grouped into two: remdesivir and placebo groups. It was found that remdesivir could speed recovery time compared to the placebo group (10 days vs 15 days).

Supplementary oxygen therapy is recommended as a first-line therapy to treat respiratory disorders due to COVID-19 based on the severity of the disease. In a study by Beigel et al remdesivir may be able to prevent the development of more severe respiratory disease as indicated by a lower number of patients requiring higher oxygen support. In Group 3, constant supplementary oxygen support before and after remdesivir administration. However, this does not mean that remdesivir is ineffective. After first checking, the patients did not receive high supplementary oxygen therapy anymore such as non-rebreather masks (NRB) but nasal canula 3-4 lpm or simple mask 6-8 lpm (Table 3).

### Table 3: Parameters effectiveness of remdesivir in COVID-19 patients with pneumonia

<table>
<thead>
<tr>
<th>Therapy Status</th>
<th>PCR/Rapid Ag Swab</th>
<th>Oxygen saturation</th>
<th>Chest X-rays imaging *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>1 C</td>
<td>7%</td>
<td>20%</td>
<td>7%</td>
</tr>
<tr>
<td>NC</td>
<td>5%</td>
<td>28%</td>
<td>3%</td>
</tr>
<tr>
<td>2 C</td>
<td>3%</td>
<td>0%</td>
<td>3%</td>
</tr>
<tr>
<td>NC</td>
<td>3%</td>
<td>17%</td>
<td>5%</td>
</tr>
<tr>
<td>3 C</td>
<td>3%</td>
<td>3%</td>
<td>2%</td>
</tr>
<tr>
<td>NC</td>
<td>2%</td>
<td>9%</td>
<td>0%</td>
</tr>
<tr>
<td>Total</td>
<td>23%</td>
<td>77%</td>
<td>17%</td>
</tr>
</tbody>
</table>

**Notes:**

6 chest X-rays imaging without follow-up are not counted

C: comorbid

NC: non comorbid

### Side effects

Remdesivir likely has side effects such as increasing liver enzymes (SGOT,SGPT) and kidney function (BUN, SCr). The increase in liver enzymes is also identified in the study of Grein et al, a nucleotide analogue prodrug that inhibits viral RNA polymerases, has shown in vitro activity against SARS-CoV-2. METHODS We provided remdesivir on a compassionate-use basis to patients hospitalized with Covid-19, the illness caused by infection with SARS-CoV-2. Patients were those with confirmed SARS-CoV-2 infection who had an oxygen saturation of 94% or less while they were breathing ambient
air or who were receiving oxygen support. Patients received a 10-day course of remdesivir, consisting of 200 mg administered intravenously on day 1, followed by 100 mg daily for the remaining 9 days of treatment. This report is based on data from patients who received remdesivir during the period from January 25, 2020, through March 7, 2020, and have clinical data for at least 1 subsequent day. RESULTS Of the 61 patients who received at least one dose of remdesivir, data from 8 could not be analyzed (including 7 patients with no post-treatment data and 1 with a dosing error) where 23% of patients had higher liver enzymes (ALT, AST, and transaminase). In this study, some patients had high SGOT and SGPT values before remdesivir therapy. This is associated with hepatic impairment caused by SARS-CoV-2 binding to the ACE-2 receptor on hepatocytes and a spike in inflammatory cytokines. An abnormal increase in liver enzyme values was seen after the administration of remdesivir. Four out of five patients had normal SGPT/SGOT levels, and they then increased by 85.30% and 79.88%, respectively after the initial remdesivir administration which causes hepatocellular toxicity. Therefore, it is necessary to observe liver function test results, especially in patients with previous hepatic dysfunction.

The therapy also affected renal function (BUN and serum creatinine). Previous research showed 6% of patients receiving remdesivir had an increase in BUN. The incidence of acute kidney injury (7%) could be triggered by elevated serum creatinine. The results of increased kidney function tests are associated with drug vehicles that are toxic to the kidneys. Remdesivir is administered intravenously, but it has low water solubility. Hence, a cyclodextrin drug vehicle is used. Sulfobutyl-ether-beta-cyclodextrin (SBEC) is a solvent for remdesivir in the formulation process. Each 100 mg vial of remdesivir lyophilized powder contains 3 g of SBEC and each 100 mg/20 mL vial of remdesivir solution contains 6 g of SBEC. Drug vehicles are filtered by the glomerulus and excreted rapidly in patients with normal renal function. On the other side, patients with moderate or severe renal impairment are at risk for SBEC accumulation. A critical point in treatment with remdesivir is that it may not be known to cause nephrotoxicity. However, SBEC which is excreted in the kidneys needs to be checked for estimated glomerular filtration rate (eGFR) when remdesivir is administered. Discontinuation of remdesivir is required if the eGFR level decreases to half of the baseline. Remdesivir is contraindicated in patients with eGFR of < 30 mL/min.

Table 4: Side effects of remdesivir in COVID-19 patients with pneumonia

<table>
<thead>
<tr>
<th>Side effects</th>
<th>Total Patients (%)</th>
<th>Percentage Increased (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased liver function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SGOT/AST</td>
<td>17 (28%)</td>
<td>85.30%</td>
</tr>
<tr>
<td>SGPT/ALT</td>
<td>28 (47%)</td>
<td>79.88%</td>
</tr>
<tr>
<td>Increased renal function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BUN</td>
<td>9 (15%)</td>
<td>74.23%</td>
</tr>
<tr>
<td>Serum Creatinine</td>
<td>8 (13%)</td>
<td>43.33%</td>
</tr>
</tbody>
</table>

Conclusion

Remdesivir with loading dose (1 x 200mg) IV followed maintenance dose (1 x 100 mg) IV for five to nine days could be antiviral in COVID-19 patients with pneumonia. It has clinical improvement with increased level of liver and kidney function.

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Ethical Clearance: This study has been approved by the Ethics Commission of Bhayangkara Hospital, Surabaya Universitas Airlangga (Number: 02/IV/2021/KEPK/RUMKIT).

Statement conflict of Interest: There is no conflict of interest

Funding: There is no sponsorship on this research

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Medical, Legal and Social Problems Faced by Medical Officers Working in Primary Health Centers

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Abstract

Healthcare facilities are getting costly, and becoming non-accessible for the poor. The medical officers working in primary health centers are facing medical, legal and social problems by lack of resources and infrastructure. This study was conducted on 150 primary health centers during the period of six months from July - December 2021 in and around Srikakulam district of Andhra Pradesh. Medical professionals are facing problems mostly due to primary health centers are located far away from headquarters. Improving primary health care facilities in rural areas may give good results in health care system.

Key words: Primary health centers, Autopsy, Medico legal, Social.

Introduction

Every profession has several obligations and responsibilities. Medical profession is unique and different from other professions because of need of scientific knowledge, technical skills and understanding of human being for providing necessary patient care. Medical negligence is a concern, but it is obvious that no doctor wishes to harm their patient. Today, those involved in saving in other’s life themselves at risk because of increasing rates of assaults on medical professionals or litigations and some doctors have faced violence at the workplace.

Annually there is 110% increase in the number of medical negligence cases in India. In medical profession, success cannot be guaranteed in every case, as it depends on several factors which are beyond doctor’s control. Moreover, medicine is ever changing science. There can awareness about rights of the patient, vulnerability of medical profession for litigation is increasing day by day. Successful clinical practice is balance between medico-legal requirements

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and excellence in patient care. Moreover, healthcare professionals have many ethical, moral and legal obligations. Medical Negligence, Disaster in patient-physician relationship, Inadequate documentation, Lapses in data storage and Method of consent process are some of Medico-Legal aspects. A survey among doctors from different specialties showed knowledge gaps in obtaining informed consent. \(^1\)

Good medical practice requires that medical graduates can demonstrate in practice knowledge and understanding of the law. \(^2\) Public health infrastructure has been referred to as “the nerve center of the public health system.” \(^3\) According to a report as of 2019 there was an estimated shortage of 60,000 doctors and 2 million nurses in the country. The report also stated that in India there is 1 bed for 2239 persons, which is way lesser than WHO recommendation of 3 beds per 1000 population. \(^3\)

The healthcare facilities are highly skewed towards urban areas observed that while about 70% of India’s population lives in rural areas, only 20% of hospital beds are located in rural areas. \(^3\) Primary Health Centers are the bedrock of rural health services and play a prominent role in the production of healthcare services in rural areas of the country. \(^3\)

One of the biggest problems in rural India is that the public health facilities are facing difficulty in attracting and holding on the presence of quality and trained medical professionals may be due to lack of basic facilities in rural areas. \(^3\) Even if the manpower is there, their participation level in healthcare production is lower than the required due to deficient supply, insufficient equipment, poor monitoring, etc. as a result the major proportion of India’s population, even the poor, choose expensive healthcare services largely provided by unregulated private sector. \(^3\)

Every doctor under law has to serve his patient and cannot refuse treatment making the health care services more complicated. Also as defined under law, every doctor has to fulfill certain legal requirements during his service by compulsion or voluntarily. Apart from routine and usual clinical cases, a doctor will come across certain Medico-Legal problems at one time or the other during the practice of his/her profession. \(^4\)

The main problem in primary health centers to execute medico legal issues is not possible because they are not recognized as medico legal centers.

**Material and Methods**

A cross sectional questionnaire based survey was conducted in Primary Health Centers in Srikakulam district in the state of Andhrapradesh. All the medical officers participated. The information collected included Age, Sex, Workplace, Recreation facilities, Interpersonal relationships, Medical facilities, Basic amenities, Medicolegal issues, Preventive measures taking for medical negligence were recorded in this study. Approval of institution ethics committee was obtained prior to the study.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Recreation</th>
<th>Satisfactory</th>
<th>Unsatisfactory</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Garden, gymnasium</td>
<td>71 (47.2%)</td>
<td>79 (52.8%)</td>
</tr>
<tr>
<td>2</td>
<td>Schools</td>
<td>49 (32.8%)</td>
<td>101 (67.2%)</td>
</tr>
<tr>
<td>3</td>
<td>Travel time from head quarter</td>
<td>&lt;2 hrs 84 (55.7%)</td>
<td>&gt;2hrs 66 (44.3%)</td>
</tr>
</tbody>
</table>

**Table 1: Perception of participants about recreational facilities at headquarter town (n=150)**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Factors</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Provision for triage in mass disaster</td>
<td>99 (65.71%)</td>
<td>51 (34.28%)</td>
</tr>
<tr>
<td>2</td>
<td>Regular Biomedical waste collection</td>
<td>114 (75.71%)</td>
<td>36 (24.28%)</td>
</tr>
<tr>
<td>3</td>
<td>Availability of Color coding bin for bio medical waste</td>
<td>135 (90%)</td>
<td>15 (10%)</td>
</tr>
<tr>
<td>4</td>
<td>Specialist doctors</td>
<td>25 (17%)</td>
<td>125 (83%)</td>
</tr>
<tr>
<td>5</td>
<td>Blood bank distance</td>
<td>&lt;10 KM 121 (81%)</td>
<td>&gt; 10 KM 29 (19%)</td>
</tr>
<tr>
<td>S. No</td>
<td>Factors</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>-------</td>
<td>---------------------------------------------</td>
<td>----------------------</td>
<td>----------</td>
</tr>
<tr>
<td>6</td>
<td>Tertiary care centre</td>
<td>&lt;10 KM 117(78%)</td>
<td>&gt;10 KM 33(22%)</td>
</tr>
<tr>
<td>7</td>
<td>Ambulance services</td>
<td>130(87%)</td>
<td>20(13%)</td>
</tr>
<tr>
<td>8</td>
<td>Equipped Ambulance</td>
<td>105(81%)</td>
<td>25(19%)</td>
</tr>
<tr>
<td>9</td>
<td>Autopsy center</td>
<td>&gt;10 km 135(90%)</td>
<td>&lt;10km 15(10%)</td>
</tr>
<tr>
<td>10</td>
<td>Preservation facilities for ML evidences, Skin Marking pencils</td>
<td>51(34.28%)</td>
<td>99(65.71%)</td>
</tr>
</tbody>
</table>

**Table 3: Medico legal issues and availability of medico legal certificates**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Factors</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Domestic violence + Child abuse + Poison cases</td>
<td>90(60%)</td>
<td>60(40%)</td>
</tr>
<tr>
<td>2</td>
<td>Cooperation of patients for Consent in medico legal cases</td>
<td>116(77%)</td>
<td>34(22.85%)</td>
</tr>
<tr>
<td>3</td>
<td>Recording of Dying Declaration</td>
<td>30(20%)</td>
<td>120(80%)</td>
</tr>
<tr>
<td>4</td>
<td>Issuing of Drunkenness Certificate</td>
<td>15(10%)</td>
<td>135(90%)</td>
</tr>
<tr>
<td>5</td>
<td>Availability of Certificates for willing and unwilling Autopsy, Specialized form for PLWH, proformas for sexual offences</td>
<td>24(15.78%)</td>
<td>126(84.28%)</td>
</tr>
<tr>
<td>6</td>
<td>Availability of Card for referral center, MCCD Certificate</td>
<td>129(86%)</td>
<td>21(14%)</td>
</tr>
<tr>
<td>7</td>
<td>Maintaining of medico legal Registers</td>
<td>109(72.85%)</td>
<td>49(27.14%)</td>
</tr>
</tbody>
</table>

**Table 4: Medical negligence and preventive measures**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Factors</th>
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</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Primary health centers recognized as medico legal centers</td>
<td>0(0%)</td>
<td>150(100%)</td>
</tr>
<tr>
<td>2</td>
<td>Violent against medical negligence</td>
<td>24(15.71%)</td>
<td>126(84.28%)</td>
</tr>
<tr>
<td>3</td>
<td>Security guard at Primary health centers</td>
<td>105(70%)</td>
<td>45(30%)</td>
</tr>
<tr>
<td>4</td>
<td>Medical Indemnity Insurance</td>
<td>34(22.85%)</td>
<td>116(77.14%)</td>
</tr>
<tr>
<td>5</td>
<td>In brought dead cases : -Refer to higher center+ ECG + Death declaration + Police intimation + MLC registration</td>
<td>60(40%)</td>
<td>90(60%)</td>
</tr>
<tr>
<td>6</td>
<td>Patient satisfactory survey</td>
<td>62(41.42%)</td>
<td>88(58.57%)</td>
</tr>
</tbody>
</table>

**Table 5: Measures taking to prevent medico legal problems**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Factors</th>
<th>Yes</th>
<th>No</th>
</tr>
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<tbody>
<tr>
<td>COPRA : (consumer protection act)</td>
<td>Education, Communication, Relations with patients</td>
<td>64(42.85%)</td>
<td></td>
</tr>
<tr>
<td>MTP : (Medical termination of pregnancy)</td>
<td>Conducting 58(38.57%)</td>
<td>Not Conducting 92(61.42%)</td>
<td></td>
</tr>
<tr>
<td>HOTA: (Human organ transplant act)</td>
<td>Registered 3(2%)</td>
<td>Not Registered 147(98%)</td>
<td></td>
</tr>
</tbody>
</table>
Observations and Discussion

General characteristics of study participants:

In present study, 103 (68.5%) participants were more than 35 years of age out of which 84 (56%) of participants were male and 66 (44%) of participants were female.

Majority of participants 79 (52.8%) complained about lack of recreation facilities like malls, gardens, gymnasiums at their headquarter town. Majority of participants 101 (67.2%) felt that schools were of not satisfactory standards at their headquarter town.

Most of the participants 100 (67%) felt that they had satisfactory social interaction in their neighborhood. Majority of participants 120 (80%) felt that they have satisfactory relationship with Lower cadre employees like ANM, MSW, UDC etc. whereas 60 (40%) participants complained of unsatisfactory relations with local politicians. 92 (61.4%) participants complained of political pressure while discharging duties. This pressure was in the form of command for priority in treatment, prompt and fast treatment, pressurizing for giving fitness certificates, age certificates etc. 67 (44.3%) of participants had to travel more than 2 hours per day to reach their head quarters.

Regarding storage of medical record, almost (n=135) 90% doctors believed that the records of medico-legal cases must be maintained till the judgment of the case. But these results vary slightly from the study on medico-legal awareness amongst health professionals in Sudan where 94.5% agreed that hospitals must maintain records until the case is judged. Legally, physician written records carry more weight than patient’s recollections. According to Medical Council of India medical records should maintain 3 years from commencement of treatment. Record Storage facility is absent in (n=81) 54% of primary health centers where 63% of medico legal records are unsafe among stored. This may be due to fewer funds provided by the government for the healthcare system to maintain.

In our study n=114 (75.71%) of primary health centers maintain biomedical waste collection and 90% among them are having color coding bins for bio medical waste. This study contrast with Divya rao et. al where 50% of doctors only have enough knowledge for biomedical waste management. The waste generation rate ranges between 0.5 and 2.0 kg bed-1 day-1. At many places, authorities are failing to install appropriate systems for a variety of reasons, such as non-availability of appropriate technologies, inadequate financial resources and absence of professional training on waste management.

Specialists are not available in (n=125) 83% of primary health centers. Now irrespective of the ability to pay people in India increasingly seek private healthcare even for minor illness like cold fever diarrhea due to non availability of doctors.

Only (n=15) 10% of primary health centers located very near to blood banks and tertiary care centers. (n=130) 87% primary health centers have ambulance services where 81% are well equipped for Basic Life Support and Advanced Life Support. This may be due to 108 ambulance services provided by the government. Basic Life Support ambulances have an oxygen cylinder, blood pressure apparatus and a stethoscope. In Advanced Life Support ambulances, in addition to the above equipment, there is a defibrillator monitor, electrocardiogram, syringe pump, pulse oximeter, resuscitation kit, suction machine, and nebulizer.

Among medico legal cases 60% (n=90) cases are like domestic violence, child abuse and poison cases, and (n=116) 77.14% of doctors believed that patients are very much cooperative for giving consent.

Majority (n=120) 80% of medical officers are not recording dying declarations. That means 80% of medical officers was not aware of this concept. A dying declaration forms a basis for conviction without any corroborative evidence if it is reliable and truthful. A dying declaration recorded by a competent magistrate has a significant reliability or acceptability than oral evidence or a dying declaration recorded by the investigating officer. This study contrasts with padmakumar.K et.al which shows 32.50 per cent graduates were not aware of this concept.

Drunkenness certificates are not being issued in (n=135) 90% of primary health centers may be due to ethanol testing becomes a medico legal issue, special documentation and specimen handling are required
and the clinical laboratory often does not have firsthand knowledge that the ethanol test result will become a medico legal issue.13

Very few (n=15)10% of primary health centers are located less than ten kilometers distance to the autopsy centers. In (n=114)75.71% of primary health centers there is no facilities to preserve medico legal evidences and skin marking pencils. Government has to put efforts to increase facilities in primary health centers.

In (n=126) 84.28% of primary health centers there are no medico legal certificates like consent for autopsy, forms for PLWH and proformas for sexual offence cases. But in (n=129) 85.71% of primary health centers there is card for referral center MCCD certificates and medico legal registers are maintained by (n=109)72.85%of primary health centers.

Violence against medical negligence is very less (n=24)15.71%. This may be because (n=105)70% of primary health centers there is good security guards. This study contrast with Richa singh et. al. where 75% of doctors face violence at workplace.1

In (n=116)77.14% of medical officers working in primary health centers they do not know about medical indemnity insurance.

As the supreme court the services provided by the medical profession under consumer protection act (COPRA)14 the doctors should follow preventive measures but only (n=64)42.85% doctors following and under MTP act 1971 trained doctors can conduct MTP in primary health centers 14 but only (n=58)38.57% doctors are conducting MTPs and most of them are following (85.18%) measures under MTP act. Most of the doctors n=90 (60%)are not following measures for brought dead cases only 40% doctors following measures like taking ECG ,informing police . and most of the medical officers in primary health centers s(58.57%) patient satisfactory survey not done.

Under the human organ transplantation act 1994 the registration of hospitals must for the removal of organs.14 but only n=3(2%) of primary health centers registered and n=147(98%) are not registered for this.

All primary centers 100 %( n=150) primary health centers are not recognized as medico legal centers.

**Conclusions**

1. Majority of Primary health centers are located far away from Head quarters.
2. Most of the doctors working in Primary health centers are facing problems with lack of recreation facilities and high standard schools.
3. Majority of the doctors in Primary health centers have unsatisfactory relations with local politicians.
4. Most of the Primary health centers are maintaining Medico legal registers but lack proper record storage facility.
5. Satisfactory number of Primary health centers are following proper waste disposal under the guidelines of Biomedical waste management
6. All most all Primary health centers have ambulance services but very few have Specialists doctors.
7. As no Primary health center is recognized as a Medico legal center there is no facilities to preserve medico legal Evidences and lack Medico legal certificates & proformas and not issuing drunkenness certificates.
8. Most of the doctors are unaware of medical indemnity insurance and the concept of recording Dying declaration.
9. Most of the Primary health centers are located very far to autopsy centre and blood bank facilities.
10. Hardly 2% of Primary health centers are registered under Human Organ Transplant Act [HOTA].

**Suggestions and Recommendations**

1. The Government should make some principals to decrease the pressure on medical officers by politicians
2. Training program need to focus on empowering the health care professionals on biomedical waste management with broad scope and practical knowledge in all aspects.8
3. Health facilities should be developed in rural sector by establishing labs blood banks
and appointing specialist doctors for better quality of medical professional to serve masses.\textsuperscript{15}

4. A coordinate approach between national rural health mission and state medical services may give good results.\textsuperscript{15}

5. Urgent need to educate the medical professionals on medical indemnity insurance by conducting seminars.

6. Government should recognize primary health centers as medico legal centers to prevent medico legal problems.

7. Organ donation awareness programs should be conducted to register primary health centers under HOTA.

Acknowledgement: I am thankful to Dr. Kulakarni Professor Department of social and preventive medicine, NRIIMS, for giving valuable suggestions during the study period.

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References


A study on Knowledge and Awareness on Duties and Responsibilities of a Doctor in a Teaching Hospital Hyderabad Telangana

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Abstract

Medical practitioner has a tremendous role in the society in saving the life of human beings; this can be achieved when they practice their profession with utmost care and responsibility. Doctor’s duties are regulated by law enforcement authorities and the National medical council, any breach of their duties can lead to a medico legal issue. The aim of the study is to analyze the knowledge and awareness of doctors regarding their duties and responsibilities towards their patients.

A cross sectional study on knowledge and awareness on duties and responsibilities of a doctor was conducted in a tertiary care teaching hospital, a total of 100 doctors have participated in the study, doctors who are registered in Medical Council of India and working in the institution as postgraduates, tutors, senior residents and assistant professors were included in this study. Data was collected in the form of a questionnaire after obtaining an informed consent from all the participants. Doctors who are not willing to participate in the study are excluded. Each duty and responsibility was assessed by grading based on their knowledge and awareness. Data analysis was done with the help of Microsoft Excel software.

Study results revealed that 12.9% of doctors are having poor knowledge, 19.3% of doctors have fair knowledge and 67.8% of doctors are having good knowledge on their duties and responsibilities. Periodical Continuous medical education programs can achieve better results.

Key words: Medical practitioner, Duties, knowledge and awareness.

Introduction

“Every right implies a responsibility, every opportunity is an obligation and every possession is a duty” these words hold true in every Physician’s life. Every doctor mainly has two important duties; one is medical and another is legal. Saving the life of the patient should be the first and foremost priority of a
The duties and responsibilities of a doctor are laid down since ancient days which came into light after Nazis atrocities on war prisoners during the Second World War.

The World Medical Association (WMA) was established in the year 1945 and started functioning officially in Paris since 1947. Its aim is to establish standards among the doctors in relation to human ethics, duties and responsibilities all over the world. The World Medical Association of 3rd general assembly at London in 1949, 20th world medical assembly Sydney in August 1968, 35th world medical assembly Italy in October 1983 and the 57th WMA general assembly South Africa in October 2006 formulated guidelines on duties of a medical practitioner towards their patients, community and colleagues.

Medical Council of India prescribed rules and regulations on professional conduct, ethics, etiquette and duties of a registered medical practitioner by Indian Medical Council (Professional conduct Etiquette and ethics regulation 2002) act which was amended in 2009. As per this act every doctor is supposed to follow the rules and regulation given by medical council of India from time to time, any violation of code of ethics and etiquette is considered as infamous conduct which is a punishable offence by penal erasure. Any violation of a duty by a medical practitioner can result into a damage to the patient is considered as medical negligence, where patient can file a legal case against the doctor which attracts imprisonment, fine or both.

From the inception of consumer protection act the number of cases filed against the doctors in relation to medical negligence are increasing day by day, this indirectly represents the dereliction of duties by a registered medical practitioners are increasing. Most of the time dereliction of duty occurs either by the act of omission or lack of awareness.

The aim and Objectives of this study is to assess the knowledge and awareness among the doctors on their duties and responsibilities and to explain how insufficient knowledge can lead to medical negligence. This study will enlighten the doctors on their duties and responsibilities to make them more vigilant and minimizes the cases filed against the doctors and the institutions.

**Material and Methods**

A cross sectional prospective study was conducted on knowledge and awareness among the doctors on their duties and responsibilities. Study was conducted in a tertiary care teaching hospital, Hyderabad. 100 doctors have participated in the study conducted from 1st January 2022 to 30th June 2022. Data was collected in the form of a questionnaire after obtaining an informed consent from all the participants before commencement of the study. The participants were explained that the name of the doctor participating in this study will remain anonymous.

Doctors who are registered in Medical Council of India and working in the institution as a postgraduate’s, tutors, senior residents and assistant professors were included in this study. Doctors who are not willing to participate in the study are excluded.

A questionnaire on duties and responsibilities of a doctor formulated by the guidelines issued by World medical association and Medical council of India are as follows.

1. Duty to exercise reasonable degree of skill and knowledge.
2. Duties with regard to attendance and examination.
3. Duty to furnish proper and suitable medicines.
4. Duty to give proper instructions.
5. Duty to prescribe generic name of the drug in capital letters.
6. Duty to control and warn.
7. Duty to display recognized medical degrees, diplomas and honors to his name as suffix as recognized by the Medical council of India.
8. Duties towards third parties.
10. Duty to obtain informed consent.
11. Duty to notify certain notifiable diseases.
13. Duties in regard to consultation.
14. Duty to maintain professional secrecy.
15. Duty to update knowledge and skill for renewal of license.
16. Duty with regard to medical records maintenance.
17. Duty to expose unethical conduct of a professional colleague.
18. Duty to display professional fee and charges.
19. Duty to display registration number.
20. Duty to follow laws and regulations in the interest of public health.

Each duty and responsibility was assessed by grading as poor, fair, good, and excellent, based on their knowledge and awareness. Data was collected for all 20 duties from each individual participant. Poor is considered as unaware and fair, good and excellent are considered as aware. The data was taken into MS excel sheet and was analyzed by using computer software, MS Excel.

**Results**

A cross sectional study on knowledge and awareness on duties and responsibilities of doctors in health care system was conducted in a territory care hospital, the following results were found.

Table 1: Percentage of result of duties and responsibilities.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Duties and responsibilities</th>
<th>Poor</th>
<th>Fair</th>
<th>Good</th>
<th>Excellent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Duty to exercise reasonable degree of skill and knowledge.</td>
<td>10</td>
<td>15</td>
<td>47</td>
<td>28</td>
</tr>
<tr>
<td>2</td>
<td>Duties with regard to attendance and examination.</td>
<td>6</td>
<td>26</td>
<td>35</td>
<td>33</td>
</tr>
<tr>
<td>3</td>
<td>Duty to furnish proper and suitable medicines.</td>
<td>3</td>
<td>18</td>
<td>39</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>Duty to give proper instructions.</td>
<td>5</td>
<td>6</td>
<td>33</td>
<td>56</td>
</tr>
<tr>
<td>5</td>
<td>Duty to prescribe generic name of the drug in capital letters.</td>
<td>8</td>
<td>27</td>
<td>29</td>
<td>36</td>
</tr>
<tr>
<td>6</td>
<td>Duty to control and warn.</td>
<td>5</td>
<td>16</td>
<td>43</td>
<td>36</td>
</tr>
<tr>
<td>7</td>
<td>Duty to display recognized medical degrees, diplomas and honors to his name as suffix as recognized by the Medical council of India.</td>
<td>7</td>
<td>21</td>
<td>40</td>
<td>32</td>
</tr>
<tr>
<td>8</td>
<td>Duties towards third parties.</td>
<td>23</td>
<td>26</td>
<td>31</td>
<td>20</td>
</tr>
<tr>
<td>9</td>
<td>Duties towards children and adults incapable of taking care of themselves.</td>
<td>5</td>
<td>29</td>
<td>37</td>
<td>29</td>
</tr>
<tr>
<td>10</td>
<td>Duty to obtain informed consent.</td>
<td>2</td>
<td>15</td>
<td>30</td>
<td>53</td>
</tr>
<tr>
<td>11</td>
<td>Duty to notify certain notifiable diseases.</td>
<td>13</td>
<td>21</td>
<td>24</td>
<td>42</td>
</tr>
<tr>
<td>12</td>
<td>Duties under Geneva Convention.</td>
<td>94</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>13</td>
<td>Duties in regard to consultation.</td>
<td>13</td>
<td>33</td>
<td>33</td>
<td>21</td>
</tr>
<tr>
<td>14</td>
<td>Duty to maintain professional secrecy.</td>
<td>7</td>
<td>7</td>
<td>31</td>
<td>55</td>
</tr>
<tr>
<td>15</td>
<td>Duty to update knowledge and skill for renewal of license.</td>
<td>9</td>
<td>12</td>
<td>28</td>
<td>51</td>
</tr>
<tr>
<td>16</td>
<td>Duty with regard to medical records maintenance.</td>
<td>6</td>
<td>28</td>
<td>35</td>
<td>31</td>
</tr>
<tr>
<td>17</td>
<td>Duty to expose unethical conduct of a professional colleague.</td>
<td>18</td>
<td>21</td>
<td>29</td>
<td>32</td>
</tr>
<tr>
<td>18</td>
<td>Duty to display professional fee and charges.</td>
<td>13</td>
<td>23</td>
<td>31</td>
<td>33</td>
</tr>
<tr>
<td>19</td>
<td>Duty to display registration number.</td>
<td>4</td>
<td>13</td>
<td>30</td>
<td>53</td>
</tr>
<tr>
<td>20</td>
<td>Duty to follow laws and regulations in the interest of public health.</td>
<td>7</td>
<td>26</td>
<td>27</td>
<td>40</td>
</tr>
</tbody>
</table>
Overall, 2000 data was collected from 100 participants for 20 duties, among them; 258 were poor, 386 were fair, 634 were good, and 722 were excellent.

Discussion

A cross-sectional study on duties and responsibilities of doctors in medical practice was conducted in a tertiary care teaching hospital of Telangana. 100 doctors including post graduates, tutors, senior residents, and assistant professors working in both clinical and non-clinical departments have participated in the study. We found that the majority of doctors are well aware of their duties and responsibilities.

A doctor must exercise a reasonable degree of skill and knowledge; it can be achieved by learning skills and by attaining higher degrees, the skills and knowledge of an MBBS doctor cannot be compared with specialist and super-specialist doctors. In our study, we observed only 10% are unaware. A practitioner is not liable because some other doctor of greater skill and knowledge would have prescribed a better treatment or operated better in the same circumstances.
In regard to duty of attendance and examination, when a practitioner agrees to attend a patient he is under an obligation to attend as long as it requires attention, failure to attend amounts to breach of duty. In our study we noticed that 94% are aware and 6% are poor in awareness. It is the duty of the doctor to furnish proper and suitable medicines to their patients; doctor must write legible prescription and give detailed instructions. Illegible prescription or wrong medication can lead to danger to the health of the patient, we observed only 3% of doctors are not having sufficient knowledge. Doctor should prescribe the drug by its generic name and in capital letters, only 8% of the doctors are not aware.

Doctor must give proper instructions regarding the health conditions, medication prescribed and proper diet to their patients, failure to give proper instructions can be danger to their patient and amounts to medical negligence. In our study we noticed 95% of doctors are aware of this duty. A physician has a duty to warn patients regarding the dangers involved in medical condition and in the use of prescribed medication under the concept of ‘reasonable foreseeability’, failure can result into danger to the life of the patient and amount to be negligence. Study revealed that 95% of doctors are aware of this duty. Medical practitioner can display recognized medical degree, diploma and honors to his name as suffix as recognized by medical council of India. Our study shows 93% of doctors are aware.

Doctors not only have duty towards their patient but also towards third parties, failure to give proper instructions of a disease may spread to the other members of the family or community. Study revealed that 23% are not aware of this duty. Doctors have duty towards children and adults who are incapable of taking care of themselves doctor should take an extra care in providing services to these patients. In our study we found 95% are well aware.

Doctor must obtain an informed consent before any procedure, section 2(11) of consumer protection act states that not taking consent is considered as a deficiency in medical service. Our study revealed that 98% of the doctors are aware of this duty.

Doctor should notify certain notifiable diseases to public health authority to avoid community spread of diseases, failure can be a great danger to the community, and study revealed that 13% are not aware. Every medical practitioner must follow the duties under Geneva Convention, which lays down that it protects the wounded or sick of the armed forces (first convention), ship-wrecked persons (second convention), prisoners of war (third convention), or civilians of enemy nationality (fourth convention), are to be treated without any adverse distinction. Our study reveals that 94% of doctors are unaware of this duty. Physician has a duty to take consultation other than his specialty, whenever a serious complication develops by his patient. Failure to provide services of higher specialty when it require, amounts to be negligence. 13% of the participants are not aware of this duty.

Doctor must maintain professional secrecy, should not divulge the health conditions of patient to anyone without consent, violation of this duty tantamount to infamous conduct, patient can file a defamation case against the doctor under section 499 IPC. 93% of doctors are aware of this duty. Every medical practitioner must renew his license once in 5 years; doctors should update their professional knowledge and skills regularly from time to time. 91% of the doctors are aware of this duty.

Physicians must maintain records of their patients, both in-patient and out-patient records must keep in custody for a period of 3 years in case of non medico legal cases. In case of MLC records, there is no time limit. Our study shows that only 6% doctors are not aware. Doctors suppose to follow medical ethics guidelines issued by National Medical Council of India and it is their duty to expose and inform to the NMC regarding unethical practice of their fellow doctors. 82% of the doctors are aware of this duty. Doctor must display his professional fee and charges in their consultation room to avoid future financial litigations. In our study we observed 13% of doctors are unaware. Doctor should display their medical registration number on their name boards and in their prescription letter head to avoid fraudulent medical practice, 4% of doctors are unaware of this duty. Doctors should obey the Laws and regulations prescribed by the Government from time to time, any violation attracts legal punishments and it is their duty to obey the Law of land. In our study we noticed 93% of doctors are aware.
Our study results revealed that 12.9% of doctors are having poor knowledge, 19.3% of doctors have fair knowledge and 67.8% of doctors are having good knowledge on their duties and responsibilities. We noticed the poor grading was observed in post graduate participants.

There were not many studies in regard duties and responsibilities of a doctor found in India and abroad. Studies in relation to ethical duties of a medical practitioner were found.

Study conducted by Surjith Singh et. al. on Knowledge, awareness and practice of ethics among doctors in tertiary care hospital at AIIMS Jodhpur, the study was different and focused on ethical practice, one of the components in their study is professional secrecy which is considered as a duty in our study, they found 9.6% of doctors were unaware whereas it is 7% in our study, which is almost similar.  

**Conclusion**

Duties of a medical practitioner were laid down after Nazis atrocities against war prisoners during 2nd world war, World Medical association formulated various duties for a doctor to follow on their day to day practice. Any deviation of the duty is called dereliction, if it is resulting into damage is called negligence. Many negligence cases are reporting against the doctors are due to their breech of duties. In our study we observed 67.8% of doctors are having good knowledge on their duties and responsibilities whereas 19.3% are fair and only 12.9% of doctors are having poor knowledge. This can be overcome by periodical trainings and Continuous medical education programs at the institutional level.

**Conflict of interest:** Nil

**Source of fund:** Self

**Ethical Clearance:** Taken from Institutional ethics committee.

**References**

Evaluation of Prescription Writing Skill of Dental Students and Professionals Working in Dental Teaching Institute in Haryana

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Abstract

Background: Prescription is an integral part of practice in the field of dentistry. Errors in prescription can be either knowledge or writing related. Understanding the lacunae gives an insight to improvement in this vital clinical skill.

Aim: To evaluate prescription writing pattern in a dental teaching Institute in Haryana with primary objective to identify the most common errors in prescription writing among students and faculty and secondary objective to assess overall performance and prescription writing skills.

Methods and Material: The hospital based cross-sectional, descriptive survey was conducted with 194 participants with a problem based approach requiring response in the form of complete prescription. Undergraduate students of third and final year, interns along with post graduate students and faculty were included. Each prescription was analysed for 28 parameters. Six prescriber related, five patient related, eleven drug related, and seven Miscellaneous parameters. Each parameter was scored as one if present and zero if absent. Total score for each parameter and total score of each prescription was calculated and graded as poor score 0-7, medium or fair score 8-14, good score 15-22, and 22-30 excellent.

Results: The patient and doctors related parameters were most deficient followed by miscellaneous and drug related parameters. Maximum prescription were of fair quality. Undergraduate prescription was well written in comparison to other groups.

Conclusions: There is a need to emphasis, audit and revise the art of prescription writing throughout the curriculum and later stages too.

Key-words: Prescription writing errors, Undergraduate students, Interns, Faculty, Dentistry

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Introduction

Prescription is an instruction written by a practitioner that authorizes a patient to be issued a medicine or treatment. It is an important document in treatment of patient.\(^1\) Pre means before and script means writing or written and it reflects that it is an order that must be written down before a compound drug is prepared. In today’s era of preformed drugs it is an important document for dispensing drug by a pharmacist and for patient to follow instructions. Thus error in prescription can majorly affect appropriate drug intake and proper treatment of the patient. Prescription errors can be knowledge related or writing or transcription related.\(^2\) Right decision and then correct transcription are essential for ideal prescription. Prescribing drugs is integral to clinical practice of dentistry. The very limited literature concerning prescription writing errors in dentistry particularly involving undergraduate students in North India motivated us to take up this study.\(^3\),(4)\) Moreover understanding the flaws give great insight to self improvisation and direction to the points to be emphasised in correct writing process.

Aim

The aim of study was to evaluate prescription writing pattern in the institute with primary objective to identify the most common errors in prescription writing among dental students and faculty and secondary objectives to assess overall performance and quality of prescription writing skills among undergraduates, interns, postgraduate and faculty.

Subjects and Methods

The hospital based cross-sectional, descriptive survey was conducted in Department of Oral Medicine and Radiology, in a dental teaching institute in Haryana. Ethical clearance was obtained from institutional ethical committee.

The participants included were dental students i.e. third years, final years, interns, post-graduate students and faculty members. An informed consent was obtained from the willing participants. A self-designed problem based questionnaire along with information regarding level of education to be mentioned on front page with blank back page was provided to write prescription. The validity of same was established by a group of expert and also a pilot survey was done on 15 participants. The sample of 190 was calculated on basis of 68% moderate to good quality prescription. To adjust for 20% no response and on basis of pilot study 240 forms were distributed.

After required modification final Performa a total of 28 parameters were analyzed as per various national and international formats and WHO guidelines. Parameters were categorized under 4 headings for simplicity and orientation i.e. six prescriber related parameters i.e., name, hospital address, telephone no, signature, professional degree and registration number, five patient related parameters (5) i.e. name, age, gender, address and diagnosis. Eleven drug related parameters (11) i.e. brand name, generic name, form, dose, route of administration, frequency, duration, direction of drug use, symbolic representation of drug dosage, quantity to be dispensed and refill information and six miscellaneous parameters (6) i.e. date on prescription, legible handwriting, symbol Rx, use of blue ink, use of indelible ink and signature near the last drug prescribed.

**Scoring:** Each parameter was given a score of 1(Present), 0(Absent). It was done by a single examiner to avoid any bias. Data was entered in excel sheet and SPSS 17 was used. Total score for each parameter in all 4 groups was assessed by frequency table and chi square test. Total score of every parameter and each prescription was also calculated and score of all five groups were calculated and graded as poor i.e. score 0-7, fair score 8-14, good score 15-22.and 22-29 excellent.

Results

Total of 240 survey form were distributed of which 219 form were returned back and among these 19 (empty performa received back), five interns thought there was no need to prescribe for given situation while one final year responded that physician should be consulted. Rest 194 received survey form were analysed for 28 criteria. Total distribution of participants in 5 different groups was –third year (63), final year (32), interns (30), postgraduate (39) and faculty (30). Total score for each parameter was calculated among five groups to analyse frequency of representing each parameter.
In Prescriber related (6) information name was present in 12.4% (24), hospital address in 14.9% (29), telephone number in 0.5% (1), signature in 17% (33), professional degree 7.6% (15), registration no. in 3.6% (7). Detail distribution in various groups mentioned in [Table 1].

Table 1: Analysis of Prescriber related information parameters among all five groups

<table>
<thead>
<tr>
<th>Group</th>
<th>MDS faculty</th>
<th>PG student</th>
<th>Intern</th>
<th>BDS final year</th>
<th>BDS III year</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Count</td>
<td>Count</td>
<td>Count</td>
<td>Count</td>
<td>Count</td>
<td></td>
</tr>
<tr>
<td>Name</td>
<td>Absent</td>
<td>29(14.95%)</td>
<td>39(20.1%)</td>
<td>30(15.46%)</td>
<td>27(13.92%)</td>
<td>45(23.2%)</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>1(0.52%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>5(2.58%)</td>
<td>18(9.28%)</td>
</tr>
<tr>
<td>Hospital Address</td>
<td>Absent</td>
<td>30(15.46%)</td>
<td>39(20.1%)</td>
<td>30(15.46%)</td>
<td>28(14.43%)</td>
<td>38(19.59%)</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>4(2.06%)</td>
<td>25(12.89%)</td>
</tr>
<tr>
<td>Telephone Number</td>
<td>Absent</td>
<td>30(15.46%)</td>
<td>39(20.1%)</td>
<td>30(15.46%)</td>
<td>31(15.98%)</td>
<td>63(32.47%)</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>1(0.52%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Signature</td>
<td>Absent</td>
<td>26(13.4%)</td>
<td>37(19.07%)</td>
<td>29(14.95%)</td>
<td>28(14.43%)</td>
<td>41(21.13%)</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>4(2.06%)</td>
<td>2(1.03%)</td>
<td>1(0.52%)</td>
<td>4(2.06%)</td>
<td>22(11.34%)</td>
</tr>
<tr>
<td>Professional Degree</td>
<td>Absent</td>
<td>30(15.46%)</td>
<td>39(20.1%)</td>
<td>30(15.46%)</td>
<td>27(13.92%)</td>
<td>53(27.32%)</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>5(2.58%)</td>
<td>10(5.15%)</td>
</tr>
<tr>
<td>Registration Number</td>
<td>Absent</td>
<td>30(15.46%)</td>
<td>39(20.1%)</td>
<td>30(15.46%)</td>
<td>31(15.98%)</td>
<td>57(29.38%)</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>1(0.52%)</td>
<td>6(3.09%)</td>
</tr>
</tbody>
</table>

In patient related criteria (5) name was present in 41.20% (80), age in 39.7% (77), gender 38.7% (75), address in 6.2% (12), diagnosis in 11.3% (22) [Table 2].

Table 2: Analysis of Patient related information parameters among all five groups

<table>
<thead>
<tr>
<th>Group</th>
<th>MDS faculty</th>
<th>PG student</th>
<th>Intern</th>
<th>BDS final year</th>
<th>BDS III year</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Count</td>
<td>Count</td>
<td>Count</td>
<td>Count</td>
<td>Count</td>
<td></td>
</tr>
<tr>
<td>Name</td>
<td>Absent</td>
<td>26(86.7%)</td>
<td>36(92.3%)</td>
<td>19(63.3%)</td>
<td>26(81.2%)</td>
<td>7(11.1%)</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>4(13.3%)</td>
<td>3(7.7%)</td>
<td>11(36.7%)</td>
<td>6(18.8%)</td>
<td>56(88.9%)</td>
</tr>
<tr>
<td>Age</td>
<td>Absent</td>
<td>26(86.7%)</td>
<td>36(92.3%)</td>
<td>22(73.3%)</td>
<td>26(81.2%)</td>
<td>7(11.1%)</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>4(13.3%)</td>
<td>3(7.7%)</td>
<td>8(26.7%)</td>
<td>6(18.8%)</td>
<td>56(88.9%)</td>
</tr>
<tr>
<td>Gender</td>
<td>Absent</td>
<td>26(86.7%)</td>
<td>36(92.3%)</td>
<td>24(80%)</td>
<td>26(81.2%)</td>
<td>7(11.1%)</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>4(13.3%)</td>
<td>3(7.7%)</td>
<td>6(20%)</td>
<td>6(18.8%)</td>
<td>56(88.9%)</td>
</tr>
<tr>
<td>Address</td>
<td>Absent</td>
<td>29(96.7%)</td>
<td>39(100%)</td>
<td>30(100%)</td>
<td>31(96.9%)</td>
<td>53(84.1%)</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>1(3.3%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>1(3.1%)</td>
<td>10(15.9%)</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>Absent</td>
<td>30(100%)</td>
<td>35(89.8%)</td>
<td>29(96.7%)</td>
<td>31(96.9%)</td>
<td>47(74.7%)</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>0(0%)</td>
<td>4(10.2%)</td>
<td>1(3.3%)</td>
<td>1(3.1%)</td>
<td>16(25.3%)</td>
</tr>
</tbody>
</table>
In drug related (11) brand name was present in 90.2% (175), generic name in 10.8% (21), form in 70.6% (137), dose in 100% (196), route of administration 100% (196), frequency in 82% (159), duration in 77.8% (151), direction of drug use 4.6% (9), symbolic representation of drug dosage 45.9% (89), quantity to be dispensed in 2.6% (5) and refill information in 0.15% (3) [Table 3].

Table 3: Analysis of Drug related information parameters among all five groups

<table>
<thead>
<tr>
<th>Group</th>
<th>MDS faculty</th>
<th>PG student</th>
<th>Intern</th>
<th>BDS final year</th>
<th>BDS III year</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brand name</td>
<td>Absent</td>
<td>0(0%)</td>
<td>1(2.6%)</td>
<td>6(20%)</td>
<td>1(3.1%)</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>30(100%)</td>
<td>38(97.4%)</td>
<td>24(80%)</td>
<td>31(96.9%)</td>
<td>52(82.5%)</td>
</tr>
<tr>
<td>Generic Name</td>
<td>Absent</td>
<td>30(100%)</td>
<td>38(97.4%)</td>
<td>30(100%)</td>
<td>29(90.6%)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>0(0%)</td>
<td>1(2.6%)</td>
<td>0(0%)</td>
<td>3(9.4%)</td>
<td>17(27%)</td>
</tr>
<tr>
<td>Form</td>
<td>Absent</td>
<td>8(26.6%)</td>
<td>5(12.8%)</td>
<td>12(40%)</td>
<td>2(6.2%)</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>22(73.3%)</td>
<td>34(87.2%)</td>
<td>18(60%)</td>
<td>30(93.8%)</td>
<td>33(52.4%)</td>
</tr>
<tr>
<td>Dose</td>
<td>Absent</td>
<td>30(100%)</td>
<td>39(100%)</td>
<td>30(100%)</td>
<td>32(100%)</td>
<td>63(100%)</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Route of Administration</td>
<td>Absent</td>
<td>30(100%)</td>
<td>39(100%)</td>
<td>30(100%)</td>
<td>32(100%)</td>
<td>63(100%)</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Frequency</td>
<td>Absent</td>
<td>3(10%)</td>
<td>8(20.5%)</td>
<td>12(40%)</td>
<td>7(21.9%)</td>
<td>5(8%)</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>27(90%)</td>
<td>31(79.5%)</td>
<td>18(60%)</td>
<td>25(78.1%)</td>
<td>58(92%)</td>
</tr>
<tr>
<td>Duration</td>
<td>Absent</td>
<td>5(16.6%)</td>
<td>11(28.2%)</td>
<td>11(36.7%)</td>
<td>5(15.6%)</td>
<td>11(17.4%)</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>25(83.3%)</td>
<td>28(71.8%)</td>
<td>19(63.3%)</td>
<td>27(84.4%)</td>
<td>52(82.5%)</td>
</tr>
<tr>
<td>Direction of drug Use</td>
<td>Absent</td>
<td>28(93.3%)</td>
<td>33(84.6%)</td>
<td>30(100%)</td>
<td>32(100%)</td>
<td>62(98.4%)</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Symbolic Representation of Drug dosage</td>
<td>Absent</td>
<td>2(6.6%)</td>
<td>6(15.4%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>1(1.5%)</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>7(23.3%)</td>
<td>13(33.3%)</td>
<td>18(60%)</td>
<td>8(25%)</td>
<td>59(93.7%)</td>
</tr>
<tr>
<td>Quantity to be dispensed</td>
<td>Absent</td>
<td>23(76.6%)</td>
<td>26(66.6%)</td>
<td>12(40%)</td>
<td>24(75%)</td>
<td>4(6.3%)</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>27(90%)</td>
<td>37(94.9%)</td>
<td>30(100%)</td>
<td>32(100%)</td>
<td>63(100%)</td>
</tr>
<tr>
<td>Refill information</td>
<td>Absent</td>
<td>3(10%)</td>
<td>2(5.1%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>30(100%)</td>
<td>39(100%)</td>
<td>30(100%)</td>
<td>31(96.9%)</td>
<td>61(96.8%)</td>
</tr>
</tbody>
</table>

In miscellaneous parameters (6) date on prescription was present in 27.3% (53), legible handwriting 92.3% (179), symbol Rx 88.11% (171), use of blue ink 91.8% (178), use of indelible ink 96.4% (187), signature near the last drug prescribed 30.9% (60) participants. The group wise distribution mentioned in [Table 4]
Table 4: Analysis of Miscellaneous parameters among all five groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Group</th>
<th>MDS faculty</th>
<th>PG student</th>
<th>Intern</th>
<th>BDS final year</th>
<th>BDS III year</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date on Prescription</td>
<td>Absent</td>
<td>25(83.3%)</td>
<td>37(94.9%)</td>
<td>29(96.7%)</td>
<td>25(78.1%)</td>
<td>25(39.7%)</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>5(16.7%)</td>
<td>2(5.1%)</td>
<td>1(3.3%)</td>
<td>7(21.9%)</td>
<td>38(60.3%)</td>
<td></td>
</tr>
<tr>
<td>Legible Handwriting</td>
<td>Absent</td>
<td>1(3.3%)</td>
<td>2(5.1%)</td>
<td>9(30%)</td>
<td>1(3.1%)</td>
<td>2(3.2%)</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>29(96.7%)</td>
<td>37(94.9%)</td>
<td>21(70%)</td>
<td>31(96.9%)</td>
<td>61(96.8%)</td>
<td></td>
</tr>
<tr>
<td>Symbol RX</td>
<td>Absent</td>
<td>2(6.7%)</td>
<td>8(20.5%)</td>
<td>9(30%)</td>
<td>2(6.2%)</td>
<td>2(3.2%)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>28(93.3%)</td>
<td>31(79.5%)</td>
<td>21(70%)</td>
<td>30(93.8%)</td>
<td>61(96.8%)</td>
<td></td>
</tr>
<tr>
<td>Use of Blue ink</td>
<td>Absent</td>
<td>2(6.7%)</td>
<td>1(2.6%)</td>
<td>6(20%)</td>
<td>1(3.1%)</td>
<td>6(9.5%)</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>28(93.3%)</td>
<td>38(97.4%)</td>
<td>24(80%)</td>
<td>31(96.9%)</td>
<td>57(90.5%)</td>
<td></td>
</tr>
<tr>
<td>Use of indelible Ink</td>
<td>Absent</td>
<td>0(0%)</td>
<td>1(2.6%)</td>
<td>6(20%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>30(100%)</td>
<td>38(97.4%)</td>
<td>32(100%)</td>
<td>32(100%)</td>
<td>63(100%)</td>
<td></td>
</tr>
<tr>
<td>Signature as near the last drug prescribed</td>
<td>Absent</td>
<td>21(70%)</td>
<td>28(71.8%)</td>
<td>26(86.7%)</td>
<td>26(81.2%)</td>
<td>33(52.4%)</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>9(30%)</td>
<td>11(28.2%)</td>
<td>4(13.3%)</td>
<td>6(18.8%)</td>
<td>30(47.6%)</td>
<td></td>
</tr>
</tbody>
</table>

Comparison of mean score third year prescription had greater share of good prescription followed by final year, faculty, postgraduate students and interns. On analysing the overall scores and grouping them in poor, medium and good, majority of prescription were of medium rank. Total 10.82% prescriptions were of poor quality, maximum i.e. 66.49% prescriptions were of medium quality and 22.68% were of good quality. The third year prescriptions were having maximum share of good prescription.
Discussion

Prescription writing errors are most preventable errors which by means of careful introspection can lead to improved quality care to patient as it is an important document between health care professional and patient. Dental professional regularly write prescription. As per Dental council of India curriculum which is followed in all the dental colleges in India, Pharmacology subject is introduced in second year. Knowledge about drugs and prescription writing is gained in second year and gradually third year onwards students under supervision start prescribing as they enter clinics in third year. Therefore in our study we included the participants third year onwards. For the sake of uniformity and as we wanted to evaluate skills of students who in clinical setup write under supervision we followed a problem based approach rather than studying the OPD prescriptions.

In Prescriber related (6) information lacunae were in order telephone number 0.5%, registration no 3.6%, professional degree 7.6%, name 12.4%, hospital address 14.9%, signature 17%. Majority of prescription showed major lacunae in mentioning these parameters. Our results were in agreement with results of Siddharth et. al.6, Ballal et. al.7, Wali et. al.8 but in contrast with Sujatha et. al.9 who represented better representation of name (21.4%), hospital address (99.4%) professional (13.6%) though parameters like registration number and telephone number were less mentioned similar to our study. Signature which is important for validation of prescription was less represented in our study i.e. 17% in contrast with Siddharth et. al.6 (96.7%) and Sujatha et. al.9 (86.4%).

The variation can be explained by difference in methodology as this study was done in tertiary care centre where they analysed prescription written on hospital pad collected from hospital pharmacy while we provided blank page where all these parameters needed to be mentioned. Prescriber related information is important for patient and pharmacist to contact back in case of any discrepancy and for validation of prescription awareness about them should be well emphasized. In patient related information lot of lacunae were observed with least mentioned being address, diagnosis followed by gender, age and name. All the parameters are important for correct dispensing, record maintenance of pharmacist and as per the WHO guidelines.10 In consensus with our observation Wali A et. al.8 and Ballal et. al.7 also observed major lacunae in diagnosis and address parameters followed by other parameters like gender, age and name while in contrast Siddharth et. al.6 in tertiary care setup who observed better representation i.e. diagnosis in 97% and address in 64.7% and Sujatha et. al.9 who reported that name, hospital number and address were well mentioned in medical prescription studied. Low representation of these important parameters can be due to heavy clinical workload and multitasking in clinical setting or sheer negligence and need to be reinforced thoroughly. Parameters like exact age are utmost important required for adult and child dose decision. In our study numerical representation only was observed unlike a Nigerian study11 that reported mentioning of adult/child data in age column.

Drug related information received maximum representation. This also reflects that prescription writing as such is considered to be drug knowledge though we didn’t analysed accuracy of factors but in general there was an increased awareness about mentioning these parameters. The most common flaws were lack of refill information, quantity to be dispensed, symbolic representation of frequency, generic name followed by form, duration. Well mentioned parameters include route, dosage, frequency, brand name. Our results were in consensus with Siddharth et. al.6, Wali A et. al.8 though Ballal et. al.7 reported lower representation of duration, dose and form.

Among generic and brand names variation is a big concern. It was advocated by Medical Council of India (MCI) as well as Dental Council of India (DCI) to mention generic names in prescription13,14. It gives patient an economical alternative. In our study we observed that 90.2% prescription had brand names and only 10.8% had generic names. Sneha K et. al. observed brand name mentioned in 72.6% and 27.4% by generic name (2014)12. Sujatha et. al. observed 99.8% prescription had brand names. Generic names were mentioned only in 1.63% prescription studied by
Siddharth et al. 6 and in 4% prescription by Wali A et. al. 8. More awareness and representation and giving patient the alternative need to be well emphasized and inculcated in prescription writing.

Among the Miscellaneous factors performance was better, similar results were reported by Wali A et. al. (2012)8, J Kumar et. al. (2010)15. The common errors were in date on prescription followed by signature near last drug prescribed parameters. While symbol Rx, use of indelible ink, legible handwriting, use of blue ink were well represented. Date on prescription which is an important parameter for record, validity and follow up and to prevent misuse especially in habit forming and narcotic drugs prescription. Our results were not in agreement with Sujatha et al. (2016)9 who analysed OPD prescriptions.

Signature near the last drug prescribed is also important to prevent unauthorised addition of any drug and was not well represented i.e. only in 30.9% prescription. Importance and relevance of same should be well stated in teaching the art of prescription writing.

The parameters like use of indelible ink, use of blue ink, signature near the last drug prescribed which were included in our study are relatively less frequently studied.

Among the various category third year students scored better while postgraduate and interns scored low. Similar results were reported by Sujatha et al. (2016)9 and Varghese-N J et al16.

Prescriptions written by undergraduate students are well scrutinized and same is reflected by their writing pattern. Moreover faculty prescription were also more in medium range which could be explained by habit of allotting juniors and students to complete the prescription especially in relation to patient and prescriber related factors or multitasking. Moreover use of pre-printed form and computerised prescription can also enhance omitting lot of errors. There is need of continuous and progressive training and regularly revisiting this essential and important clinical skill.

Limitation

Sample size was small and it was a unicentric survey so results cannot be generalised to entire population. Overall knowledge based errors and number of drugs prescribed were not analysed.

Conclusion

As we observed majority of prescription, required further improvement, it’s an urgent and important field of concern. Prescriber and patient related information was less represented. Drug related information was comparatively well mentioned though mentioning the generic drugs should be also emphasised. Prescription audit can help us to improve and reinforce our writing skill thus imparting better patient care. Our study emphasis this essential art should be regularly reinforced and revisited throughout the clinical training and afterwards.

Key Message: Prescription writing errors are common. It’s an essential skill and should be re evaluated and revisited throughout the curriculum and revisited and reinforced later also to provide better patient care too.

Source of Funds support: Self funded

Conflict of interest: None

Ethical clearance: Ethical clearance was taken.

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Quick Response (QR) Codes In Dentistry: A Review

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Abstract

Quick Response (QR) codes are the two dimensional barcodes which store a large amount of data in the form of black square modules (dots). They connect physical objects with digital world by providing links to videos, websites and maps. Use of QR codes saves time, energy, paper and enhances the learning process. The purpose of this review is to provide information about structure, types and applications of QR codes in dentistry.

Key Words: QR code, Barcode, Scanner, Dentistry, Mobile, Education

Introduction

Quick Response (QR) code is a two dimensional barcodewith enhanced data storage capacity than traditional (1D) barcode. It was invented in 1994 by the Denso Wave (Toyota Motors subsidiary) to track vehicles and parts during the manufacturing process. The QR code consists of black square dots (modules) arranged in a square grid on a white background. The data/information encoded may contain numeric, alphanumeric, byte / binary, Kanji orthrough supported extensions (URLs).

The amount of data that can be stored in QR code depends on mode, version and error correction level. The maximum data that can be stored in a typical barcode is 20 digits in a horizontal direction while a QR code can hold great amount of information in both horizontal and vertical directions. The maximum data capacity for QR code depends on the type of data like Numeric[7089 characters (0,1,2,3,4,5,6,7,8,9)], Alphanumeric [4296 characters (0-9, A-Z, space, $, %,*,+)], Binary/Byte [2953 characters (8-bit bytes)] and Japanese Kanji (1817 characters).

The version of the QR code indicates its size which relates to the number of black square dots (modules) present in the QR code. There are about 40 versions; higher version means more modules, bigger size and more information. Version 1 has the minimum size 21 x 21 modules while version 40 has the maximum size of 177 x 177 modules. The QR code increases in size by four modules for each increment in its version.

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QR code has a unique capability that it is possible to read it even after damage or distortion to some extent. The error correction level indicates the percentage of symbol area that can be restored. There are four error correction levels for QR codes, Low(7%), Medium(15%), Quality (25%) and High(30%). The data storing capacity decreases as the error correction level of the QR code increases. The error correction level has to be selected while creating the QR code based on the damage that can occur in the environment in which it will used.

Structure Of QR Code

QR code consists of two regions, (1) Function patterns and (2) Encoding region. Function patterns include position patterns, alignment patterns and timing patterns. Encoding region consists of data and error correction zones. QR code also consists of separators, format information and quiet zone (Figure 1).

Figure 1: Structure of QR code

Position/FinderPatterns: These are three identical patterns located in three corners of the QR code. These help in identifying the position, size and orientation (angle) of the QR code. Any alterations in these patterns can prevent decoders from reading the code.

Alignment Pattern: If the QR code is distorted, the alignment patterns help the scanners to decode the code by identifying the central coordinate. No alignment pattern is available in version 1 of QR codes while Version 2 contains one alignment pattern. As we move to higher versions, the size of QR code increases and so is the number of alignment patterns.

Timing Pattern: In this pattern, white and black modules are alternately arranged to determine the coordinate. The timing pattern is placed between two position patterns in a QR code.

Encoded data: Data is first converted into a stream of binary numbers of 0 and 1 and then converted into black and white modules. It consists of Reed-Solomon codes to provide error correction function.

Error Correction: These are 8-bit code-words (binary) stored in the error correction section which help in reading the damaged QR codes.

Separators: These are used to separate the position patterns from the actual data and thus improve the visibility of the position patterns.

Format Information: The error correction level of the QR code and the masking pattern (distribution of black and white modules) are the information stored in this section.

Quite zone: It is the white margin surrounding the QR code which helps in better detection of the QR code.

QR codes can be easily generated using free online or offline QR code generator software. These can be printed on a normal paper with an ordinary printer and can be attached to any surface. A QR code is read by an imaging device, such as a mobile phone camera, BarcodeScanner and QR Scanner. These scanners can read and decode data from a QR code. Previously, QR code scanner software applications (Apps) have to be installed in mobile phones to read the QR codes but now most of the mobile phones are coming with inbuilt QR code scanner software making it easier to read QR codes.

Types Of QR Codes:

Micro QR Code:

It has only one position pattern to achieve more efficient data encoding in smaller size (Figure 2a). It is suitable for small data/space applications. The largest version of Micro QR code is M4 (17 x 17 modules) with a maximum storage of 40 numerals.

SQRC (Security QR Code):

QR Code is currently being used in many applications and the information encoded in them includes personal information of the users. As these QR codes can be read anywhere and anytime using a
mobile phone, there is threat for the loss of personal data from QR codes. So, DENSO WAVE developed a “Security QR Code (SQRC)”, which has the ability to prevent the data from being disclosed to the public.

**Custom QR code:**

The QR code can be customized by changing the shapes of the position patterns and data modules. Position pattern can be square, round, rhomboid and grooved. Data modules can be square, round or star shaped. Different colors and photo can be incorporated like colored frame QR code (Figure 2d).

**Other Two Dimensional Bar codes:**

Two dimensional bar codes include Datamatrix code, PDF417 and AZTEC codes other than QR codes.

**Datamatrix codes:**

Datamatrix codes are small sized and can store up to 2335 alphanumeric characters. They have two solid black adjacent borders in ‘L’ shape (finder pattern) and other two borders contain alternating black and white modules (Figure 3a).

**PDF417:**

PDF417 are used when there is huge amount of information to be stored. It consists of quiet zone, start pattern, left row indicator, data code words, right row indicator and stop pattern (Figure 3b).

**AZTEC codes:**

AZTEC codes are used in transportation industry over airlines tickets and train tickets. It consists of a central finder pattern surrounded by data in black and white modules (Figure 3c).

**Applications Of QR Code:**

QR codes are used to maintain the repair and maintenance records for buildings and equipment.
They are used to describe about the equipment components of complex systems. QR codes on business cards provide information like address, phone number and even website. QR codes on broachers will provide URLs or links to YouTube videos giving description about the product or building in the broacher. QR code on books will guide the users to book reviews and additional resources.

QR codes containing bank account information can be used for online payments of bills, booking of train, airlines & movie tickets and money transfer through wallets. AADHAR card or The Unique Identification Cards (UID) issued by Government of India to its citizens has a QR code printed on it which contains the basic information of the cardholder.

QR codes on groceries, food packages and fruits & vegetables provide information like ingredients, expiry date and cooking recipes. QR codes on jewelry tags provide information about name, weight and production date. QR codes on biological specimens provide links to photographs, maps, ecosystem notes and citations.

In medical hospitals, QR Codes are used for patient identification by printing it on patient’s wrist band which contains information like patient’s name, identification number, date of birth, sex, ward and bed numbers. In pathological diagnostic labs, QR codes are attached to the test tubes during testing with automated counting machines. Physiotherapists give QR codes to the patient containing information about the postoperative physical exercises and links to multimedia sources on the internet showing video demonstrations of the exercises.

**QR Code Applications In Dentistry:**

QR codes can be used in various departments of Dentistry to help patients, doctors and undergraduate students. The protocol for the usage of these QR codes is

**Step 1:** Data to be encoded in the QR code is prepared, like case history, radiographic interpretation, treatment plan, patient details, equipment information and material manipulation.

**Step 2:** QR code of size 1x1 cm is generated after incorporating data in it by using an offline or online QR code generator software.

**Step 3:** QR code is laminated to prevent its damage or distortion from moisture, dust and acrylic materials.

**Step 4:** QR code is attached to the object like radiographic cover, model, appliance or medical equipment.

**Step 5:** QR code is read or scanned using a mobile phone camera or a QR code scanner.

**Step 6:** QR code can be stored in a computer or over a paper for documentation or can be sent to the patient through e-mail or mobile.

**Prosthodontics:**

A laminated QR code encoding patient details is generated. Then it is placed in the recess created in the widest part of the prosthetic appliance (removable partial/complete denture) and covered with clear auto-polymerizing resin (Figure 4a). This QR code can also be attached over the models. These help in identifying the dentures in senior citizen centers and models in prosthetic labs.

![Figure 4a: Complete Denture with QR code](image.png)

![Figure 4b: Histopathological slide with QR code](image.png)

![Figure 4c: Mandible with QR code](image.png)

![Figure 4d: Skull with QR code](image.png)

QR codes are printed on ceramic crowns (palatal/lingual surface) and implant abutments using laser
markers. These withstand high temperatures without undergoing distortion/damage. Micro QR code or Data matrix code are used for these.9,10

Orthodontics:

QR code encoding for case history, radiographic interpretation (cephalometrics) and treatment plan is generated and laminated. This QR code is incorporated into orthodontic appliance in its widest part or attached to the study models and radiographs. QR codes help in patient’s subsequent visits, retrospective studies and to study the tooth movement patterns.11

Oral Pathology:

QR code encoding for the case history is generated and laminated. Then it is attached to the case history form, specimen bottle, cassettes containing the grossed specimen, embedded block and to the slide after staining. A second QR code is generated encoding for biopsy report and attached to the reverse side of the slide (Figure 4b). First QR code helps in identification of the specimen and block during retrospective studies while the slide containing both first & second QR codes provides both case history and histopathological report of that particular slide.12 These QR codes may contain URL leading to a website where the digital histopathological picture of the slide is available.

Pedodontics:

The information like case history, radiographic interpretation and treatment plan are encoded in a QR code. This QR code is printed and laminated and attached to the radiographic cover or incorporated into the pedodontic appliances like habit breaking appliances or space maintainers. Tooth eruption sequences can also be encoded in the QR codes.13

Oral Radiology:

Radiographs like Intra Oral Peri-Apical radiograph (IOPA), Orthopantomogram (OPG) & Lateral Cephalogram are taken and interpreted. QR code encoding for the patient details and radiographic interpretation are generated. These are laminated and attached to the radiographic covers in IOPA (adult/pediatric) and to the x-ray film in case of OPG & Lateral Cephalogram. These help in retrieving the patient’s information during subsequent visits and in retrospective studies.14

Dental Education:

QR codes can enhance the dental education by connecting the paper based teaching methods with digital sources making it m-learning (mobile learning). Students can use QR codes containing video links and journal articles to get detailed information of the seminar or lecture they have attended from an online source. QR codes can also be used for online surveys, self assessment tests for students and feedback for faculty.15

QR codes encoding for video links and images can be attached to skull, mandible which will provide information like the anatomical landmarks and muscle attachments making the anatomy learning more interesting (Figure 4c, 4d). QR codes attached to the instruments like Sphygmomanometer and Neubauer’s chamber will provide information regarding experimental procedure, normal values.

QR codes encoding for histological features can be attached to the ground sections and histological slides. QR codes attached to the mixed dentition models will help the students in identifying the correct age and eruption sequence of the patient. QR codes containing information about dental material composition, material manipulation and stepwise procedures for impression taking can be given to the students. QR codes containing information about indications, contraindications and fabrication procedure can be incorporated in various appliances like Prosthodontic, Orthodontic and Pedodontic appliances.

Oral Medicine:

Patient’s case history, investigations and treatment were planned. Recording these details electronically using QR codes will save paper and energy as they are easy to retrieve during subsequent visits or for retrospective studies.

Community Dentistry:

Patient details attending community health programs, dental health awareness camps, mass screening of oral diseases are usually saved on the paper. Recording these details electronically using
QR codes will save paper, energy and time as it easy to access the details during subsequent visits of the patients.

**Forensic Odontology:**

Identifying the person becomes difficult during accidents like air plane crashes and train accidents. If QR codes are used to mark the dentures, crowns and implants then the identification of the person becomes easy as these can withstand high temperatures (370°C), concentrated acids without undergoing any damage. These QR codes can be read even if they are placed at varying depths in the acrylic materials.[16]

In dental clinic, these QR codes help the dental assistant in providing information regarding tray set up for procedures like impaction, flap surgery, root canal and implant placement. Patients can use these QR codes to get information about dental procedures, post operative instructions, for booking appointments and review of the clinic. QR codes can also be used to set up a digital museum.

Inter-departmental confusion may arise while using QR codes in a dental college. This may be avoided by using colored frame QR code specific for each department. Each department will be given a specific color and logo in the frame QR code will be the short form of the department name. Eg: Blue color and letters ‘OP’ in logo of QRcode for the Department of Oral Pathology.

**Advantages:**

QR codes are easy to create, less expensive, small in size and can store large amount of data. High speed 360 degree scan and ability to read even if it is damaged by error correction adds to it benefits. Use of QR codes saves paper, time and energy during retrospective studies. It is very economical method of labeling the dentures and appliances. It turns the classroom learning into digital m-learning.

**Limitations:**

Lack of technical familiarity in creating and scanning of the QR codes is the main limitation in their usage. Privacy and confidentiality is the other limitation as the information encoded in them includes personal details, banking information, case history, diagnoses and treatment plans. Other drawbacks like inclusion of histopathologic picture, radiographic image in the QR code limits its usage. Malicious links can be present in the QR codes leading to websites hacking user mobile phones and retrieving their details.

QR codes incorporated in appliances made from acrylic materials cannot withstand temperatures above 500°C. The presence of porosity in the acrylic material overlying QR code might hinder its scanning. Fracture of the appliances during major accidents might limit QR code’s readability depending on the amount of distortion occurred to it.

**Future Scope:**

Technical advancement might help in inclusion of images in QR codes and also creates familiarity of QR codes which will ultimately increase their usage. Privacy and confidentiality of the users can be preserved by the development of highly advanced versions of QR codes with password protection.

**Conclusion**

QR codes act as a bridge between physical objects and digital world. They store information electronically which can be easily accessed from anywhere in the world. They revolutionize the learning process by augmenting the textbook method with m-learning. Technology is advancing at a rapid pace, making the usage of QR codes in modern dentistry as the need of the hour.

**Ethical clearance:** As it is a review paper, ethical clearance has not been taken.

**Source of funding:** Self

**Conflict of Interest:** Nil

**References**


Microbiological Analysis of Indoor Air in the Provincial Hospital of Sidi Kacem, Morocco

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Abstract

Background: The air environment of the hospital is often contaminated by pathogenic microorganisms of various origins, which constitute a risk of infection for hospitalized patients, health staff and visitors. Microbiological control of the hospital wards is an essential element in the prevention of these infections, it allows a bacterial diagnosis of the area in order to carry out preventive and corrective actions.

Objective: The aim of this study is to reduce the rate of nosocomial infections, through the diagnosis of bacteria present in the services area of the provincial hospital of sidi kacem in the region of Rabat-Salé-Kénitra Morocco.

Methods: 14 samples of the area were collected from different sites on the hospital wards, using the sedimentation technique, by exposing Petri plates containing nutrient agar, in a 1m² area for 30 minutes. The plates were incubated in a temperature of 37 ± 1 °C for 48 hours. The isolates were identified by the classical biochemical gallery and API (Biomerieux, France).

Results: A total of eight (08) bacterial species were isolated from the air with a predominance of coagulase-negative staphylococci (36%), Bacillus sp (25%), staphylococcus aureus (20%) and E. coli (8%) followed. One (1) sample was a negative culture with a positivity rate of 93%. The distribution of isolated bacteria by department shows a predominance of bacterial strains in the intensive care unit (24%) and the emergency department (19%).

Conclusion: These surprising results demonstrated the importance to implement a periodic monitoring of the hospital area, and a global policy for the prevention of nosocomial infections including an air treatment protocol.

Key words: hospital area, nosocomial infections, microbiological control.

Introduction

Nosocomial infection (NI) is an infection contracted in hospital after 48 hours of hospitalization. It is becoming increasingly prevalent in Morocco and abroad¹ and its occurrence is responsible for significant morbidity and mortality.²
The global frequency of these infections measured by international studies, varies from 5 to 10% of hospitalizations. And it varies greatly from one hospital sector to another, both in terms of frequency and type. 

The air quality in health care establishments has becoming an increasing concern and requires special attention to protect patients from nosocomial infections caused by micro-organisms.

The airborne micro-organisms are carried by various sized supports: dust, skin flakes, saliva droplets or micro-droplets. The latter are emitted when coughing, sneezing or speaking and remain suspended in the air and can diffuse and penetrate by inhalation to the alveoli of patients.

Several factors influence the contamination of the area, namely seasons, weather conditions, ventilation systems, humidity, the presence of airborne diseases, visitors, human activities and cleaning in hospitals can also affect the air quality.

The hospital area thus constitutes an ecological niche of microorganisms that can have a clinical significance, this contamination varies qualitatively and quantitatively from one establishment to another, within the same establishment according to the services, the patients, the care practiced and the capacity of survival of the microorganisms and to remain suspended in the area.

The microbiological monitoring of the hospital area represents a major element in the fight against nosocomial infections. It allows the identification of airborne bacteria for making a microbial diagnosis in order to carry out corrective, and preventive measures and to reduce the rate of these infections.

The direct involvement of hospital air in the transmission of infections is debatable and remains difficult to assess. Some consider its role to be negligible, others believe that, the covid 19 pandemic has approved to the whole world the importance of prevention against airborne diseases.

The aim of our study is to investigate the pathogenic bacteria found in hospital air in order to assess the degree of aerocontamination with the aim of reducing the rate of nosocomial infections.

Materials and Methods

Place and type of study:

This prospective study of microbiological control of the indoor air of the services in the provincial hospital of Sidi Kacem with a total bed capacity of 202 beds was carried out over a period of six months, from January 1, 2020 to June 30, 2020.

Sampling sites and technique:

Fourteen (14) samples were collected in seven (7) departments of the provincial hospital of Sidi Kacem. The departments studied were: intensive care, medicine, emergency, operating theatre, surgery, pediatrics and maternity. The selection of sampling sites was based on the most critical and representative sites in each hospital department. The samples were prepared using the qualitative sedimentation technique based on the exposure of 90 mm diameter Petri plates containing nutrient agar in a 1 m² area for 30 minutes. The samples were quickly transported an ice box in a temperature of 4°C to the Provincial Laboratory of Epidemiology and Environmental Hygiene for analysis in coordination with the provincial delegation of Sidi Kacem.

Sample analysis:

In the laboratory, Petri dishes previously exposed to the air in the seven departments were incubated in a temperature of 37 ± 1 °C. The results reading was done after 48 hours of incubation. The number of colonies, their appearance, size and color were noted and the purification of the different types of colonies was carried out by exhaustion on Plate Count Agar (PCA) medium. The isolated bacterial strains were identified by both the classical biochemical gallery and API (Biomerieux, France). The culture media, distilled water and reagents, materials and equipment, sterilization and other conditions were regularly checked according to the requirements of NM ISO 17025.

Results

Fourteen (14) air samples from different points of the hospital wards were analyzed in the laboratory (LPEHM) during a period of six (6) months. One (1) sample was negative. A total of eight (08) bacterial
species were isolated from the indoor air of the wards. The distribution of the bacterial strains isolated according to the hospital wards is shown in table 1. We have noted the presence of the following bacterial flora: coagulase-negative Staphylococci (36%) and Bacillus (25%) were predominant, followed by Staphylococci aureus (20%), E. coli (8%) and Klebsiella pneumoniae (4%). Finally, Pseudomonas aeruginosa with a proportion of 3%, Serratia rubidaea (2%), and streptococcus with 2%.

The Microbiological analysis of the air in the hospital wards revealed the presence of Bacillus sp with a percentage ranging from 17% to 26%, coagulase negative staphylococci with a percentage ranging from 25% to 31%, Staphylococcus aureus with a percentage ranging from 11% to 30%, and E. coli with a variation ranging from 11% to 26%, these four bacterial strains are present in the air in all the wards studied. Klebsiella pneumoniae was isolated in all wards except pediatrics and the operating theatre with a percentage variation between 6% and 23%. Serratia rubidaea was isolated only in the
emergency department with a percentage of 4% and in the intensive care unit with 5%. Pseudomonas aeruginosa was isolated only in the intensive care unit with a percentage of 12%. Streptococcus was isolated in two departments, emergency and surgery, with a percentage of 3% and 11% respectively.

Table 1: Percentage of bacteria isolated from the air by department

<table>
<thead>
<tr>
<th>Services</th>
<th>emergency</th>
<th>Medicine</th>
<th>Pediatric</th>
<th>Surgery</th>
<th>Maternity</th>
<th>Intensive care</th>
<th>Operating room</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus.</td>
<td>13%</td>
<td>18%</td>
<td>23%</td>
<td>16%</td>
<td>20%</td>
<td>11%</td>
<td>30%</td>
</tr>
<tr>
<td>Serratia rubidaea</td>
<td>4%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5%</td>
<td>-</td>
</tr>
<tr>
<td>E.coli.</td>
<td>20%</td>
<td>11%</td>
<td>26%</td>
<td>13%</td>
<td>15%</td>
<td>18%</td>
<td>15%</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>12%</td>
<td>-</td>
</tr>
<tr>
<td>Bacillus sp</td>
<td>24%</td>
<td>19%</td>
<td>26%</td>
<td>20%</td>
<td>22%</td>
<td>17%</td>
<td>24%</td>
</tr>
<tr>
<td>streptococcus</td>
<td>3%</td>
<td>-</td>
<td>-</td>
<td>11%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Klebsiela pneumoniae</td>
<td>6%</td>
<td>23%</td>
<td>-</td>
<td>13%</td>
<td>11%</td>
<td>12%</td>
<td>-</td>
</tr>
<tr>
<td>coagulase negative staphylococci</td>
<td>30%</td>
<td>29%</td>
<td>25%</td>
<td>27%</td>
<td>32%</td>
<td>25%</td>
<td>31%</td>
</tr>
<tr>
<td>Total</td>
<td>100%</td>
<td>100%</td>
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<td>100%</td>
</tr>
</tbody>
</table>

Discussion

The hospital is a place where treatment is provided but it is also a place where the risk of infection is very high and where germs are becoming increasingly resistant. As a result, hospital-acquired infections are recognized as major public health problems because of their frequency, their socio-economic cost and their severity, which affects patients and their families as well as all health professionals.14,15

These acquired infections are a worrying reality, especially in high-risk departments that recruit patients who are extremely vulnerable to colonization and consequently to infection.16

The scientific evidence for the responsibility of air contamination in nosocomial infections is often difficult to demonstrate because we don’t know if this contamination is the cause or the consequence of the infection.13 However, some studies have reported the incrimination of environmental reservoirs in the genesis of these infections for example Dagata and al. 1999,17 Rampling and al. 200118 Zeana and al. 2003,19 Kac and al. 200420, and Carling and al. 200821

Microbiological air monitoring is part of the policy for the control of nosocomial infections and is essential for assessing the quality of air in hospitals. However, these controls are only justified in controlled environment areas. These controls are quality indicators of infectious risk management and require a rigorous and standardized methodology, from sampling methods and analysis techniques to the interpretation of results.22

The aim of our study is to research the microorganisms and more specifically the pathogenic bacteria found in hospital air in order to investigate the microbiological quality of hospital air, to evaluate the degree of bio-contamination, and to identify the bacteria that are often the cause of nosocomial infections, and to verify the effectiveness of air treatment processes.

In our study, a total of fourteen (14) microbiological samples were taken and analyzed in the context of the microbiological control of the air in the provincial hospital of Sidi Kacem. A positivity rate of 93% was detected with one (n = 1) sample being found negative. Other studies have shown a similar percentage of contamination for example:

Bekkari and al (2016) for example reported a positivity rate of 92%,23 kunwar and al (2019) at Kathmandu District Hospital reported a positivity
rate of around 95%. Cabo verde and al (2015) found a percentage of 86.29%.

Abbas Lamia and al (2017) revealed a contamination percentage of 60% in a study conducted in the public hospital establishment mohamed boudiaf in the wilaya of bouira in Algeria. Benmahdi and al (2014) in Algeria found a positivity rate in the order of 96.30%.

In our study, the most contaminated areas were the care rooms and patient wards. This could be related to the fact that these rooms are commonly used by medical staff, patients and visitors and to a lack of air treatment practices.

The results found in our study show a predominance of bacterial species coagulase negative staphylococci with a percentage of 36%, and Bacillus sp with 25%, followed by S. aureus with a percentage of 20%, Klebsiela pneumoniae (4%). Finally, Pseudomonas aeruginosa with 3%, E. coli (8%), Serratia rubidaea (2%), and Streptococcus with 2%. The classification according to gram staining showed an equality between the two types with a percentage of 50% for Gram (+) and Gram (-).

Several studies have found similar results to ours for example: Bekkari and al (2016) who found as results coagulase negative Staphylococci (48%), followed by Bacillus sp (14%), Staphylococcus aureus and Aeromonas salmonicida (8%) for each, Gram negative cocci (7%) and Pseudomonas vesicularis (5%). Other bacteria such as Lactobacillus, Pseudomonas putrificiens, Streptococcus, Aeromonas hydrophila, Serratia liquifaciens, putrificiens, Serratia rubidaea and Stenotromonas maltophilia were isolated in small proportions.

Benmahdi and al (2014) found The bacteria isolated were coagulase negative staphylococci CNS (84.38%). Acinetobacter baumannii (5.47%), Klebsiella pneumoniae (3.13%), S. aureus (3.13%), E. coli (2.34%) and Pseudomonas aeruginosa (1.56%).

Cabo verde et. al. (2015) found the following results: Gram-positive cocci with (88%) Staphylococcus (51%), Micrococcus (37%), Gram-negative cocci (5%), Neisseria (4%) Gram-positive rods (2%), Gram-negative rods (2%), Shigella (3%), Proteus (2%) and other (3%).

Abbas Lamia and al (2017) found results as follows: 33.33% for hemolytic Streptococcus sp, a similar rate of 22.22% for S. aureus and S. epidermidis, 16.66% for E. coli and lastly K. pneumoniae with a rate of 5.55%.

Several studies have shown that bacterial strains; Bacillus sp, coagulase negative staphylococci, Staphylococcus epidermidis, Staphylococcus aureus, Escherichia Coli, and Pseudomonas aeruginosa, Acinitobacter (especially A. baumani), and Klebsiella pneumoniae are omnipresent in the hospital environment and can generate a risk of infection for patients, health professionals and visitors. The particularity of these germs is that they have often acquired resistance to antibiotics, such as MRSA (methicillin-resistant staphylococcus aureus) or EBLSE (extended-spectrum beta-lactamase-producing Enterobacteriaceae).

In our study, the distribution of germs by department showed a predominance of bacterial strains in the intensive care unit (24%), and in the emergency department (19%). The other services of medicine and surgery with (16%) and (14%) respectively, maternity (13%), pediatrics (10%), and the central block (4%). In this context other studies have shown similar results: Bekkari and al (2016): All hospital departments studied had a diverse microbial load: trauma (20%), surgery (17%), intensive care (16%), central operating room (14%), neonatology (13%), kitchen (10%), cardiovascular gastrology (7%) and emergency (4%).

Abbas Lamia and al (2017) found that among the strains isolated in hospital setting, the pediatric department occupied the first place with 33.33% of the strains isolated, followed by 27.78% in oncology, and the operative bloc with 23.45%.

Kunwar and al (2019) found that the bacterial load was found to be high in the emergency department (55.8%) compared to the general department (44.2%). There was no significant difference between the bacterial load of the two departments (general and emergency) of different hospitals.

Several studies have shown the existence of pathogenic bacterial strains in hospital air that can cause serious infections for patients, health professionals and visitors. From this reality, we can
see that microbiological control of hospital air is a strategic element in the fight against nosocomial infections. This makes it necessary to develop standardized and periodic procedures and protocols for the management of aerocontamination risks through: a periodic treatment of the air in high-risk areas, a training programme for health professionals in this sense, and periodic microbiological monitoring of the air.

**Conclusion**

Our study of the microbiological analysis of the air in the seven hospital wards showed a significant colonization of the air by bacterial species of human and environmental origin that have the potential to infect hospitalized patients, health professionals and visitors. This makes it necessary to carry out periodic microbiological control of hospital air, the principal bacterial strains isolated being: coagulase negative staphylococci 36%, bacillus sp 25%, staphylococcus aureus 20% and E. coli (8%). The overall contamination rate is around 93%, with a variation between departments, the most contaminated places being the care rooms and the hospitalization rooms of patients. The aim of our study is to reduce the rate of nosocomial infections. Our results show the importance of installing a risk management system based on preventive measures.

**Conflicts of Interest:** All authors have no conflict interest to declare.

**Source of Funding:** The source of the research cost from self.

**Ethical Clearance:** Nil

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Acceptance of Covid 19 Vaccine in Terms of Perception and Knowledge: A Cross-Sectional Study

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Abstract

The Covid-19 pandemic had an impact on various sectors. One of the efforts to prevent the spread of the virus on a large scale is the Covid-19 vaccine. This study aimed to investigate people’s knowledge and perception of acceptance regarding Covid-19 vaccines. Quantitative data were collected using an online survey (n = 299) with accidental sampling. This survey was conducted from March-May 2021 and distributed through social media. Data were collected using questionnaires. The result shows that almost all respondents (93.3%) accept the Covid-19 vaccine with good knowledge (63.2%), sufficient knowledge (6.7%), and a lack of knowledge (13.4%). The OR value (3.78, p = 0.026) means people with good knowledge will receive three times more vaccines than people with less knowledge. Perception of knowledge about the Covid-19 vaccination program led to 13.7 times of acceptance Covid-19 vaccine compared to those who did not know this program (OR 13.7, p=0.01). Knowledge and perception about the Covid-19 vaccine will affect accepting the Covid-19 vaccine. This study recommended this study is expected to be a reference or literature review for determining government policies to implement the Covid-19 Vaccine program in the community.

Keywords: knowledge, perception, acceptance, Covid-19 vaccine

Introduction

The Indonesian government has designated Coronavirus Disease 2019 (Covid-19) as a non-natural disaster in the form of an outbreak/pandemic. This determination was followed by efforts to prevent the spread of the coronavirus through social restrictions, including restrictions on crowds, travel restrictions, implementation of isolation, postponement and cancellation of events, as well as the closing of facilities and public service arrangements. Covid-19 in Indonesia was first reported on March 2, 2020, in several two cases. Data on March 31, 2020, showed 1,528 confirmed cases and 136 deaths. The Covid-19
mortality rate in Indonesia is 8.9%, this figure is the highest in Southeast Asia\textsuperscript{1}. Currently, Covid-19 is a major concern of the world. The rapid spread of the disease accompanied by the addition of cases still increasing, including in Indonesia.

The various clinical manifestations of Covid-19 can cause the health system to collapse\textsuperscript{2–4}. One of the efforts to prevent the spread of the virus on a large scale is the Covid-19 vaccine. The Covid-19 vaccine is the best hope for suppressing the coronavirus transmission. However, many ordinary people still ask about the benefit of the Covid-19 vaccine, how it works, or perhaps the side effects. The public is worried about the Covid-19 vaccine. This Covid-19 vaccine is focused on a national effort towards independent access to safe and effective vaccines. The development of a Covid-19 vaccine is raising public concern about the Covid-19 vaccine program and is worsening as the pandemic progresses. A September survey conducted by the Pew Trust of more than 10,000 Americans showed that intentions to get a Covid-19 vaccine have declined 72\% from May 2020 across all major political and demographic groups. The significant decline in public confidence in the Covid-19 vaccine amid the ongoing pandemic signals that greater attention and communication are needed in providing information about the safety and effectiveness of the Covid-19 vaccine in preventing the spread of Covid-19\textsuperscript{5}. Although vaccine development is still slower than the spread of the Covid-19 virus, it is hoped that a safe and effective vaccine will be obtained to prevent the spread of this virus. The Covid-19 vaccine may be too late to reduce the number of victims in the first wave around the world, but it will be beneficial later to avoid further waves after this pandemic is over\textsuperscript{6}.

This study aimed to investigate people’s knowledge and perception of acceptance regarding Covid-19 vaccines.

**Methods**

This cross-sectional study was conducted between March – May 2021 and distributed through social media. The sample was 299 people with accidental sampling. In this study, the knowledge and perception variables were correlated with the acceptance of the Covid-19 vaccination in the community.

The data collection tool was a questionnaire regarding sociodemographic characteristics. The sociodemographic characteristics questions were conceconcerning age, gender, education, occupation, and monthly expenses. The researchers developed the questionnaires using items based on the Covid-19 vaccine acceptance survey in Indonesia by the Ministry of Health (2020). A knowledge instrument based on the Technical Instructions for the Implementation of Vaccination in handling the Covid-19 Pandemic from the Ministry of Health. Based on the results of the reliability and validity test of Cronbach’s Alpha, with a df of 78 at 5\%, it was found that the critical point of the correlation coefficient ($r$) was 0.222. The reliability test results showed the results of 0.413, so it can be concluded that the questionnaire is reliable and valid.

To analyze the relationship between variables with the acceptance of Covid-19 vaccines in the community, it was tested using logistic regression with 5\%.

**Result**

From the result shows that the sociodemographic data of 299 respondents, 74.6\% are female, and the majority are adults (95.7\%). 63.9\% of respondents have high education, and 35.5\% have middle education. In the occupational, 47.2\% of respondents do not work because of school or joblessness, while most of the respondents’ monthly expenditures are moderate (59.9\%).

Respondents’ perceptions show from 299 respondents, 97\% knew that the government was currently holding a Covid-19 vaccination program. Judging from the willingness to pay, 34.4\% said they were willing if the Covid-19 vaccination had to be paid for, while 39.5\% said they didn’t know. The locations chosen to implement vaccinations were in public health centers (24.7\%) and hospitals (29.1\%). The socialization media chosen to disseminate the vaccination program were through social media (71.7\%), and 14.3\% directly. There is a significant effect in the perception variable between respondents knowing the Covid-19 vaccination program and receiving the Covid-19 vaccination, indicated by a p-value of 0.01 with an OR of 13.7. This explains
that respondents who know about this vaccination program will be willing/receive 13.7 times more Covid-19 vaccination than those who don’t know. Willingness to pay is also significantly related to vaccine receipts, with a P-value of 0.000 which there is a relationship between willingness to pay and receipt of Covid-19 vaccination. The knowledge showed that 66.6% of respondents’ knowledge about the covid-19 vaccination is good, and 16.1% lack knowledge.

Table 1: Perception’s Participants about Covid-19 vaccine (N =299)

<table>
<thead>
<tr>
<th>Variables</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Know that vaccines are a government program</td>
<td>Know</td>
<td>290</td>
</tr>
<tr>
<td></td>
<td>Don’t know</td>
<td>9</td>
</tr>
<tr>
<td>2 Willingness to pay for vaccines</td>
<td>Willing to</td>
<td>279</td>
</tr>
<tr>
<td></td>
<td>Unwilling</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>Not know</td>
<td>118</td>
</tr>
<tr>
<td>3 Place of Vaccines</td>
<td>Primary Health Care</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>Hospital</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>Office</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>School institution</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td>14</td>
</tr>
<tr>
<td>4 Socialization Media</td>
<td>Social media / Internet</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>Electronic/print media</td>
<td>34</td>
</tr>
</tbody>
</table>

Table 2 shows that according to the chi-square test, demographic data in terms of gender, age, education, occupation, and monthly expenditure do not significantly correlate with the acceptance of Covid-19 vaccination. In the perception variable, there is a significant effect between respondents knowing about the Covid-19 vaccination program and receiving Covid-19 vaccination indicated by a p-value of chi-square test 0.01 with an OR of 13.7.

Table 2: Knowledge’s Participants about Covid-19 vaccine (N=299)

<table>
<thead>
<tr>
<th>Variables</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good knowledge</td>
<td>199</td>
<td>66.6</td>
</tr>
<tr>
<td>Moderate knowledge</td>
<td>52</td>
<td>17.4</td>
</tr>
<tr>
<td>Lack of knowledge</td>
<td>48</td>
<td>16.1</td>
</tr>
</tbody>
</table>

Table 3: Distribution of Frequency of Public Acceptance of Covid-19 Vaccination in 2021

<table>
<thead>
<tr>
<th>Variables</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccines Acceptance</td>
<td>Accept</td>
<td>279</td>
</tr>
<tr>
<td></td>
<td>Un Accept</td>
<td>20</td>
</tr>
</tbody>
</table>

Reason to not vaccines

| Not sure be safe | 13 | 65 |
| Not sure be effective | 3 | 15 |
| Afraid of side effect | 3 | 15 |
| Religious prohibition | 1 | 5 |

Table 4: Distribution of Public Acceptance Frequency about Covid-19 Vaccination in terms of Demographics, Perceptions, and Public Knowledge in 2021

<table>
<thead>
<tr>
<th>Variables</th>
<th>Vaccines Acceptance</th>
<th>P-Value</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Participant’s Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>Yes</td>
<td>266</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Old</td>
<td>Yes</td>
<td>13</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>Yes</td>
<td>70</td>
<td>92.1</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>7</td>
<td>7.9</td>
</tr>
<tr>
<td>Female</td>
<td>Yes</td>
<td>209</td>
<td>93.7</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>14</td>
<td>6.3</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basic</td>
<td>Yes</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Variables</td>
<td>Vaccines Acceptance</td>
<td>P-Value</td>
<td>OR</td>
</tr>
<tr>
<td>--------------------</td>
<td>---------------------</td>
<td>---------</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>Middle</td>
<td>98</td>
<td>92.5</td>
<td>8</td>
</tr>
<tr>
<td>High</td>
<td>180</td>
<td>94.2</td>
<td>11</td>
</tr>
<tr>
<td><strong>Job</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jobless</td>
<td>128</td>
<td>90.8</td>
<td>13</td>
</tr>
<tr>
<td>Civil servant</td>
<td>54</td>
<td>98.2</td>
<td>1</td>
</tr>
<tr>
<td>Private employee</td>
<td>58</td>
<td>92.1</td>
<td>5</td>
</tr>
<tr>
<td>Entrepreneur</td>
<td>18</td>
<td>94.7</td>
<td>1</td>
</tr>
<tr>
<td>Others</td>
<td>21</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td><strong>Salary</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>70</td>
<td>89.7</td>
<td>8</td>
</tr>
<tr>
<td>Middle</td>
<td>170</td>
<td>95</td>
<td>9</td>
</tr>
<tr>
<td>High</td>
<td>37</td>
<td>94.9</td>
<td>2</td>
</tr>
<tr>
<td>Very high</td>
<td>2</td>
<td>66.7</td>
<td>1</td>
</tr>
<tr>
<td><strong>Perception</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Know that vaccines are a government program</td>
<td>274</td>
<td>94.5</td>
<td>16</td>
</tr>
<tr>
<td>Don't know</td>
<td>5</td>
<td>55.6</td>
<td>4</td>
</tr>
<tr>
<td>Willingness to pay for vaccines</td>
<td>0</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Willing to</td>
<td>103</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Unwilling</td>
<td>77</td>
<td>98.7</td>
<td>1</td>
</tr>
<tr>
<td>Not know</td>
<td>99</td>
<td>83.9</td>
<td>19</td>
</tr>
<tr>
<td><strong>Knowledge</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good knowledge</td>
<td>189</td>
<td>95</td>
<td>10</td>
</tr>
<tr>
<td>Moderate knowledge</td>
<td>50</td>
<td>96.2</td>
<td>2</td>
</tr>
<tr>
<td>Lack of knowledge</td>
<td>40</td>
<td>83.3</td>
<td>8</td>
</tr>
</tbody>
</table>

This explains that 13.7 times more respondents who know about this vaccination program will be willing / receive covid-19 vaccination than those who do not know. Willingness to pay is also significantly related to vaccine acceptance, with a P-value of 0.000 means a relationship between willingness to pay and receiving covid-19 vaccination. According to the chi-square test, in the knowledge variable, the p-value is 0.026, which means that the respondent’s knowledge significantly affects the acceptance of the Covid-19 vaccination. An OR of 3.78 shows that respondents with good knowledge will receive Covid-19 vaccination 3.78 times more than those with less knowledge.

### Discussion

**Relationship between knowledge and acceptance of the Covid-19 vaccine.**

The OR value (3.78, p = 0.026) was obtained based on the research. It means people with good knowledge will receive vaccinations three times more than people with less knowledge. Vaccines are considered an effective way to combat Covid-19 infection, so several developers from various companies are trying to produce vaccine development programs against the coronavirus⁷. Vaccination needs to be carried out immediately, and socialization from the government is required about the importance of vaccines in breaking the chain of Covid-19 spread.
With the continuous socialization about the Covid-19 vaccination, it is hoped that the public will know more about the vaccine’s benefits. This pandemic that has occurred more than one year is a long time for the community, so efforts to overcome this pandemic are highly expected. One of the efforts made by the government in Indonesia and in various other countries is by giving vaccinations. Based on research data, almost all respondents have good knowledge about Corona Virus Disease, the efforts made to prevent transmission, and an overview of Covid-19 vaccinations. This good knowledge may be obtained from socialization from various social media sources, information from people around, and individual efforts to dig up more information about Covid-19 and vaccinations. Most of the respondents are adults, where the more mature a person’s mindset is, the more open they will be to adding new information and are good at receiving information. This is in line with what was expressed by 8 that the more mature a person’s ages, the level of maturity and ability to receive information are getting better. The level of education of the majority of respondents in higher education where this level of education factor can affect a person’s level of knowledge and experience because it is in line with what was expressed by Carter (2011) in (Mujiburrahman, Riyadi, and Ningsih, 2020) that the higher a person’s education, the easier it is to receive information, it will also increase.

**Relationship between perception and acceptance of the Covid-19 vaccine.**

Perceptions in this study relate to respondents’ knowledge of the Covid-19 vaccination program, willingness to pay, the place for the Covid-19 vaccination, and socialization media. From these variables, respondents’ understanding of the Covid-19 vaccination program and willingness to pay were positively correlated with receiving Covid-19 vaccinations. The results showed that respondents’ perceptions of knowledge about the Covid-19 vaccination program led to 13.7x acceptance of Covid-19 vaccination compared to those who did not know this program (OR 13.7, p=0.01). Perception of willingness to pay for the Covid-19 vaccination is positively correlated with the receipt of Covid-19 vaccination (p=0.000). However, 16.1% of respondents are still unsure and do not know whether they will join the Covid-19 vaccination program if the program has to be paid for one day. Positive perception will encourage conscious behavior towards something. Every individual has different feelings, thinking abilities, and experiences. Therefore, the perception results will differ from one individual to another in perceiving a stimulus.

In this study, respondents’ perceptions of accepting the existence of the Covid-19 vaccination well. Respondents perceive that this vaccine is a program from the government that needs to be followed and the government provides this vaccine free of charge. This is following the aspects behind the formation of perceptions, which is seen from the way of thinking of the respondents, almost all of the respondents are adults with reasonable knowledge that programs from the government are undoubtedly good for the community and of course the ultimate goal of government programs is in line with the thoughts of respondents so that the Covid-19 pandemic condition could end soon. Based on the results of the study, it was also found that respondents were familiar with vaccines as evidenced by the respondents’ good knowledge of the covid 19 vaccine. Respondents interpreted that the high number of cases of covid 19 was a problem that required cooperation from all parties, namely between the government and the community10,11. If the community supports the government’s program, namely the Covid-19 vaccine program, it is hoped that the number of Covid-19 sufferers can be reduced.

**Ethical Approval**

The research passed the ethical approval as evidenced by issuing an ethics approval certificate by the Health Research Ethics Commission of Universitas Pesantren Tinggi Darul Ulum Jombang with the number 027-KEP-Unipdu/5/2021.

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**Conflict of Interests:** The authors have declared that no conflict of interests

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Reference


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Study of the Medicolegal aspect of Trauma, Cases came to Emergency Department at Beni-Suef University Hospital “Egypt”

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Abstract

Trauma is the 6th leading cause of death worldwide resulting in 5 million of all deaths. It is the 5th leading cause of significant disability. In Egypt, injuries the fifth leading cause of death and the most common cause of hospitalization and account for at least 1/4 of all inpatients. The aim is to identify the medico-legal importance of different causes of orthopedic trauma to patient admitted at Beni-Suef university hospital and identify their relation with different demographic data of the patient. Data of 207 patients with orthopedic trauma admitted at Beni-Suef university hospital period from September 2016 to December 2016. Age, sex, residence, cause, site, number of trauma, type of fracture, investigation done, treatment and also associated other injuries are reported. The most common cause of trauma is motorcycle accidents (25.6%) followed by fall on ground (25.1%). Lower extremities are the most common site of trauma (44.9%). Most of cases have single fracture (71.5%) with simple type (50.3%). X-ray is the most common investigation done to patients (71%). (68.1%) of cases need operative treatment. (84%) of cases came with orthopedic trauma only not associated with other injury. While the most common associated injury is wounds over skin (5.4%). Limb injuries are a common problem among the physically active, and such injuries may result in diminished performance, reduced participation, and in the longer term loss of function, chronic joint disease, and disability. Limb injuries represent the commonest form of injury involved in road traffic accidents

Keywords: Admissions; fracture; infection; orthopedics; trauma.

Background

In Egypt, injuries are a significant source of morbidity and mortality. They’re the fifth leading reason behind death and also the leading cause of hospitalization and account for a minimum of one quarter of all inpatients3. It’s the leading reason behind mortality and incapacity during the first four decades of life. Trauma is the sixth leading cause of death worldwide leading to 5 million or ten present of all deaths. It is the fifth leading reason behind total incapacity19. Orthopedic trauma represent the most common sort of injury concerned in RTA (Road Traffic Accidents). it is a common drawback for the physically active and such injuries could lead to diminished performance, reduced participation.
and, within the long run, loss of perform, chronic joint illness, and disability. The World Health Organization has predicted that traffic fatalities will be the sixth leading cause of death worldwide and the second leading cause of disability-adjusted life-years lost in developing countries by the year 2020.

**Subjects and Methods**

This is a prospective study. For statistical purposes. Data of all cases who were exposed to different types of trauma lead to orthopedic trauma and have been report at Beni-Suef university hospital (admitted at Beni-Suef university hospital) were collected and analyzed. At period from September 2016 to December 2016.

**Statistical analysis:**
- Computer software package SPSS 24 was used in the analysis.
- Frequency and percentages were presented for categorical variables.
- Chi-square and Fischer’s Exact tests were used to estimate differences in qualitative variables. P value showed significance below 0.05.

**Results**

The total patients’ number during the period of the study was 207 patients.

Table 1 showed the demographic data of the studied patients which revealed that males (68.6%) outnumbered females (31.4%) with male to female ratio 2:1. The most common age group is (31-60y) years old (30.4%), followed by age group (0-18y) years old (30.0%). Many of injured cases came from urban areas (54.1%).

Table 2 showed the data of trauma which revealed that the most common cause was motorcycle accidents (25.6%), followed by fall on ground (25.1%), then car accidents (20.8%). The most common site of trauma was lower extremities and pelvis (44.9%), followed by upper extremities (26.0%), then skull (18.8%). The most common type of fracture is simple fractures (50.3%) followed by comminuted fractures (30.9%). The most common number was single fracture (71.5%). Most of cases require operative treatment (68.1%) followed by intervention treatment (27.1%) and least cases need conservative treatment only (4.8%). Most of cases had no other injuries (84.0%), wounds were the most common associated injuries (5.4%).

Table 3 showed there is a significant relation between age and sex as in all age groups males were more than females except for age groups above 60 yrs. (P value <0.000). Also there is a significant relationship between age and cause of trauma (P value <0.000) as motorcycle accident was most common in age group (31-60y) and least number of cases was in age group (>60y). The significant relationship between age and treatment of cases (P value <0.028) as cases who need operative treatment are more in age group (31-60y). While cases who need intervention are more in age group (0-18y).

<table>
<thead>
<tr>
<th>Table 1: The demographic data of the patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>0-18y</td>
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<tr>
<td>19-30y</td>
</tr>
<tr>
<td>31-60y</td>
</tr>
<tr>
<td>&gt;60y</td>
</tr>
<tr>
<td>Residence</td>
</tr>
<tr>
<td>Urban</td>
</tr>
<tr>
<td>Rural</td>
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</table>

<table>
<thead>
<tr>
<th>Table 2: The data of trauma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cause of trauma:</td>
</tr>
<tr>
<td>Motorcycle accident</td>
</tr>
<tr>
<td>Fall on ground</td>
</tr>
<tr>
<td>Car accidents</td>
</tr>
<tr>
<td>Fall from height</td>
</tr>
<tr>
<td>Direct trauma</td>
</tr>
<tr>
<td>Fall down stairs</td>
</tr>
<tr>
<td>Pedestrian</td>
</tr>
<tr>
<td>Gun shot</td>
</tr>
<tr>
<td>Train accident</td>
</tr>
</tbody>
</table>
### Table 3: Relationship between age and (sex, cause of trauma and treatments)

<table>
<thead>
<tr>
<th></th>
<th>Age groups n (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-18y</td>
<td>19-30y</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>43 (69.3)</td>
<td>50 (84.7)</td>
</tr>
<tr>
<td>females</td>
<td>19 (30.7)</td>
<td>9 (15.3)</td>
</tr>
<tr>
<td><strong>Cause of trauma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motorcycle accident</td>
<td>8(15.09)</td>
<td>20(37.74)</td>
</tr>
<tr>
<td>Fall on ground</td>
<td>17(32.69)</td>
<td>3(5.77)</td>
</tr>
<tr>
<td>Car accidents</td>
<td>14(32.56)</td>
<td>19(44.19)</td>
</tr>
<tr>
<td>Fall from height</td>
<td>10(58.81)</td>
<td>3(17.65)</td>
</tr>
<tr>
<td>Fall down stairs</td>
<td>4(26.67)</td>
<td>6(40)</td>
</tr>
<tr>
<td>Pedestrian</td>
<td>4(50)</td>
<td>3(37.5)</td>
</tr>
<tr>
<td>Gun shot</td>
<td>1(50)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Train accident</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Treatments</td>
<td>Age groups n (%)</td>
<td>P value</td>
</tr>
<tr>
<td>--------------</td>
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</tr>
<tr>
<td></td>
<td>0-18y</td>
<td>19-30y</td>
</tr>
<tr>
<td>Operative</td>
<td>33(53.3)</td>
<td>48(81.4)</td>
</tr>
<tr>
<td>Intervention</td>
<td>24(38.7)</td>
<td>11(18.6)</td>
</tr>
<tr>
<td>Conservative</td>
<td>5(8)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
<td>59</td>
</tr>
</tbody>
</table>

Figures of some patient admitted showing different types of fractures and the cause of them.

Figure 1: Show CT-scan 3D skull, male patient, 37y, and showing lateral wall fractures of Right orbit and anterior wall of maxilla after car accident.
Figure 2: Show CT-scan 3D skull, male patient, 37y, and showing fracture of Right angle of mandible & Left parasymphyseal fracture after car accident.
Figure 3: Show X-ray of lower limb, male, 22y, and show infected proximal femoral plate after car accident for more than two years.

Discussion

The present study revealed that males comprised most of the cases, as males represent 68.6%. Men are much more exposed to trauma possibly due to their active life styles and their relatively aggressive attitudes both in business and in their private lives. This result is agree with that reported by 6,8&10 which show that sex distribution was heavily skewed towards males.

The incidence of trauma in age group (31-60 years old) is higher than others because they are workers and more liable to trauma in work. As regard age group (0-18 years old), they came on the second and this could be explained by the fact that this has the age of adolescence and early youth where individuals start a more independent life thus exposing themselves to all sorts of violence. This result is in agreement with that reported by (Devarshi et al., 2014) that show the lowest incidence of orthopedic trauma in old age. The result is against to that reported by (Gaurav et al., 2014) that show the maximum incidence in age group 19-30 years old.

There is increase in incidence among urban than patients in rural area as urban. This may be due to increase the activity in urban, most of work places are in urban area and they use different means of transportation that were the leading cause of orthopedic trauma. This result is against to that reported by

The most common cause of trauma is motorcycle accidents followed by fall on ground then car accidents. This is mainly due to lack of safety while driving motorcycle and high speed while driving. This result is agreed with that reported by 17. But it is against the result reported by Gaurav et al., 2014 which show that car accident is the most common cause of trauma.

As regard site of trauma, it was found that the most common site of orthopedic trauma is pelvis and lower extremities followed by upper extremities then skull injury. This is may be due to mode of trauma (mainly motorcycle accidents) that mainly affect lower extremities and cause fractures to them. This result is agreed with that reported by Taylor and Young which show that lower extremities is the most common site of trauma followed by lower extremities injury. But it is against by that reported by Harry et al., 2011 which show that upper extremities is the most common of orthopedic trauma11.

The most common type of fracture is simple fractures followed by comminuted fractures. This result coincide with the work of that show the simple fractures are more common in orthopedic fractures.

As regard number of fractures, single fracture is the most common number of fracture occur after orthopedic trauma (71.5%) and the remaining is the multiple fractures (28.5%). This result coincide with the work of14.

Most of cases require operative treatment followed by intervention treatment and least cases need conservative treatment only. This result is in agreement with that reported by Somersalo et al., 2014 that show most of cases of orthopedic trauma admitted to hospital need operative treatment2. This result is against to the data reported by Marc et al.,
2013 as in their analysis of extremity fractures might partially have remained classified under conservative treatment just because patients never reached surgery due to cases of high mortality\textsuperscript{14}. 

As regard cases had orthopedic trauma associated with other injuries, most of cases had no other injuries, and wounds were the most common associated injuries followed by neurological injuries then abdominal injuries. This affected mainly by the modes of trauma which were different from case to another and as motorcycle accidents were the most common cause of fractures which lead to isolated fractures in most of cases. This result is in agreement with that reported by Hassan and Emara, 2015 as they told that most of cases had orthopedic fractures followed by general surgery as associated injury\textsuperscript{16}.

In all age groups males were more than females except age group (>60y). This mainly due to increase incidence of osteoporosis in female patients after menopause which lead to increase fragility of bones and bone fracture from minor trauma. This result is in agreement with that reported by Sadat v et. al. 2015 & Taylor and Young 2015) as they show that there is an increasing incidence of elderly osteoporotic fractures in females\textsuperscript{17\&20}.

In fall from height, it was more common in age group (0-18y). As reported by\textsuperscript{12} in their study about pediatric musculoskeletal injuries falls have been the most common mechanism of pediatric trauma and also reported by. In car accident, it was common in age group (19-30y) followed by age group (0-18y). This is in agreement with data reported by (Ganveer and Tiwari 2005) as they show that the majority of the victims after road traffic accidents were in the age group of 18–37 years. Direct trauma is equal in both age groups (19-30y) (31-60y) because they were the age groups of workers who were more susceptible to direct trauma during work. The most common age group came due to motorcycle accident was age group (31-60y) and least number in age group (>60y)\textsuperscript{9}. This is against the data reported by (Amin et. al., 2011) as they show that the most common age group came with motorcycle accident is age 20-29y and there is increase in percentage of cases in age above 50y. In fall on ground, it was common in age group (0-18y)\textsuperscript{1}. Pedestrian trauma was more common in age group (0-18y) this is against that reported by (Mishra et. al., 2010) who show that Pedestrians represented the majority of victims sustaining injury as a result of being knocked by a vehicle, motorbike or cycle\textsuperscript{15}.

It was found in the relation between age and treatment, cases who need operative treatment are more in age group (31-60y). While cases who need intervention are more in age group (0-18y) and they are also more in conservative treatment. There were different factors to determine treatment modality as site of fracture, type of fractures and general state of the patients. This result is coincide with the work of (Terje et. al., 2009) as they found that the Pediatric fractures were more likely to be treated conservatively with only (8\%) requiring internal fixation, compared to (56\%) internal fixation in those ≥16 years of age in their study on incidence of traumatic long-bone fractures requiring in-hospital management\textsuperscript{21}.

**Conclusion**

Trauma can affect different parts of the body: head, chest, abdominal region, extremities, face, spinal cord, the genitourinary system, pelvis, and soft tissues. Limb injuries are a common problem among the physically active, and such injuries may result in diminished performance, reduced participation, and in the longer term loss of function, chronic joint disease, and disability. Limb injuries represent the commonest form of injury involved in road traffic accidents.

**Abbreviations:** Not applicable.

**Financial support and sponsorship:** Nil.

**Conflicts of interest:** There are no conflicts of interest.

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Jatropha Curcas L As Anti-Fertility Agent: A Review

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Abstract

Introduction: The use of natural ingredients as contraception has long been known to the Indonesian people, especially in rural areas, where people still inherit the habits of their ancestors. The plants used as contraception contain compounds that are antifertility, antiimplantation, and antiestrogenic for both women and men. The purpose of this study was to determine the effectiveness of the Jatropha curcas L as an antifertility agent with the approach of several previous studies.

Methods: The method of this review used Prisma guidelines with scientific article searches using the Pubmed database, Google Scholar, and Science direct. A search was done on Pubmed with keywords Jatropha curcas [MeSH Terms] AND antifertility, Google scholar with keywords Jatropha curcas AND antifertility, Science direct with keywords Jatropha curcas AND antifertility

Findings: The results of this review of several research articles that have been reviewed indicated that the effectiveness of Jatropha (Jatropha curcas L) has an effect as an antifertility agent.

Conclusions: In short, there is a significant effect of using Jatropha curcas L as antifertility in the research finding. The implications of the research can be implemented as the contraception for male.

Keywords: Jatropha curcas L, Anti-fertility, Agent.

Introduction

One of the problems that the Indonesian government has not been able to overcome until now is the problem of population density. It is because the population continues to grow every year. The increase in population is increasingly troubling because it is not in line with the increase in welfare. The increase in population will not only complicate efforts to increase and equalize people’s welfare in the food sector, but also education, health, employment, and housing. Therefore, the government makes the Family Planning program a part of national development.

One of the Sustainable Development Goals (TPB) 3 is to reduce the global maternal mortality ratio to less than 70 per 100,000 live births, with no country

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having a maternal mortality rate more than twice the global average. Severe postpartum hemorrhage, infection, high blood pressure during pregnancy (pre-eclampsia and eclampsia), complications from childbirth, and unsafe abortion are the main complications that cause almost 75% of all maternal deaths. The indicator to be achieved in improving public health status is a decrease in the maternal mortality rate from 359 per 100,000 live births in the 2012 IDHS to 306 per 100,000 live births in 2019.

The use of contraception is closely related to the success of family planning. Family planning has become less effective due to the lack of male participation. There are still many doubts about the potential of male contraceptives. Currently, there are no male contraceptive products that meet the requirements, namely safe, comfortable, effective, cheap, reversible, and acceptable. Efforts to obtain male contraceptive materials derived from plants have been widely studied, but the results have not been satisfactory, so that its application to humans is still in doubt. Therefore, the priority of research still refers to the use of plant materials considering that medicinal materials derived from plants have advantages such as low toxicity, less side effects, easy to obtain, and cheap in price. Various types of plants have been studied. One of which is Jatropha (Jatropha curcas L) which has an effect on the reproductive system of male animals, so it can be used as a male contraceptive.

Figure 1: Plants, leaves, flowers, fruits and seeds of Jatropha curcas L [Sources: 4]

Jatropha (Jatropha curcas) is a plant that belongs to the Euphorbiaceae family. This plant has a high economic value because the seeds produced can be useful for medicinal raw materials and producing oil. According to Laxane et al. (2013) Jatropha curcas (Jatropha curcas) has various pharmacological activities including castor seeds used for disinfectant, anti-parasitic, wound treatment, antifertility agent, and so on. According to Ariaodion et al. (2020), Jatropha curcas reduces sperm quality and lowers levels of male reproductive hormones. Therefore, the writer wanted to know the effectiveness of Jatropha curcas L as an antifertility agent.

Methods

The method of this review used Prisma guidelines, with scientific article searches using data based on Pubmed, Google Scholar and Science Direct. A search was done on Pubmed with the keywords Jatropha curcas [MeSH Terms] AND antifertility, Google scholar with the keywords Jatropha curcas AND antifertility, Science direct with the keywords Jatropha curcas AND antifertility, then screened for publication of articles from 2011-2021, full text, research articles, recurring and theme-related publications detailed.
Findings

After finding the articles from PubMed search, Science Direct and Google Scholar, then they were filtered according to the inclusion criteria. It was begun with the identification of the articles. A total of 5436 articles were screened by looking at complete text articles becoming 1991 articles. Then there were 1869 articles which were not research content not used, so they became 122 articles. Of the 122 articles, there were 85 articles not selected because they were the repeated publication carried out again with criteria related to the theme. So that the final number obtained was 27 articles. The research was carried out in several countries such as: Indonesia, India, Nigeria, Mexico and others who discussed the effect of Jatropha curcas L as an antifertility agent.

Research conducted by Arini (2012) aims to see the antifertility effect of 70% ethanol extract of Jatropha (Jatropha curcas) seeds in vivo. Giving ethanol extract of Jatropha seeds with doses of 5mg/kg BW, 25mg/kg BW, and 50mg/kgBW for 48 days to male rats can reduce spermatozoa, testicular weight and seminiferous tubule diameter statistically when compared to control animals. In addition, the administration of extracts at doses of 5mg/kg BW, 25mg/kg BW, and 50mg/kgBW for 48 days in male rats can affect the process of spermatogenesis, which is indicated by a decrease in the number of spermatocytes and the number of Sertoli cells.

Empirically it is reported that several countries such as Cambodia, Vietnam and India have used castor seeds as an ingredient that can cause abortion, while in Sudan castor seeds are used as contraceptives. Compounds found in castor seeds that have the potential as antifertility ingredients are known as jatrophones. Research from Puspitadewi (2007) stated that oral administration of jatropha seed powder at a dose of 0.2 g/head/day in mice with a treatment period of 14 days showed that the antifertility effect of castor bean did not have a significant effect on changes in uterine profile, both the weight of uterus and the thickness of endometrial. Jatropha curcas fruit is also able to reduce sperm motility and count and has an abortive activity.

The mechanism of antifertility agents found in Jatropha (Jatropha curcas) has not been known with certainty. There are two possible mechanisms of action of these antifertility agents, namely through the hormonal system or directly reacting to the reproductive organs. Based on the previous research, it is shown that there was effectiveness of jatropha curcas as an antifertility agent.

1. Effects of Jatropha curcas on hormones

Jatropha seed ethanol extract has antifertility activity in female rats at which steroid activity was reported. The chemical constituents of Jatropha seeds are fatty acids, sterols, campesterol, stigmasterol, beta-sitosterol, delta 5-avenasterol. Beta-sitosterol is the largest component contained in Jatropha seeds. In another study, the serum testosterone concentration was tested on day 0 and day 49. Testosterone concentration test results show that there is a decrease in testosterone concentration at low doses which can cause a decrease in the quality of spermatozoa because the function of the hormone testosterone, among others, affects the maturity of spermatozoa.

The hormone testosterone is very important in men for the development and maintenance of male reproductive tissues, namely the testes, epididymis, seminal vesicles, and penis. Consumption of phytosterol compounds in excess amounts can cause an increase in plasma testosterone levels because the phytosterols in the body will be converted into testosterone. Beta-sitosterol compounds have a chemical structure similar to the hormone testosterone, which is a cyclopentane perhydrophenantrene-core hydrocarbon compound. An ingredient can work as a hormone because it contains substances whose molecular structure is similar to that of a hormone. Thus, it is suspected that beta-sitosterol also acts like testosterone.

Saponins and alkaloids are the raw materials for steroid hormones. It is suspected that this compound is involved in the steroid hormone biosynthesis pathway, resulting in the formation of a compound with a structure similar to testosterone. This compound is anti-testosterone, binds to testosterone receptors in the seminiferous tubules so that testosterone does not function and causes disruption of spermatogenesis. The same thing was reported by Nurliani, Anni.; Rusmiati and Heri Budi Santos...
that saponins and alkaloids are used as raw materials for the synthesis of steroid hormones, and triterpenoids have a biogenesis link with steroids. It is suspected that saponins, alkaloids and triterpenoids are involved in the biosynthetic pathway of steroids, especially testosterone, resulting in the production of materials with a structure similar to testosterone. Anti-androgen agents act competitively at target tissue receptor sites to block the action of androgen steroids. Allegedly these compounds are anti-androgenic.

Increased testosterone in the blood will result in negative feedback on the hypothalamus. This feedback mechanism is a way of working hormonal contraception that can inhibit the process of spermatozoa maturation. High testosterone levels cause a negative feedback mechanism to the hypothalamus and pituitary. Testosterone will inhibit the hypothalamus to produce GnRH so that GnRH levels fall and inhibit the anterior pituitary to produce FSH and LH. When FSH decreases, there is a disturbance in the Sertoli cells which causes a reduction in nutrients needed for proliferation, differentiation and maintenance of spermatogenic cells. When LH levels drop, the testosterone produced is also reduced. The sperm count and testosterone concentration are kept constant by a feedback mechanism. If a negative feedback mechanism occurs, the levels of FSH and LH in the blood circulation decrease and the next result is that the spermatogenesis process stops and the number of spermatozoa produced will decrease.

### 2. Effects of Jatropha curcas on reproductive organs

Jatropha has the activity of terminating pregnancy, anti-inflammatory, anti-metastatic, anti-coagulant, disinfectant, wound healing. Jatropha leaf extract induces multiple contractions in the uterus of pregnant rats after in vivo and in vitro administration. Jatropha curcas fruit has abortion and contraceptive activity. Giving castor seed powder solution is thought to affect the reproductive organs, especially changes in the uterine profile.

The administration of a solution of jatropha seed powder containing the antifertility agent jatrophone with a subchronic dose of 0.2 g/head/day did not have the potential to affect changes in the uterine profile of Swiss Webster mice. Jatropha seeds are used as contraception or trigger abortion in South Sudan. The antifertility effect of them is thought to be due to the presence of jatrophone as a cytotoxic agent. Jatrophone action is known through inhibition of the protein kinase C pathway.

### Conclusion

Based on the theoretical description and Literature research findings above, it can be concluded that: There are two possible mechanisms of action of these antifertility agents, namely through the hormonal system or directly reacting to the reproductive organs. Based on the previous research, it is shown that there is the effectiveness of jatropha curcas as an antifertility agent.

### Suggestion

Regarding to the results of our findings in this review, it is necessary to do a further research on the isolation of compounds to determine the structure of compounds that play a role in antifertility activity in Jatropha curcas L. It can be developed as a basic ingredient of traditional male or female contraceptive.

### Source of Funding

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### Conflict of Interest

The authors have declared that there is no conflict of interest in term of results of publication as well.

### Ethical Clearance

Not required

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Digital Pocketbook Increase Mother's Knowledge about Covid-19 Transmission Prevention

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Abstract

Mothers and infants are at an increased risk of COVID-19 transmission. One of the preventive efforts that can be done is to use technology as a medium for health promotion, namely digital pocketbooks. Digital pocketbooks in accordance with the conditions of the COVID-19 Pandemic, where there is a policy of transmission efforts by limiting interaction and staying away from crowds. To determine the effect of using a digital pocketbook on a mother’s knowledge about reducing COVID-19 transmission to pregnant women, new mothers, postpartum mothers, and newborns. This study is a quasi-experimental study with an uncontrolled, pretest and posttest approach conducted at the Posyandu Tangkolo (Melati 2) in the Ciamis District. The sampling method used was purposive sampling, and a total of 30 samples. The digital pocketbook is given to increase mothers’ knowledge about to raise moms’ awareness of COVID-19 prevention strategies for pregnant women, mothers in labor, postpartum mothers, and infants. Questionnaires were used to measure the mother’s knowledge before and after being given a digital pocketbook. Paired T-test was applied for effect analysis.

The results showed that there was a significant effect of digital pocketbooks on mothers’ knowledge about preventing the Covid-19 transmission in pregnant women, mothers giving birth, postpartum mothers, and newborns (p=0.000). Digital books make it easy for mothers to access information without having to leave the house, practically, whenever and wherever they want to open and read them easily.

The use of digital pocketbooks can help mothers have a better understanding of how to prevent the transfer of COVID-19 to pregnant women, new mothers, postpartum mothers, and infants. It is recommended that digital pocketbooks can be used as a means to carry out health promotion.

Keywords: Digital pocketbook, knowledge, mother, covid-19.

Introduction

Covid-19, also known as Coronavirus illness 2019, is a contagious disease caused by Coronavirus 2 Severe Acute Respiratory Syndrome (SARS-COV-2). SARS-CoV-2 is a novel coronavirus type that humans have never seen.¹ COVID-19 infection is often associated with acute respiratory distress syndrome symptoms such as fever, coughing, and shortness of breath. COVID-19 incubates for an average of 5-6 days, with the longest incubation duration being 14 days. COVID-19 infection in severe conditions may result in pneumonia, acute respiratory syndrome, renal failure and even death.²

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COVID-19 has spread to all of Indonesia’s province, with an escalating mortality toll. It affects all spheres of life, including political, economic, social, cultural, defense, security, and the welfare of the Indonesian people.1

COVID-19 can be transmitted to anyone, including mothers and babies. Mothers and infants are at an increased risk of COVID-19 transmission. It is anticipated that may result in an increase in mother and newborn mortality and morbidity.3

The restriction is one of the consequences of the COVID-19 pandemic, implemented to almost all services, especially maternity and infant health. For example, pregnant women are hesitant to visit the Puskesmas or other health care facilities as the are afraid of contracting the disease, the recommendation for pregnancy check-ups is limited, class activities for mothers are terminated indefinitely, and Posyandu activities are postponed so that mothers and babies do not go to have their health checked, as well as service unpreparedness. In terms of personnel and infrastructure, one of them is personal protective equipment.1

Based on data in September 2020, children aged 0-5 years accounted for 2.4 percent of all infected patients, and 1.3 percent of them perished. Pregnant women were proven positive for COVID-19 at a rate 4.9 percent. Pregnant women, maternity moms, postpartum mothers, and babies are all targets for COVID-19 transmission, and this condition is expected to increase maternal and child mortality and morbidity.1

The data of positive confirmed patients in the Ciamis district is increasing and is included in the red zone. In a day, it can reach 30 cases; even within three days, there are cases of death of COVID-19 patients, which are quite high. This can pose a risk to the health of mothers and children.4

The government has carried out socialization and directives on preventing COVID-19 to the entire community. But not a few of the people who do not really understand how to prevent it. There are still many people who have not done what is directed by the government. This happens because the people’s literacy skills are still low, and there are still many people who do not have access to information media so that the knowledge possessed by the community is still minimal. It is critical to have a thorough understanding of the Covid-19 disease in order to avoid contributing to a rise in the number of Covid-19 cases.5

At the moment, technological advancement have driven many changes in human existence transitioning us from the information age to the digital almost every sector. Utilization of technology as a medium of instruction is one of the inventive ways to expand one’s knowledge.6

Technological advances have brought major changes in the field of health promotion, especially during the COVID-19 Pandemic, where at this time, in an effort to control and prevent the Covid-19 transmission, a policy of limiting interactions and crowds was carried out and maintaining physical distance from one another. Based on this, direct face-to-face interactions are required to adapt to pandemic situations and conditions, one of which is online interaction (in the network). One of the technological advances is the use of digital books to increase knowledge (Kementerian Kesehatan RI, 2020b). Digital books are learning media that are innovative as the use of technology that contains material that is more concise and practical to use and is usually in pdf format.8 Digital books are easier to access on mobile devices. So digital books are easier to understand and apply, and there is no need to face to face to avoid the Covid-19 transmission.9

Government policies in order to reduce the Covid-19 transmission through efforts to limit interactions and crowds greatly impact the learning process, these conditions lead to changes in learning patterns. The use of digital platforms is very effective in increasing knowledge about preventing the Covid-19 transmission.10

Ciamis District has 26 sub-districts, one of the sub-districts with the highest incidence of COVID-19 is in Ciamis District, which can be a high risk to the health of mothers and babies. One of the problems in the Ciamis District is that information on preventing the Covid-19 transmission has not been evenly distributed, especially to mothers and children. One
of the centres for public health activities, especially for mothers and children in the Ciamis District, is the Tangkolo Posyandu (Melati 2). Based on a survey asking about the prevention of Covid-19 transmission to pregnant women, mothers giving birth, postpartum mothers and newborns to mothers who come to the Posyandu, they only know about prevention in general.

**Materials and Methods**

**Study Design:** This quantitative study uses a quasi-experimental approach with a single group pretest-posttest. The goal of this research was to investigate the influence of using a digital pocketbook on a mother’s knowledge regarding COVID-19 prevention in pregnant women, mothers in labour, postpartum mothers, and newborns.

**Instrument:** This research used a questionnaire as its data collection tool. This questionnaire was used to measure mothers’ knowledge regarding COVID-19 transmission prevention in pregnant women, mothers giving birth, postpartum mothers, and newborns. This questionnaire contains 44 questions regarding efforts to prevent Covid-19 transmission in general for pregnant women, mothers in labour, postpartum mothers and newborns. The questionnaire is in multiple-choice questions with correct and incorrect answer choices. Knowledge is divided into three categories, namely good if 75% of respondents answered correctly, sufficient if 56-70% of respondents answered correctly, and less if 55% of respondents answered correctly. In addition to instruments, this study also uses a Digital Pocket Book, which contains guidelines for preventing the Covid-19 transmission in pregnant women, mothers in labour, postpartum mothers, and newborns.

**Research Procedure:** The research process begins with filling out the consent form to become a respondent. Furthermore, respondents filled out the first questionnaire as a pre-test by taking into account the health protocol. Then the respondent was given treatment in the form of giving a digital pocketbook containing how to prevent the Covid-19 transmission to pregnant women, maternity mothers, postpartum mothers and newborns. The book is distributed in the form of a link via Whatsapp. Respondents were given the opportunity to study digital pocketbooks for 1 month. The next meeting was conducted post-test when the respondent returned to the Posyandu the following month.

**Data Analysis:** The normality test of the data was carried out using Kolmogorov Smirnov. The data obtained were normally distributed because the significance value was > 0.05. Paired T-Test to determine the effect of digital pocketbooks on mother’s knowledge about preventing the Covid-19 transmission in pregnant women, maternity mothers, postpartum mothers and newborns, the significance test was carried out using α = 0.05 and CI. 95%. As a result of the statistical test, the p-value was 0.000.

**Result**

In the study of digital pocketbook increase mother’s knowledge about covid-19 transmission prevention can be seen in the following table:

**Table 1: Frequency Distribution of Respondents Characteristics**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Frequency (n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>22</td>
<td>73.3</td>
</tr>
<tr>
<td>Old</td>
<td>8</td>
<td>26.7</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>3</td>
<td>43.3</td>
</tr>
<tr>
<td>Intermediate</td>
<td>14</td>
<td>46.7</td>
</tr>
<tr>
<td>Low</td>
<td>13</td>
<td>10.0</td>
</tr>
</tbody>
</table>

Table 1 shows that the majority of respondents are mostly young women aged 20-35 years at 26.7%, with a secondary education level of 46.7%, the average is high school education.

**Table 2: Frequency Distribution of Prior and Knowledge After being given a Digital Pocketbook**

<table>
<thead>
<tr>
<th>Mothers’ Knowledge</th>
<th>Frequency (n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>7</td>
<td>23.3</td>
</tr>
</tbody>
</table>
Table 2 shows that most mothers’ knowledge before being given a digital pocketbook was sufficient at 56.7%. After being given a digital pocketbook, it seemed to have increased, where the majority had good knowledge of 73.3%.

Table 3: The Effect of Digital Pocketbook on Mother’s Knowledge about COVID-19 Prevention

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before</th>
<th>After</th>
<th>Value of p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mothers Knowledge</td>
<td></td>
<td></td>
<td>0.000*</td>
</tr>
<tr>
<td>Mean</td>
<td>1.9</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>0.548</td>
<td>0.563</td>
<td></td>
</tr>
</tbody>
</table>

Note: * T test paired

Table 3 shows that the digital pocketbook variable has a statistically significant effect on mothers’ knowledge about preventing the Covid-19 transmission to pregnant women, mothers giving birth, postpartum mothers, and newborns. Based on the results of the questionnaire analysis, there was an increase in knowledge about preventing the Covid-19 transmission in pregnant women, mothers giving birth, postpartum mothers, and newborns. Previously, they only knew about prevention generally. With the digital book, they can read about prevention specifically, namely preventing the Covid-19 transmission for pregnant women, mothers in labor, postpartum mothers, and newborns.

The consequences that can occur if the mother’s knowledge about preventing the Covid-19 transmission is low is that it can increase the risk of maternal and child morbidity and mortality. This is the basis for the need for efforts to spread knowledge about preventing COVID-19. This is a preventive effort that can be done with the aim of preventing transmission.

One of the results of the study stated that the lack of information about the impact of the pandemic on mothers and children could lead to a declining trend in the use of maternal and child health services. Many mothers did not know about the flow of health checks during the COVID-19 pandemic, causing many to be rejected when they were about to check with health workers because they had not previously made an appointment.

The results of statistical tests using the t-test obtained a p-value 0.000 < 0.05, which means that there is an effect of giving digital books to increasing maternal knowledge in the Tangkolo Posyandu (Melati 2) Pawindan Village, Ciamis District. Based on the results of the study, digital books have proven to be effective in increasing mothers’ knowledge, especially in preventing the Covid-19 transmission. Digital books are very appropriate to use, especially during the COVID-19 pandemic, where there are...
policies to prevent the Covid-19 transmission by limiting interactions and crowds. Digital books are able to increase mothers’ knowledge about COVID-19 prevention without mothers having to meet face-to-face with health workers to get information.

Digital books are publications consisting of text, images, and audio, published in digital format, and can be read on computers and other electronic devices. This digital book is more environmentally friendly because it reduces paper usage, is durable, can be easily distributed via short messages, WhatsApp, or the website. These digital pocketbooks are usually more interesting, interactive, easier to understand the material, easy to carry, not obsolete, easy to process, and physically small in size. This digital book is one of the alternative learning media which is a very appropriate choice to improve knowledge, because whenever and wherever they want to open and read it easily.  

The development of digital technology is currently so rapid. One of the mobile devices currently used is a cellular phone. Android-based digital pocketbook application is one of the developments of mobile learning (m-learning). The advantages of a digital-based digital pocketbook application are user-friendly, which is easy to use in operation, practical to use, and does not take up too much space in the system to install this application.

Digital media provides women with ease and color in great detail, and it is very easy to access the information needed to build and maintain social connections and relationships with other women. Pregnant women and those with young children are in dire need of digital information to add information about health.

This research is also in line with Coral et. al., who stated that the use of digital books provides a greater ability to increase knowledge, attitudes, and skills. These findings suggest that there are other capabilities or colors offered by the technology, which are not available in paper-based books.

Other studies suggest that the use of technology can increase maternal knowledge and also increase awareness of health literacy. This health literacy can bridge the health status of mothers. The use of technology can also make it possible for women to involve themselves in empowering their environment, for example, by informing the wider community of what has been obtained, especially information on preventing the Covid-19 transmission.

The utilization of information media is very influential on the delivery of health messages, especially on maternal and child health. O’Higgin’s research (2013) states the role of digital media will make it easier for mothers to access information about pregnancy to increase knowledge in prenatal care services.

Health technology is developing rapidly and is everywhere. There is a lot of potentials for digital solutions to extend health services to hard-to-reach communities. Digital health can help meet health information and improve health. But before implementing digital health or other digital technology, you must consider the availability of mobile phone facilities, internet connectivity, and others. So that it will be effective in providing health information. It is found in this that some mothers who visited the Posyandu did not have a mobile phone with Android facilities, so they could not be given a digital pocketbook.

**Conclusion and Acknowledgment**

**Conclusion:** There is an influence of digital books on mothers’ knowledge about preventing the Covid-19 transmission. It is recommended that digital pocketbooks can be used as a means to promote health, and mothers can disseminate information obtained from digital pocketbooks to the general public.

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**Ethical Clearance:** This research has received approval from the STIKes Muhammadiyah Gombong Ethics Committee by obtaining an ethical approval letter from the STIKes Muhammadiyah Gombong Health Research Ethics Committee with the number: 198.6/II.3.AU/F/KEPK/IV/2021.
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Early diagnosis of acute kidney injury by measurement of Interleukin-6 (IL-6) and Hepcidin levels in patients following cardiac catheterization

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Abstract

Acute kidney damage is a severe condition common in patients who have undergone heart surgery (catheterization) and secondary injury is also referred to as being synonymous with surgery. The goal of this research is to determine the rate of serum urea, Interleukin-6 (IL-6) and Hepcidin levels in patients with acute renal injury (AKI) following cardiac catheterization. The study included 81 patients (64 males and 17 females) ranging in age from 40 to 75 years. Data from most patients is reported in the form of an age, gender and smoking background questionnaire. The results indicate a significant increase in serum urea, Interleukin-6 (IL-6) and Hepcidin levels in patients with severe renal insufficiency after cardiac catheterization in (79%) males versus (21%) females. According to the outcomes of this study, improved risk prediction could improve patient monitoring and treatment after surgery, as well as direct patient treatment and decision making. Also, the findings show that they enhance participation in AKI interventional trials.

Keywords: Serum Interleukin-6(IL-6), Hepcidin, AKI, Chronic kidney disease, Cardiac surgery

Introduction

Chronic kidney disease (CKD) is one of the most common diseases afflicting people worldwide. The relative rise in the number of cases is due to old age and related conditions such as diabetes and high blood pressure¹. Recently, CKD cases have been reported to have risen by 10-15% worldwide². Statistics in the United States (US) show that more than 10% of the adult population has CKD. The number of cases of chronic kidney disease in Asian countries ranges from 13 to 17.5%³. One of the most important risk factors, such as cardiovascular disease, which is associated with other chronic diseases, is a common factor in patients with chronic renal inflammation. The risk drivers of CKD can be classified into three major categories: chronic, behavioral and biomedical. The fixed group includes family history, age of the patient, prior cases of kidney failure, low birth weight, and sex of the patient⁴. Behavioral risk factors consist of the patient’s daily activities, such as alcohol and unhealthful food⁵. It is worth noting that

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smoking raises the accumulation of urinary albumin. In a sample of 40,619 people aged 28 to 75 years, concentrations of albumin increased below the level of micro albuminuria.

Even in non-diabetic and hypertensive people, smoking has been independently linked with micro albuminuria. A cross-sectional analysis of 7,476 people without diabetes found that the amount of urinary albumin excretion is related to the amount of cigarette smoking, lethargy, and malnutrition. The biomedical section covers people with diabetes, elevated blood pressure, cardiovascular disease, chronic renal inflammation, overweight and obesity. In addition to risk factors, the most important of which are renal artery disorder, diabetic renal dysfunction, glomerulonephritis and inherited renal disease are common triggers of chronic kidney disorder. Surgery is one of the major causes of acute kidney injury, the most important of which is cardiac surgery, and its risks, such as inflammation and elevated toxicity to the kidneys as they interact with each other and inflict acute kidney injury.

One of the most important reasons for not detecting chronic kidney disease early is that it has no signs and is known as a silent disease, as it is detected after a patient loses about 90% of kidney function and symptoms begin to emerge. Among the major signs associated with chronic kidney disease are elevated blood pressure, changes in urine (decreased or increased urinary frequency during the night), peripheral edema, kidney discomfort, weakness, loss of appetite, difficulty sleeping, fever, lack of focus, itching and restlessness. Respiration, nausea and diabetes-like symptoms of loss of appetite. Acute renal injury (AKI) is a complex condition that arises in a number of clinical conditions. Its signs include a mild rise in creatine in the blood and gradual renal failure. AKI is an expression of the full spectrum of renal disease, in addition to acute renal failure (ARF), the primary symptom of which is a drastic decline in renal function (within 48 hours). Renal dysfunction is characterized by a significant increase in serum creatine of > 0.3 mg/dL or > from baseline or a decrease in urinary volume of 0.5 ml/kg/hour. One of the most common issues is the failure to clinically identify patients with renal insufficiency because long-term effects are dangerous and occur immediately. The number of cases and deaths in intensive care units due to AKI has grown from 5% to 30%, relative to other diseases. Despite technical advances in clinical treatment, acute nephritis-related deaths remain high as the practitioner can only give preventive care such as hemodialysis due to elevated amounts of creatine in the blood. In general, several studies have found that CI-AKI and possibly AKI have declined in patients who have undergone heart surgery over the past 10 years, with the exception of those who had postoperative coronary angiography and tested positive for AKI that required dialysis (AKI-D).

Many biomarkers are helpful in the detection of acute renal dysfunction at different stages of the condition, from the onset of symptoms to the progression of symptoms, such as serum creatine and urea concentration. However, this method is deemed inadequate to detect and diagnose these diseases and the complex aspects of AKI. Creatine and urea analyses are critical markers for the detection of diseases as well as for clinical assessment. However, this criterion is resistant to extreme changes in renal activity and can differ depending on several factors, such as age, gender and diet. In cases of intact kidneys, an increase in this criterion could be due to renal ischemia. This criterion is often assumed to be insufficient because, in most clinical cases, its meaning is unclear, which makes it impossible to diagnose. New diagnostic strategies (for example, renal biomarkers and enzymes) can be useful for early diagnoses, and Hepcidin may be one of the biomarkers. Several laboratory studies have shown that Hepcidin has distinct and overlapping roles; the effects of Hepcidin can cause systemic iron deficiency, reduced availability of iron for erythropoiesis, and resistance to endogenous and exogenous erythropoietin. Along with impaired kidney production of erythropoiesis, Hepcidin mediated iron restriction contributes to anemia of chronic kidney. The second marker is an Interleukin-6 (IL-6) cytokine that is one of the key factors controlling the defense mechanisms of the body through multi-directional actions. IL-6 activity plays a significant role in its inclusion in the immune response, hematopoiesis, and inflammatory processes. This marker is thought to be one of the key proinflammatory human cytokines and is also active.
in those processes, which may explain the rise in the serum level of this cytokine during chronic damage to the kidney and acute kidney.

According to the above survey, a lot of studies have employed different types of tests, such as blood tests that reveal the percentage of waste products in the blood, such as creatine and urea. But there are a few studies using the rate of Hepcidin and Interleukin-6 (IL-6) cytokines to diagnose AKI. As a result, the study’s goal is to follow the condition of patients before and after heart surgery, rather than relying on traditional methods of monitoring “kidney function” and measuring the level of creatine in the blood.

Materials and Methods

This study was conducted on (81) patients (64 males and 17 females) whose ages ranged between (40-75) years, mean (58.56) years, and attended the Medical City hospital and Ibn Al-Bitar hospital and underwent cardiac catheterizations during the period from January to July 2020. The patients included in the study developed acute kidney injury (AKI) after catheterization. Data from all the patients is recorded in a questionnaire form regarding age, gender and smoking history. The body mass index of patients is calculated from their height and weight. Laboratory investigations, including estimation of serum IL-6 and Hepcidin, are performed at private laboratories in Baghdad, Iraq. Venous blood samples are collected by means of disposable syringes from each patient before and after cardiac catheterization. Blood samples were collected in clean, dry tubes and left for 15 min, after which the coagulated part was separated from the clear solution using a centrifuge (Win-com Company Ltd., China) at a speed (min / 10000 g) for a period of 15 min. The laboratory investigations for (serum IL-6 and Hepcidin) are immediately performed by the ELISA technique using a (Cloud clone corp., USA) ELISA kit.

Statistical analysis

Statistical analysis was performed using the SPSS Statistical Package for Social Sciences (version 20). Data is presented as a mean ± SD or number and percentage, as applicable. A Paired Student’s t-test was used to compare before and after the catheterization procedure. The Unpaired Student’s t-test and the ANOVA test were used to study the effect of gender, other complications and smoking. A P-value of 0.05 was deemed statistically significant.

Result

The results in Table 1 showed that the mean age of the patients with AKI was 58.56 years, while the mean BMI was 34.87 years. Gender distribution findings among AKI patients revealed that the number and percentage of males were 64 (79%) and the number and percentage of females were 17 (21%). As a result, as shown in table (2), the number and percentage of cigarette smokers patients were 33 (40.7%) compared to non-smokers 31 (38.3%) and 17 (21%), respectively. Table (3) indicates a significant increase in mean serum Interleukin-6 (IL-6) levels from (22.08) to (71.21) (P=0.005) following cardiac catheterization. There was also a significant rise in mean serum Hepcidin levels (114.21) and was (47.94) (p=0.005) among AKI patients after cardiac catheterization.

Discussion

Before and after cardiac surgery, two biomarkers, Interleukin-6 (IL-6) and hepcidin, were examined and evaluated in relation to the issues associated with acute kidney injury. The results revealed that the number and percentage of male patients were higher than females and obese, with an average BMI of 34.87. The results of our analysis also revealed a slight increase in the number and percentage of smokers. As shown in Table (1), depending on the age of patients with severe renal impairment, the mean age of the patients was (58.56) years, indicating that damage is roughly related to ageing. Ageing is associated with a decrease in renal function and an associated decrease in the estimated glomerular filtration rate, which reduces normal kidney function and increases the predisposition to severe renal impairment after surgery. As a result, improvement of renal function after acute renal injury in elderly individuals is at risk and “age” should be considered as a possible diagnostic rate after acute renal impairment. These findings are consistent with a study in a pediatric patient group that recorded a potential 3- to 5-year follow-up of 245 children with acute renal impairment treated at Texas Children’s Hospital between January
1998 and June 2001. As shown in Table 2, the results revealed the majority of male cases were in patients with severe renal insufficiency. This finding differs from other research that showed a greater risk of females developing renal dysfunction compared to males. The effects of gender distribution were inconsistent with our findings as males were more prominent in our sample, and this could be due to the presence of patients with rheumatoid arthritis as well as environmental and health factors in the study area. As shown in Table 1, the body mass index (BMI) is high in the infected people studied, as it is thought to be an important predictor of many disease cases. For examples, chronic obstructive pulmonary disease, chronic renal impairment and cancer have increased mortality in underweight patients. The findings of increased body mass index (BMI) in patients with significant renal impairment were in line with previous research.

Woersching, J. (2019) Reported the association between BMI and the incidence of acute renal injury in 445 patients undergoing cardiac surgery. Also, the findings refer to how a higher BMI was separately associated with an increased risk of developing acute renal impairment, as well as that obesity was found as an independent risk factor for developing CKD. In an analysis of conditions that help increase the occurrence of acute renal failure, the proportion of smokers was higher than non-smokers, as seen in the Table 2. As smoking raises the accumulation of urinary albumin, even in a group with albumin concentrations below the threshold of micro albuminuria, this is consistent with the research involving 40619 people aged 28 to 75 years. It was noted that smoking was a significant influence on people without diabetes or hypertension and was independently associated with albumin structure.

According to a report performed by Pinto et. al. (8) on 7,476 non-diabetic participants, the rate of excretion of urinary albumin is related to the amount of cigarettes smoked. Table (3) shows significant differences in IL-6 levels before and after cardiac catheterization procedures. The inverse correlation of glomerular filtration rate (GFR) with circulating levels of pro-inflammatory markers, including IL-6, is additional evidence of a connection between IL-6 and kidney disease, as seen in Fig.1.

In patients with diffuse inflammatory marker levels such as IL-6, which were higher than those of healthy subjects. The GFR incidence was slightly lower; elevated serum interleukin (IL)-6, IL-8, IL-1β, and IL-10 levels were observed. Also, in patients with acute renal dysfunction of AKI, tumour necrosis factor alpha (TNF-alpha). The results indicate an increase concentration of Hepcidin in the urine of patients with acute kidney inflammation following cardiac surgery. The increased concentration results in the isolation of iron inside the cells, which leads to renal perfusion as well as causing severe anemia due to decreased renal clearance and inflammatory state.

**Conclusion**

1. The findings suggest that the concentration of Interleukin-6 (IL-6), hepcidin are increased in the serum of adults who have undergone heart surgery and this is a sign of acute nephritis. IL-6 and hepcidin are good biomarkers of chronic kidney damage in acute-stage survivors after surgery.
2. The current study showed that the age of the patient plays a significant role in the preparation of AKI, particularly after cardiac surgery, as the rate of infection increased in adults aged 58 to 75 years.
3. Dietary and behavioral habits, such as smoking, are considered a contributing factor in raising the prevalence of infection, particularly for people who have kidney and urinary system issues in addition to respiratory disorders.
4. The Body Mass Index (BMI) is an additional cause for developing AKI due to associated complications with obesity and related disorders such as high blood pressure, diabetes and diabetic nephropathy.
5. “The findings of this analysis suggest that genetics and psychological and social influences could be the driving force behind the gender related variation found in patients with chronic kidney disease, where the proportion of adult males affected was higher than females. The proportion of adult males affected was 79% higher than females, 21% more than women and 21% of 81 samples of the patients who underwent the examination.
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Conflicts of interest: The authors declare no conflicts of interest.

Ethical clearance: Taken from College of Science, University of Tikrit.

Source of funding: Self.

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Biomedical Waste Management at Health Care Facilities in Chikkaballapur District: A Situational Analysis

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Abstract

Background: It has been almost two decades since the implementation of biomedical waste management (BMWM) rules in India, but most Indian hospitals are yet to achieve the desired standards for BMWM practices. Hence, we intended to study the status of BMWM in healthcare facilities (HCFs) of Chikkaballapur district.

Methods: A cross-sectional study was conducted in Chikkaballapur district among all 67 HCFs i.e. 46 HCFs in the private sector and 21 HCFs in the government sector. A pre-tested questionnaire was used to collect information on micro level process of healthcare waste management.

Results: Segregation practices were not followed as per BMWM Rules. In private HCFs, segregation efficiency score was very poor in 23 (50%) out of 46 HCFs, poor in 21 (45.6%) out of 46 HCFs, and satisfactory in only 3 (6.5%) out of 46 HCFs. In government HCFs, segregation efficiency score was very poor in 5 (23.8%) out of 21 HCFs, poor in 12 (57.1%) out of 21 HCFs, and satisfactory in only 4 (19.0%) out of 21 HCFs.

Conclusion: The micro-level BMWM process at the HCF under the private and government sector was found to be very poor especially the segregation practices.

Keywords: Bio-medical waste management, health care facilities, Chikkaballapur, micro-level, segregation process, private, government

Introduction

Catering to the health needs of the increasing population of the country has lead to expansion of the health care sector to a great extent ,both public as well as private health sector. Bio medical waste management (BMWM) includes life cycle i.e. segregation, disinfection before disposal, containment, storage and final disposal of waste. Some of the blood borne infections and pollution of air, water and land is predominantly due to increased quantum of waste generated and also due to mismanagement of BMW . Realizing this, Ministry of Environment & Forests(MoEF), Government of India had notified the Bio- Medical Waste (Management & Handling) Rules

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1998 under the Environment Protection Act 1971, which has been amended and notified in 2016 keeping in view of the fact that there has been unscientific disposal BMW which is a threat to the health as well as the environment and hence its treatment and disposal should be done in a scientific manner.

As per WHO report, hospital waste generated is either general waste (80%) or infectious/toxic waste (20%). The general waste is treated like a municipal waste, whereas the infectious waste requires proper handling so as to avoid any spread of infections.

As per MoEF estimates, almost 4,05,702 kg waste is generated daily, of this 28% is left untreated and this acts as a health hazard by entering the system again. The improper disposal of BMW not only predisposes the hospital staff and patients to risk of infections but also poses a risk to environment. A study by Patel DA et. al., found that the hepatitis outbreak in Gujarat, 2009 was due to poor management of BMW.

Situation Analysis is always a first step in identifying the magnitude of problem and existing practices in BMWM. Chikkaballapura is a newly carved out district from Kolar. There haven’t been many studies in the district to evaluate BMWM in the HCFs of the region. The study aims at exploring the current situation and micro-level process of BMWM i.e. the amount of waste generated, to assess segregation of waste, containers used for collection, on-site transportation, temporary storage site, disinfection before disposal and final disposal among the HCFs in private sector and government sector.

**Material and Methods**

A cross sectional study was done to study the micro level situational analysis of BMWM at HCFs in government and private sector in Chikkaballapur district, Karnataka, during the year January-December, 2017 that were registered under the district health office and Karnataka Private medical establishment (KPME) Act 2007. The study included the HCFs at different level both in the private sector as well as government. Ethical clearance from the institutional review board and permission from the district health office, Chikkaballapur. A written informed consent was taken from the management of all the health care facilities for the study, prior to data collection.

**Sample size**: Based on the INCLEN program evaluation network, the sample size for the study worked out to be 67 HCFs i.e. 46 health care facilities from private sector and 21 health care facilities from government sector with relative precision 15%, confidence level 95% and 10% non response rate.

**Sampling method**: Probability proportion to size followed by simple random sampling was applied for the selection of HCFs.

A validated questionnaire was used to collect information from the nodal officer of the HCFs regarding general description of the HCF, number of beds, process of BMWM and the quantum of waste generated by the HCF, segregation process, disinfection before disposal, management of sharps, transportation of waste, worker safety, storage of waste, liquid waste management, management of mercury and expired drugs.

The quantum of BMW generated was calculated through receipts received from the CBMWTF of the respective HCFs. The BMWM process was observed at various of sub units of the HCFs i.e. OPD, injection room, labour room, laboratory, ward and operation theatre. Segregation practices were categorized according to the tool used as mentioned. (Table 2)

Management of sharp items: This was studied by looking into how the HCFs were handling them. The current practice were put into following practices either no disinfection but contained in PPC/ Burnt using needle burner /Disfigured using hub cutter / None of the above

If any of the above existed in the HCF, it was considered that sharps were managed appropriately, if none then it was assumed that no sharp management existed in the HCF.

**Statistical analysis**: Descriptive statistics was used to summarize the quantitative variables. Data was classified according to sector wise (Private and Government) and then based on type of HCF (Large, Medium and Small). Frequency and percentages were used for presentation and making comparison of study variables across the sectors. Data was analysed using SPSS version 18 Chicago.
Results

Quantity of waste generated

In private sector, the Quantum of infected waste generated / day was 49.4 kg whereas it was 31.5 kg / day in government HCFs. Quantum of sharp waste generated / day in private HCFs was 57.8 kg and in the government HCFs it was 28.2 kg / day (Table 1).

Table 1: Distribution of total quantity of waste generated/kg/day in HCFs*

<table>
<thead>
<tr>
<th>Type of waste</th>
<th>Quantity of waste (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Private (N=45)</td>
</tr>
<tr>
<td></td>
<td>Large</td>
</tr>
<tr>
<td>Infective waste (yellow)</td>
<td>3 31.2 15.2 49.4</td>
</tr>
<tr>
<td>Plastic waste(Red)</td>
<td>2 43 16.8 61.8</td>
</tr>
<tr>
<td>Glass(blue)</td>
<td>0.5 11.3 6.5 18.3</td>
</tr>
<tr>
<td>Sharp waste(white)</td>
<td>2.0 39.1 16.7 57.8</td>
</tr>
</tbody>
</table>

*Note:

Private sector HCFs
a. Large – hospital, nursing home, maternity home
b. Medium - hospital, nursing home, maternity home
c. Small – physician offices, dental clinics, laboratories, physiotherapy clinics and small hospitals

Government sector HCFs
a. Large – District hospital, Taluk hospital
b. Medium – Community health centres
c. Small – Primary health centres

Table 2: Distribution of segregation efficiecny score among private and governemnt HCFs

<table>
<thead>
<tr>
<th>Segregation efficiency score *</th>
<th>Type of health care facility (N=68)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Private (n=47)</td>
</tr>
<tr>
<td></td>
<td>Large (1)</td>
</tr>
<tr>
<td>Very Poor (0-25)</td>
<td>0</td>
</tr>
<tr>
<td>Poor (25-50)</td>
<td>0</td>
</tr>
<tr>
<td>Satisfactory (51-75)</td>
<td>1</td>
</tr>
<tr>
<td>Good (75-100)</td>
<td>0</td>
</tr>
</tbody>
</table>

*Note: Segregation efficiency: segregation score obtained/maximum segregation score * 100

A. >75% - Colour coded bins/liners/exclusive bins (with lids) present on observation. Segregation of waste is done as per color code specified in BMW rules. No mix at all in any of the bins – GOOD

B. 51-75% - Segregation practice present and satisfactory-colour coded bins/liners/exclusive bins present on observation. Waste other than the specified category present in one bin [other than yellow (infected waste bin)] – SATISFACTORY

C. 25-55% - Segregation practice present but incomplete- Colour coded bins/liners/exclusive bins present on observation – POOR

D. <25% Segregation practice present but not satisfactory-Colour coded bins/liners/exclusive bins present on observation.Waste other than the specified category present in yellow, red and blue bin. More than one different category present other than prescribed category in yellow, red & blue bin..Waste other than the specified category present in yellow (infected waste) only or/+ red(plastic waste) or blue ( glass items )bin – VERY POOR
E. No segregation practices - No exclusive bins for segregation. All waste dumped with no criteria or classification for segregation in only one bin. Complete mix - ABSENT

Table 3: Distribution of spill outside the waste bins in private and government HCF’s

<table>
<thead>
<tr>
<th>Health care facility (N=68)</th>
<th>Private (N=46)</th>
<th>Government (N=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spill outside the waste bin</td>
<td>Large (%)</td>
<td>Medium (%)</td>
</tr>
<tr>
<td>Present</td>
<td>33.3</td>
<td>56.4</td>
</tr>
<tr>
<td>Absent</td>
<td>66.7</td>
<td>43.6</td>
</tr>
</tbody>
</table>

Table 4: Distribution of efficiency of waste storage in Private and Government HCFs

<table>
<thead>
<tr>
<th>Efficiency score (%)*</th>
<th>Type of health care facility (N=68)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Private (N=46)</td>
</tr>
<tr>
<td></td>
<td>Large n(%)</td>
</tr>
<tr>
<td>≤25</td>
<td>0</td>
</tr>
<tr>
<td>26-50</td>
<td>1 (100)</td>
</tr>
<tr>
<td>51-75</td>
<td>0</td>
</tr>
</tbody>
</table>

*Efficiency score expressed as obtained score/Maximum score possible * 100

≤25% - Very poor 26-50% - Poor 51-75% - Satisfactory 75% - Good

Segregation of Biomedical Waste

In the present study, among the 46 HCFs under the private sector, it was found that in the large HCF, the segregation was present and in par with the BMWM rules 2016 i.e. in the labour room, laboratory and operation theatres, whereas out of the 18 medium HCFs, segregation was appropriate in 77.7% of the OTs and out of 27 small HCFs, segregation was appropriate in 92.5% of the labor room.

Among 47 HCFs under private sector, only 1 HCF was found to have satisfactory segregation efficiency score and of the 21 HCFs under government sector, 19% of HCFs was found to have satisfactory segregation efficiency. (Table 2)

Spill management

In the present study, among the 46 HCFs under the private sector, it was observed that higher percentages of HCFs under private sectors had spills outside the waste bins i.e. 33.3% of the large HCF, 56.4% medium HCFs and 28.7% small HCFs had spill in the sub units when compared to the 21 HCFs under government sector i.e. 22.2% in large HCF, 50% medium HCFs and 44% in small HCFs combing the score of all sub units in the respective HCFs. (Table 3)

In the government sector only one PHC had a proper spill management protocol in place.

Disinfection process

In the present study, among the 46 HCFs under the private sector, it was observed that higher percentages of subunits of HCFs under private sectors and government sectors had not used disinfectant before disposal of waste i.e. between 60% to 85.8%.

Management of sharps

In the present study, among the 46 HCFs under the private sector, it was observed that higher percentages of subunits of HCFs under private sectors had managed sharps appropriately i.e. all large HCF, 82.5% in medium HCFs and 87.1% small HCFs as well as among the sub units under 21 HCFs under government sectors had managed sharps appropriately i.e. 61.2% large HCFs, all sub units under medium HCF and 74.1% of small HCFs.

Transportation of Biomedical Waste

In house, transportation of BMW is as important as segregation at the point of generation. Under the private sector, it was observed that of the 46 HCFs
under the private sector, none of the HCFs had trolley exclusively for transportation of BMW, only 18.3% sealed the waste bag, 67.3% of HCFs was found that waste was segregated properly and 23.9% of HCFs transported sharp waste in a puncture proof container. Similarly, among the HCFs under government sector, none of the HCFs were using wheeled trolleys for in-house transportation of waste, 52.4%. There was mixing up of waste (52.4%), only 28.6% of sharps were transported in puncture proof container and only 14.3% of waste handlers wore personal protective devices.

**Storage process of Biomedical Waste**

In private sector, among large HCF, despite a designated storage area, it was not for different types of BMW and no water supply whereas in government sector among the large HCFs, despite a designated storage area only 33.3% did not have water supply, 16.6% had no locking facility, 16.7% had separate storage area for different types of BMW and in 50% of HCFs there was mixed up of waste in storage area.

Out of 46 HCFs in private sector, only 5(10.8) HCFs had an efficiency score between 51-75 range and of the 21 HCFs in government sector, only 3(21.4) HCFs had efficiency of waste storage score between 51-75 (Table 4)

**Worker's safety**

In our study the large HCF in the private sector, observed deficiency in worker’s safety by 60%, 80%, 60% and 80% whereas in government sector, among the large HCFs, worker’s safety was found to be deficient in 40%, 43.4%, 73.3% and 43.3% at the Injection rooms, casualty, labour rooms and laboratories respectively.

In private sector, among the medium HCFs, Worker’s safety was found deficient in 95.5% of Injection rooms, 78.8% of casualty, 78.8% of labour rooms and 66.6% of laboratories; in contrast, in government sector medium HCF, Worker’s safety was found deficient in all Injection rooms, 80% of casualty, all of labour rooms and 80% of laboratories.

In private sector, among the small HCFs, Worker’s safety was found deficient in 46% injection rooms and 28.1% laboratories while in government sector small HCFs, worker’s safety was deficient in 95.7% injection rooms and 51.4% labour rooms

**Liquid waste management**

It was observed that only a few Private and Government HCFs had a mechanism to disinfect liquid waste and would prepare bleach solution every day for disinfection. It was also observed that none of the HCFs had an effluent treatment plant for management of liquid waste.

**Discussion**

Segregation of BMW at the site of generation is the key to whole hospital waste management process. Poor segregation not only results in risk to staff and public but may also considerably increase handling and disposal cost of hospital waste. Despite amendments in the BMWM rules, there exists inadequate BMWM at different types of HCFs at the country as well as globally.

In this study, majority of HCFs showed segregation efficiency to be within the grade from very poor to poor (less than 50% segregation efficiency score) in both private and government sector. However, similar observations was found in another study done in Punjab by Devi et. al. found that 21.8% and 28.7% health facilities in government and private sectors with appropriate collection and storage.

It was observed that inappropriate bins were used for waste collections in small HCFs in private sector, were using materials such as discarded bottles, paint boxes and bottles and rest of the HCFs were using bins of plastic material without lids as lack of space made it challenging for the small health care facilities to accommodate so many types of bins. A study done by Jagadeesh U et. al. in Puducherry were indiscriminately using black, brown, and yellow colored bags and urban PHC used black color bags to collect all the BMW and was mixed.

Due to absence of proper lid cover on waste bins and attitude of the health care staff it was observed that there was spill outside the bin i.e. around 20 to 50% of the HCFs and mix up of waste in the yellow bags. This is a concern as far final treatment of yellow bags which is incineration. In the study, there was
no protocol in place at any of the HCFs for spill management and spill kit availability. In a study by D’souza et al, found that they had better knowledge that 51.9% showed that they had good knowledge about spill management and hence better BMWM.8

In our study, HCFs lagged in disinfection process to prevent infection among the waste handlers. Similar observation was found in a study done by a Somaish PT et al, in a District Hospital, Kodagu, South India was seen wherein it was observed that no disinfection was done before disposal of waste.9 In another study in a tertiary hospital in Muzzafaranagar by Choudary PK et al, it was observed that only sharp items were disinfected before disposal.10 In a study by Manasi S et. al. done in Bangalore, it was observed that only sharps were disinfected, and that too only in a few HCFs with 1% hypochlorite solution.11

On a positive note, it was observed that the private HCFs managed sharps better than government HCFs. HCFs in both private and government sector did not have waste trolleys for in house transportation of health care waste and to add to the concern, the waste handlers did not don personal protective gear while transporting waste. Almost similar findings was found in a study done in Muzzafaranagar by Choudary PK et al, though trolleys existed but did not have lids.10

It was observed that the workers safety was found to be deficit (78% to 95%) in medium HCFs. Similarly in a study done in Puducherry, 86% of waste handlers had injuries while handling BMW on one or more occasions hence the recommendation to emphasize on the importance of safety while handling BMW at the HCFs.7

Liquid waste management among the HCFs in both private and government sectors, was found to be satisfactory though it was through simple mechanism of regular bleach solution preparation and disinfection and there was no effluent system existing in the HCFs. Similarly, in a study done in a district hospital, Kodagu, it was found that there was an absence of the effluent treatment plant /local treatment facility for infectious liquid waste.9

The limitation of this study was the quantum of BMW generated in HCFs was from a proxy receipt obtained from CBMWTTF. Due to feasibility of time to manually weigh the BMW was challenging which would have ensured more accurate calculation of quantum of BMW generated in HCFs.

Conclusion

In the current study, overall there is scope for improvement in the micro level areas of BMWM process at the HCFs under private and government sector, as it was found to be very poor. Hence, there is a need to emphasize on continuous training of the health care staffs, monitoring of BMWM on a regular basis at all HCFs in Chikkaballapur district in order to strengthen the life cycle of BMWM process at the point of generation in all HCFs in both private and government sector as per the current BMWM 2016 rules.

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Conflicts of interest: Nil

References


Detecting Cypermethrin Pesticide as Analyte by Capillary Action on Adsorbent Phase of Silica Gel G by Thin Layer Chromatography: A Reinstate Method for Complex and Costly techniques

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Abstract

Pesticide poisoning is one of the most common instances of poisoning deaths in every agriculture-dependent country may it be in form of suicidal or homicidal cases. Traditional classes of pesticides like the Organophosphates, Organochlorides and Carbamates have given way to the newer class of pesticides known as synthetic Pyrethroids. Some of the synthetic Pyrethroids like Cypermethrin, Deltamethrin and Fenvalerate are increasingly being encountered as routinely used poisons in rural India. In the instance of poisoning detecting the class of Pyrethroids uses a complex and costly instruments which is not very user friendly and also expensive. In the present article Thin Layer chromatography which is classical method of separation science has been used as tool to identify the class of Pyrethroids, identification of Cypermethrin pesticide is analyzed using different solvent system for qualitative analysis with suitable spray reagent to identify the movement of the spot.

Keywords: Cypermethrin, Pyrethroid, Pesticide, Thin Layer Chromatography, Pesticide poisoning

Introduction

In India more than 126 million population rely on farming for their livelihood and these farmers own 74.4 million hectares of arable land and average land that they hold is less than 0.6 hectares each which is not sufficient enough to upkeep their livelihood. Semi medium and medium land holding farmers own between 2-10 hectares of land which are almost 13% of total farmers and own up to 43.6% of crop area.¹ This research has shown that many of the farmers are poor and hold small piece of land and therefore to increase the yield of their crop farmers use chemical and its derivatives in different forms to increase the output of their crop in fields. These chemical may be in form of Pesticides, Insecticides or Herbicides and majority of them are chemically synthesized which are often poisonous in nature to human beings and if proper guidelines are not followed as to how to use them they can be fatal. Pesticides are the chemicals which are being used regularly by farmers in India to stop the manifestation of pests on crops and Cypermethrin is one such pesticide. These pesticides at times result in occupational and accidental poisoning. When such instances of poisoning happen to farmers

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either accidental or intentional, Investigation and Judicial agency comes into action and probe has to be done as to what could be the poison consumed by the deceased and to answer that there has to be performed chemical analysis of visceral organs which are preserved by the doctor during the post mortem. To identify the type of poison which the deceased has consumed there are sophisticated instruments which are routinely used like Gas Chromatography, Mass Spectrometry and High Performance Liquid Chromatography which are actually very costly and to procure these instruments for every laboratory it is not easy. Another drawback of working with these instruments is that cost for each sample to be analyzed is very high and the technique is not very user friendly. Therefore convenient and easy to use method should be developed in order to identify the Cypermethrin pesticide for which till date only the costly and complex instruments have been used. In the present study authors have made an effort to develop simple, user friendly and convenient method to detect Cypermethrin by using Thin Layer Chromatography.

Cypermethrin is a synthetic Pyrethroid having high insecticidal activity and research for the same has been done on cucumber which has shown decreased fungal and bacterial growth\(^2\) and enough photostability to use in fields\(^3\). Cypermethrin poisoning in humans can have a devastating neurological consequence as reported by doctors.\(^4\) Study has also revealed that combination of Organophosphate and Pyrethroid is more lethal as compared to any other pesticide poisoning alone.\(^5\) Cypermethrin is not only used with suicidal intent but there has also proved to be as an accidental poisoning to the farmers in India as reported in the case study done.\(^4\) In India acute poisoning have been reported by researchers\(^6\) in farmers who are growing cotton crop. Ample of awareness program and effort is being made to educate farmers about the pest management so as to overcome the act of pesticide poisoning.\(^7\) One of the study conducted in Poland on mice has shown that there is hematological changes after Pyrethroid poisoning that is increased number of leukocytes.\(^8\) As far as treatment is considered efforts have been made to use hyosciane and chlorpheniramine maleate in the treatment of pyrethroid poisoning.\(^9\) Oral intake of Cypermethrin exhibits the neurotoxin and gastrointestinal effects in human beings.\(^10\) Studies have shown that when there is increase in the occupational exposure of pesticide with farmers, the rate of suicide attempts also increases.\(^11\) Cases have been reported where there is combination of two different type of pesticide used which has led to increased toxicity.\(^12\) According to one of the research conducted in China it has been reported that Cypermethrin, Deltamethrin and Fenvalerate all three have more number of accidental poisoning as compared to any other occupational poisoning.\(^13\)

Complex and high end techniques have been routinely used to identify the Cypermethrin in the laboratory and for the same many attempts have been done. In one of the study conducted by\(^14\) has devised a High Performance Thin Layer Chromatography system for Cypermethrin detection using HPTLC-densitometry method. In another study done by\(^15\) devised a Gas Chromatography-Electron Capture Device system for Cypermethrin detection in vegetables. Thin Layer Chromatography technique cited by\(^14\) in his research article for the detection of Pyrethroid pesticides in general was able to detect Pyrethroids like Cypermethrin, Deltamethrin and Fenavalereate using solvent systems like methyl acetate and formic acid in ratio of 80:20, ethanol and hexane in ratio of 50:50, acetone and cyclohexane in 60:40 by using solution of iodine and o-tolidine as spray reagent. All the pyrethroid pesticides reacted the same way and produced pink spots with retention factor (Rf) value of 0.63. In another attempt of Thin Layer Chromatography technique developed by\(^16\) for the detection of pyrethroid pesticides in general was able to detect Pyrethroids like Cypermethrin, Deltamethrin and Fenavalereate using solvent system of acetone, methanol and water in ratio of 30:30:30 and spray reagent 1 gm % alcoholic p-nitrobenzaldehyde followed by 10% sodium hydroxide. All the pyrethroid pesticides reacted the same way and produced pink spots with Rf value of 0.67. As per the Dept. of statistics, Government of India, among the 3 common pyrethroid pesticides, Cypermethrin seems to be the greater cause of suicidal poisoning deaths in India. In 2019 Cypermethrin has accounted for 874 deaths while Deltamethrin accounted for 162 and Fenavalereate accounted for 185 deaths.\(^17\)
All the above quoted techniques are complex and time taking a simple, convenient and user friendly method has still not been developed for analysis of the Cypermethrin which can be done in less time and cost for each samples examination is less. There is an urgent need for a simple, cost-effective system for detection of Cypermethrin both in clinical and forensic contexts. With this study an effort would be made to develop new solvent systems and spraying reagents and also to standardize the same.

**Materials and Method**

Authors have adopted Thin Layer Chromatography technique to perform the experiments; Chromatography is a technique which allows separation, identification and determination of closely related components in complex solution which are non-volatile. The method of chromatography involves two phases firstly mobile phase and secondly stationary phase. Stationary phase here was the thin layer of silica gel G which was spread on the glass plate. Mobile phase is the sample to be analyzed here in our case it is Cypermethrin. The principle of chromatography lies in the fact that mobile phase is made to run on the stationary phase and during the process components of mobile phase which has more affinity to bind with stationary phase is held on the stationary phase surface and lag near to their source whereas those components which have less ability to bind with the stationary phase will travel for longer distance thus resulting in separation of different components in the complex mixture making a unique pattern for every sample.

Silica gel G has chemical composition of Silicon dioxide and G stands for Gypsum which is binder and the primary role of this binder is to hold the silica paste over the glass plate on which it is applied and the chemical composition of gypsum is Calcium Sulfate. Preparation of Thin Layer Chromatography plate was done by dissolving Silica gel G in distilled water in the ratio of 1 : 2 to make slurry and was evenly spread on the glass plate to make sure that it spreads with uniform thickness of around 0.5mm on the plate later leaving the plate at room temperature to dry. The plate once dried is kept in the hot air oven for activation at 110 degree centigrade for 30 minutes. The sample Cypermethrin standard 10% used for testing was provided by M/s. Anu Products Limited, Ahmadabad, Gujarat, India. Sample was taken in its pure form about 0.1ml using micro-capillary tubes there was no need for any solvent because the analyte was in liquid state.

**Observation**

Authors used seven different reagents to make six solvent systems with different volumetric ratios. Solvent systems like Methanol: water 65:35, Benzene: Acetone: Ammonia 50:30:20 and Ethyl Acetate: Cyclohexane 70:30 were used. Twenty five solvent systems were taken with its unique spray reagent for each combination and was used to identify if there is any positive observation on the TLC plate? For all twenty three solvent system the observation was negative except in two solvent system which gave a positive result on the TLC plate in form of white and black spot. The first solvent system which gave positive result was Benzene, Acetone and Ammonia in the ratio of 50: 30:20 when 5% Rhodamine B in Ethanol was used as sprayed over the developed plate it gave white spot with Rf value 0.92. The second solvent system which gave positive result was Benzene, Acetone and Ammonia in the ratio of 50:30:20 when the plate was treated with spray reagent 10% Molybdic Acid in 150ml Sulfuric Acid there was black spot observed with Rf value 0.89. Rf (Retention Factor) was calculated by using the formula that Rf is the ratio of distance travelled by the solute from the origin line is to distance traveled by the solvent from the origin line. After the development of TLC plate with the respective solvent systems, TLC plates were treated with total eighteen different types of spray reagents. Different spray reagents used in the experiment were namely AgNO₃ in Ethanol, AgNO₃ in Ammonia, FeCl₃ in butanol, Rhodamine B in Ethanol, Thymol Blue in water, Coomasie brilliant Blue in water, Cotton blue in water, Amido black
in water, Stannous Chloride in HCl, Ammonium Molybdate in HNO₃, Perchloric Acid in Acetone, Bromine in CCl₄, Dragendorff’s reagent, Molybdic Acid in Sulphuric Acid, Para-dimethyl Amino Benzaldehyde and HNO₃ in Ethanol.

Figure 2: White spots visualized after spraying with Rhodamine B as spray reagent, by using Benzene: Acetone: Ammonia 50:30:20 as solvent system for detection of Cypermethrin by TLC

Figure 3: Black spots visualized after spraying with Molybdic Acid as spray reagent, by using Benzene: Acetone: Ammonia 50:30:20 as solvent system for detection of Cypermethrin by TLC.

The detailed observation all twenty five solvent systems used are shown below in tabular manner.

Table 1: Primary solvent systems and spray reagents

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Solvent System</th>
<th>Spray Reagent</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methanol: water 65:35</td>
<td>1gm AgNO₃ in 90ml Ethanol and 5ml Ammonia</td>
<td>No Spot</td>
</tr>
<tr>
<td>2</td>
<td>Ammonia: Acetone 70:30</td>
<td>1gm AgNO₃ in 90ml Ethanol and 5ml Ammonia</td>
<td>No Spot</td>
</tr>
<tr>
<td>3</td>
<td>Acetone: Acetic Acid 80:20</td>
<td>2% AgNO₃ in Ammonia</td>
<td>No Spot</td>
</tr>
<tr>
<td>4</td>
<td>Acetone: Acetic Acid 80:20</td>
<td>2% FeCl₃ in n-butanol</td>
<td>No Spot</td>
</tr>
<tr>
<td>5</td>
<td>Benzene: Acetone: Ammonia 50:30:20</td>
<td>5% Rhodamine B in Ethanol</td>
<td>White Spot Rf=0.92</td>
</tr>
<tr>
<td>6</td>
<td>Ethyl Acetate: Cyclohexane 70:30</td>
<td>10% Thymol blue in water</td>
<td>No Spot</td>
</tr>
<tr>
<td>7</td>
<td>Ethyl Acetate: Cyclohexane 70:30</td>
<td>10% Coomasie brilliant blue in water</td>
<td>No Spot</td>
</tr>
<tr>
<td>8</td>
<td>Cyclohexane: CCl₄ 60:40</td>
<td>10% Thymol blue in water</td>
<td>No Spot</td>
</tr>
<tr>
<td>9</td>
<td>Cyclohexane: CCl₄ 60:40</td>
<td>10% Coomasie brilliant blue in water</td>
<td>No Spot</td>
</tr>
<tr>
<td>10</td>
<td>Ethyl Acetate: Cyclohexane 70:30</td>
<td>0.5% Cotton Blue in water</td>
<td>No Spot</td>
</tr>
<tr>
<td>11</td>
<td>Ethyl Acetate: Cyclohexane 70:30</td>
<td>0.5% Amido Black in water</td>
<td>No Spot</td>
</tr>
<tr>
<td>12</td>
<td>Benzene: Acetone: Ammonia 50:30:20</td>
<td>0.5% SnCl₂ in HCl</td>
<td>No Spot</td>
</tr>
<tr>
<td>13</td>
<td>Benzene: Acetone: Ammonia 50:30:20</td>
<td>5% Ammonium Molybdate in 35 ml HNO₃ and 65 ml Water</td>
<td>No Spot</td>
</tr>
<tr>
<td>Solvent System</td>
<td>Spraying Reagent</td>
<td>Result</td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>------------------</td>
<td>--------</td>
<td></td>
</tr>
<tr>
<td>Benzene: Acetone: Ammonia 50:30:20</td>
<td>4% Perchloric Acid in Acetone</td>
<td>No Spot</td>
<td></td>
</tr>
<tr>
<td>Benzene: Acetone: Ammonia 50:30:20</td>
<td>SnCl2 followed by Ammonium Molybdate followed by Perchloric Acid reagents</td>
<td>No Spot</td>
<td></td>
</tr>
<tr>
<td>Ethyl Acetate: Cyclohexane 70:30</td>
<td>0.5% SnCl2 in HCl</td>
<td>No Spot</td>
<td></td>
</tr>
<tr>
<td>Ethyl Acetate: Cyclohexane 70:30</td>
<td>5% Ammonium Molybdate in 35 ml HNO3 and 65 ml Water</td>
<td>No Spot</td>
<td></td>
</tr>
<tr>
<td>Ethyl Acetate: Cyclohexane 70:30</td>
<td>4% Perchloric Acid in Acetone</td>
<td>No Spot</td>
<td></td>
</tr>
<tr>
<td>Ethyl Acetate: Cyclohexane 70:30</td>
<td>SnCl2 followed by Ammonium Molybdate followed by Perchloric Acid reagents</td>
<td>No Spot</td>
<td></td>
</tr>
<tr>
<td>Benzene: Acetone: Ammonia 50:30:20</td>
<td>Bromine: CCl4 1:8</td>
<td>No Spot</td>
<td></td>
</tr>
<tr>
<td>Benzene: Acetone: Ammonia 50:30:20</td>
<td>Dragendorff’s reagent</td>
<td>No Spot</td>
<td></td>
</tr>
<tr>
<td>Benzene: Acetone: Ammonia 50:30:20</td>
<td>10gm Molybdic Acid in 150 ml H2SO4</td>
<td>Black Spot Rf=0.89</td>
<td></td>
</tr>
<tr>
<td>Benzene: Acetone: Ammonia 50:30:20</td>
<td>100mg AgNO3 in 10ml water, 10ml Phenol, 200ml Acetone and 2 drops H2O2</td>
<td>No Spot</td>
<td></td>
</tr>
<tr>
<td>Benzene: Acetone: Ammonia 50:30:20</td>
<td>1% Para-Dimethyl Amino Benzaldehyde</td>
<td>No Spot</td>
<td></td>
</tr>
</tbody>
</table>

**Results**

It was found that with two spraying reagents namely Rhodamine B in Ethanol and Molybdic Acid in Sulphuric Acid, the visualization of separated Thin Layer Chromatography plates were made possible. Spray reagents Rhodamine B in Ethanol and Molybdic Acid in sulphuric acid were replicated for experimentation thirty times each with the same solvent system to check the reproducibility of the test. The response of the separation of Cypermethrin with the same solvent system was checked and analysed. Following, the above set of results, the two different successful Thin Layer Chromatography systems were separately performed thirty times each to examine their reproducibility.

The Figure 4 below shows the reproducibility of the experiment while using solvent system Benzene, Acetone, Ammonia and Rhodamine B in Ethanol.

![Figure 4: Graphical representation of Rf value when using solvent system Benzene, Acetone, Ammonia and Rhodamine B in Ethanol](image)

The Figure number 5 below shows the reproducibility of the experiment while using solvent system Benzene, Acetone and Ammonia in ratio of 50:30:20 and using different spray reagent 10gm Molybdic Acid in 150ml H2SO4.
In the end of the analysis it was found that 5% Rhodamine B in Ethanol was giving very promising results as visualized by white spots representing the separated Cypermethrin in the solvent system Benzene, Acetone and Ammonia in ratio of 50:30:20 as shown in Figure 2. Similarly, the spray reagent 10g Molybdic Acid in 150ml Sulphuric acid was also giving a very prominent result as visualized by black spots representing the already separated Cypermethrin with the solvent system Benzene, Acetone and Ammonia in ratio of 50:30:20 as shown in figure 3. The mean value of Rf values obtained with Rhodamine B was found to be 0.92 and for Molybdic Acid 0.90.

Table 2: Statistical Analysis of Data

<table>
<thead>
<tr>
<th>Spray Reagent</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Standard Error</th>
<th>t Value</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhodamine B in Ethanol</td>
<td>0.92</td>
<td>0.025</td>
<td>0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molybdic Acid in Sulphuric Acid</td>
<td>0.90</td>
<td>0.017</td>
<td>0.003</td>
<td>3.45</td>
<td>&gt; 0.0005</td>
</tr>
</tbody>
</table>

Standard deviation is a measure of the amount of variation or dispersion of set of values. Standard deviation is calculated by using the formula:

$$\sigma = \sqrt{\frac{\sum (x_i - \mu)^2}{N}}$$

$\sigma$ = retention factor standard deviation

$N$ = the size of the retention factor

$X_i$ = each value of the retention factor

$\mu$ = the retention factor mean

Standard Error is defined as the measure of the statistical accuracy of an estimate, equal to the standard deviation of the theoretical distribution of a large sample of such estimates.

Standard Error was calculated using formula:

$$SE = \frac{\sigma}{\sqrt{n}}$$
SE = standard error of the retention factor  
σ = retention factor standard deviation  
n = number of retention factor  
T-value measures the size of the difference relative to the variation in our sample data.  
T-value is calculated using formula:

\[
\frac{\text{mean of 30 solvent system} - \text{mean of 60 solvent system}}{\text{Standard Error}}
\]

P-value is the probability of obtaining results at least as extreme as the observed results of a hypothesis test, assuming that the null hypothesis is correct.

**Conclusion**

By and large the Rf values for both Rhodamine B and Molybdic Acid trials range from 0.88-0.96 and 0.87-0.93 respectively. The difference in the mean Rf values of the Rhodamine B and Molybdic Acid trials range from 0.90 - 0.92 As expected the difference between Rf’s in Rhodamine B and Molybdic Acid trials were satisfactorily significant. Having found that there is difference in Rf value and also the difference varied from 0.88-0.96 and 0.87-0.93, an attempt was made to see whether the Rf values of the Rhodamine B trial and the Molybdic Acid trial are varying and if so whether the obtained results were showing the Mean, Standard Deviation and if so whether the obtained results were showing significance in intra-comparison. After studying the Mean & Standard Deviation, the t-value was calculated and found to be 3.45 and the statistical significance through probability was found to be greater than 0.0005.

Therefore a Thin Layer Chromatography system using solvent system of Benzene, Acetone and Ammonia in ratio of 50:30:20 and spray reagents of either 5% Rhodamine B in Ethanol or 10gm Molybdic Acid in 150 ml H₂SO₄ is a reliable Thin Layer Chromatography system for the detection of cypermethrin pesticide. The technique is robust, reliable and easy to operate as compared to its counterparts which are difficult to operate and houses complex mechanism. Advantage of using Thin Layer Chromatography with the solvent system and spray reagent developed by author has wide scope which reduces the cost of per sample to be tested and even small laboratories can easy afford it as the techniques and requirement are not very expensive. More study is required to identify its applicability in detecting Cypermethrin in viscera samples in Forensic Toxicology.

**Acknowledgement:** Not Applicable

**Conflict of Interest:** Authors declare that there is no conflicts of interest between them

**Ethical Clearance:** Not Applicable

**Source of Funding:** Not Applicable

**References**

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COVID-19 Vaccine Awareness among Dental Students: A Cross Sectional Study

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Abstract

Background: Dental professionals are frequently exposed to environments with high levels of occupational hazards by performing duties in close contact with the oral cavity prone for COVID-19 disease transmission. Further, as healthcare providers, dentists are assigned with providing health care support and encouraging their patients, and community in various activities to prevent the spread of diseases including vaccinations especially during this COVID19 pandemic.

Aim: The present study was aimed to assess the awareness of an individual towards COVID19 vaccination.

Methodology: A cross sectional online-survey was conducted using self-administered questionnaires, through Google forms among dental students of Thai Moogambigai dental college and Hospital, Chennai, Tamilnadu, India.

Results: Majority of the dental students were aware of the COVID19 Vaccines currently available. Among which 78.85% considered COVID-19 vaccines should be made mandatory for all the health care professionals. 75% felt vaccination is not recommended immediately following covid-19 infection or recovered recently. About half of the participants were unaware of vaccination protocols for patients with diabetes, immuno-compromised disease or any bleeding disorder.

Conclusion: Most of the dental students were aware of vaccines available for the COVID 19 infection however show very low vaccination rate. Thus immediate implementation of epidemiology education associated with infectious diseases and vaccination developments should be incorporated by organizing awareness programs.

Keywords: Awareness, Children, COVID-19 Vaccines, mRNA Vaccine, Pregnancy, Systemic disease

Introduction

COVID-19 disease caused by Severe Acute Respiratory Syndrome-CoV2 (SARS-CoV2) corona virus is a global pandemic that created a panic situation across the world. Vaccine hesitancy associated with low awareness has been recognized as a serious
public health threat that requires comprehensive investigation among various population groups particularly among the health care professionals to fully understand its prevalence and associated factors. Several evidence-based investigations suggested that among various health care professionals, dentists are frequently exposed to environments with high levels of occupational hazards by performing clinical cases in close contact with oro-pharyngeal region accompanied with aerosol and droplets generating procedures conferring additional risks of viral exposure and disease transmission.

Dental students represent a particular subcategory of the healthcare professional. Dental student's population play an important role in influencing their patients' and peer groups on health-related awareness attitudes and behaviors to improve the quality of life. Research on dental professionals and dental students' acceptance of vaccinations has indicated partial agreement with recommended vaccinations across several countries owing to several factors such as lack of awareness, fear of complications associated with vaccine, hesitation on new vaccines due to lack of safety data, unsure vaccination protocols for systemically compromised patients and its efficacy on new variant strains. Thus, it is important to ensure early vaccination and achieve high COVID-19 vaccination rates among this group.

Further, as healthcare providers, they are assigned with providing health care support and encouraging their patients, community, and profession, in various activities to prevent the spread of diseases including vaccinations. Thus the present study was aimed to assess the awareness of an individual towards COVID19 vaccinations amongst dental students.

Discussion

Materials and Methods

The present cross-sectional questionnaire survey was conducted among dental students of Thai Moogambigai Dental College and Hospital, Chennai, Tamilnadu, India, to assess the awareness of an individual towards COVID19 vaccinations. All the required information was collected through published scientific articles pertaining to the study and self-administered structured questionnaires, comprising of 20 questions in English language was prepared and evaluated. The questionnaire had both combination of few selected response to the certain questions and also close ended questions (Yes / No) as shown in Table 1. Since this study was conducted during COVID-19 pandemic lockdown period, online Google forms were generated and distributed through social media platforms.

The study included dental students of Thai Moogambigai Dental College and Hospital, age greater than or equal to 18 years, individuals active in social media platforms such as Facebook, Whatsapp, LinkedIn and those who can read and understand English with voluntary interest to participate in the survey. Dental professionals, practitioners, dental students of other colleges/universities were excluded from the study. The protocol was submitted to the ethical committee before the start of the study and the approval was obtained from the Institutional Review Board. All the participants were informed about the purpose of the study and assured that their participation was purely voluntary.

Non-probability random sampling technique was employed that yielded information from 156 dental students were taken into this observational study having a cross-sectional design. Responses recorded among the selected population group were evaluated for statistical analysis by SPSS software Version 19.0. On statistical evaluation it was observed all 156 samples were valid for the study with Cronbach's alpha reliability score being 0.898 (significant score).

Results

The data was analysed using Statistical Package for Social Science (SPSS) software. Descriptive analysis with frequency, percentage, mean and standard deviation was computed. Pearson Chi Square Test and Fisher’s Exact Test was used to assess the level of significance at p<0.05. In the present study, the responses were recorded and the statistical data obtained is represented in table 1. The results obtained were statistically significant as shown by Chi Square Test which is represented in Table-1. It was observed that all the participants (100%) were aware of COVID-19 vaccines though only 25%(39)
have vaccinated during the time of the study. 78.85%(123) considered COVID-19 vaccines should be made compulsory for all the health care professionals including dentists. It was observed that 85.90%(134) felt vaccinations should be taken by COVID recovered person apart from non-infected group however 75%(117) considered vaccination is not necessary immediately for a person who recently had covid-19 infection and treated with antibiotic based therapy. 85.90%(90) participants think that COVID19 vaccine contains antibiotics while 42.31%(66) responded in contrast that COVID Vaccines does not contain antibiotic, thus showing statistically insignificant value(0.05466) as shown in table-1. About 16.03%(25) participants revealed that COVID19 vaccine can alter the DNA of a person while 83.97%(131) disagreed with role of mRNA vaccine in modifying the DNA of a person. (as represented in graph -1)

Graph 1: Awareness on COVID 19 vaccine among various conditions

About 52.56%(82) of the participants were unaware of vaccination protocols for patients with bleeding disorder. 21.15%(33) agrees that vaccines will be effective against the new variant while 14.74%(23) disagrees with the same context and 64.10%(100) are not sure about the resistance of the new variant to covid 19 vaccination. (as shown in graph- 2)

Graph 2: Response among dental students on covid 19 vaccination in bleeding disorder patients/resistant against new variant

55.77%(87) recommended avoidance of COVID19 vaccines in pregnancy, thus showing statistically insignificant value of 0.14954 as shown in table-1. 80.13%(125) suggested that children...
below 10 years of age should not get vaccinated thus considered as an absolute contraindication among these groups. About 60.90% (95) responded that immuno-compromised patients can get vaccinated. 23.72% (37) participants think that COVID19 vaccine should be given for person currently having covid-19 (confirmed or suspected) while 76.28% (119) disagreed with COVID19 vaccination for confirmed/suspected cases. (as depicted in graph-3).

Graph 3: Awareness on COVID 19 Vaccine in various medical conditions

Majority of the respondents (80) strongly believe in the complications (blood clots) associated with these vaccines, revealing statistically insignificant value (0.74877) as shown in table 1. 41.03% (64) were unsure about the activities of these vaccines invented from one country will be effective for all countries. 80.77% (126) considering blood sugar control is mandatory before vaccination in diabetic patients. 89.10% (139) participants think COVID test maybe positive even after vaccination. 91.67% (143) participants were aware of black fungus among COVID recovered patients while only 8.33% (13) were unaware of black fungus attack among COVID recovered patients. (as shown in the graph 4).

Graph 4: Awareness on effectiveness and complications of COVID 19 vaccine

55.77% received information about COVID-19 vaccine through news (media) followed by google / internet (31.41%) and articles (12.18%). (as shown in Figure 1)
Around 67 out of 156 (42.95%) prefers 1 month waiting period for COVID vaccination after undergoing non-COVID vaccines and 46 participants (29.49%) opted for 2 weeks time interval, whereas 34 participants (21.79%) selected 3 weeks duration for COVID Vaccination after taking non covid vaccine. The least being 9 participants (5.77%) preferred 1 week time interval respectively. (as shown in the Figure 2)

Table 1: Table showing the Questionnaire Responses

<table>
<thead>
<tr>
<th>Questions</th>
<th>Responses</th>
<th>N</th>
<th>%</th>
<th>Chi²</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Are you aware of covid-19 vaccine?</td>
<td>yes</td>
<td>156</td>
<td>100</td>
<td>78</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Have you undergone vaccination for COVID19?</td>
<td>yes</td>
<td>39</td>
<td>25</td>
<td>39</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>117</td>
<td>75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you think covid-19 vaccines should be made compulsory?</td>
<td>yes</td>
<td>123</td>
<td>78.85</td>
<td>51.92</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>33</td>
<td>21.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Questions</td>
<td>Responses</td>
<td>N</td>
<td>%</td>
<td>Chi²</td>
<td>p value</td>
</tr>
<tr>
<td>--------------------------------------------------------------------------</td>
<td>--------------</td>
<td>-----</td>
<td>-----</td>
<td>--------</td>
<td>-----------</td>
</tr>
<tr>
<td>What is the most common information source that you refer to gather information about covid-19 vaccine?</td>
<td>News</td>
<td>87</td>
<td>55.77</td>
<td>108.92</td>
<td>&lt; .00001*</td>
</tr>
<tr>
<td></td>
<td>Articles</td>
<td>19</td>
<td>12.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neighborhood</td>
<td>1</td>
<td>0.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Google /Internet</td>
<td>49</td>
<td>31.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Is it necessary for a COVID recovered person to take the vaccination?</td>
<td>yes</td>
<td>134</td>
<td>85.90</td>
<td>80.41</td>
<td>&lt; .00001*</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>22</td>
<td>14.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Can a person who recently had covid-19 infection and treated with antibiotic based therapy get vaccinated immediately?</td>
<td>yes</td>
<td>39</td>
<td>25</td>
<td>39</td>
<td>&lt; .00001*</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>117</td>
<td>75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do COVID-19 vaccines contain antibiotics?</td>
<td>yes</td>
<td>90</td>
<td>57.69</td>
<td>3.69</td>
<td>0.05466</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>66</td>
<td>42.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Can a person with bleeding disorder get vaccinated?</td>
<td>Yes</td>
<td>23</td>
<td>14.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>51</td>
<td>32.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maybe</td>
<td>82</td>
<td>52.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Can the mRNA vaccine change the DNA of a person?</td>
<td>yes</td>
<td>25</td>
<td>16.03</td>
<td>72.03</td>
<td>&lt; .00001*</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>131</td>
<td>83.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whether pregnant women can get covid-19 vaccination?</td>
<td>yes</td>
<td>69</td>
<td>44.23</td>
<td>2.077</td>
<td>0.14954</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>87</td>
<td>55.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Can children below 10 years get vaccinated?</td>
<td>yes</td>
<td>31</td>
<td>19.87</td>
<td>56.64</td>
<td>&lt; .001*</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>125</td>
<td>80.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you think immuno-compromised patients can get vaccinated?</td>
<td>yes</td>
<td>95</td>
<td>60.90</td>
<td>7.41</td>
<td>0.00649</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>61</td>
<td>39.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Can a person currently having covid-19 (confirmed or suspected) be vaccinated?</td>
<td>yes</td>
<td>37</td>
<td>23.72</td>
<td>43.10</td>
<td>&lt; .0001*</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>119</td>
<td>76.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>People who got other non-COVID-19 vaccine should wait for _____ days for taking covid-19 vaccine?</td>
<td>1 Week</td>
<td>9</td>
<td>5.77</td>
<td>45.07</td>
<td>&lt; .0001*</td>
</tr>
<tr>
<td></td>
<td>2 Week</td>
<td>46</td>
<td>29.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 week</td>
<td>34</td>
<td>21.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 Month</td>
<td>67</td>
<td>42.95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Will the new variants be resistant to current covid-19 vaccines?</td>
<td>Yes</td>
<td>33</td>
<td>21.15</td>
<td>67.43</td>
<td>&lt; .0001*</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>23</td>
<td>14.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maybe</td>
<td>100</td>
<td>64.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Can you test positive even after vaccination?</td>
<td>Yes</td>
<td>139</td>
<td>89.10</td>
<td>95.41</td>
<td>&lt; .0001*</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>17</td>
<td>10.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whether covid-19 vaccine causes blood clots in patient’s body after vaccination?</td>
<td>Yes</td>
<td>80</td>
<td>51.28</td>
<td>0.10</td>
<td>0.74877</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>76</td>
<td>48.72</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Discussion**

Dental students as a part of health care workers are susceptible to an increased risk of contacting infectious diseases as part of their clinical training program. Development of a vaccine appears to be the most encouraging signs of restoring normalcy and initiating economic reintegration among general public⁸. Establishing public health policy for sustainable health promotion and adopting vaccination strategies to overcome the COVID-19 pandemic by health care workers is essential to increases the patient’s behavioral changes on reducing the mortality and improve the quality of life⁹.

In the present study it was observed that all the participants (100%) were aware of COVID-19 vaccines similar to studies by Riad, Zigron et al²,⁷ among which 78.85% (123) considered COVID-19 vaccines should be made compulsory for all the health care professionals including dentists even though only 25% (39) have vaccinated during the time of the study similar to studies by Alwazzan and Rieshy et al⁸,⁹ slightly higher than results obtained by Cornwall et al¹⁰, Neumann-Böhme S et. al.¹¹. This higher percentage when compared to the predicted acceptance rate of 75% among the health care population in several studies could be due to the influence of awareness programs, local health authority control on disease progression, government policies, proper reporting of number of severely ill patients, and mortality rate however lower vaccination rate could be due to past vaccine history, fear of vaccination protocols, safety and efficacy concerns, preference for physiological immunity, distrust in government and health organizations and personal attitudes toward the COVID-19 vaccines¹²,¹³.

55.77% received information about COVID-19 vaccine through news (media) followed by google/internet (31.41%) and articles (12.18%) similar to studies by Garrett, Ageyukum, Nzaji et al¹⁴,¹⁵,¹⁶. The low percentage can be attributed to conspiracy theories and media misinformation that created doubts about the nature, course of disease, prevention, mortality rate, and vaccine safety, and can promote mistrust of the government, policymakers, health authorities, and pharmaceutical companies¹⁷. Presenting accurate information on social media with evidence based research and reliable sources will help to counter this lower rate.

It was observed that 85.90% felt vaccinations should be taken by COVID recovered person apart from non-infected group however 75% considered vaccination is not necessary immediately for a person who recently had covid-19 infection and treated with antibiotic based therapy or for a person currently having covid-19 symptoms (76.28%) either suspected or confirmed. However recent guidelines by Ministry of Health and Family Welfare (MOHFW), Government of India suggested that it is advisable to receive complete schedule of COVID vaccine irrespective of past history of infection with COVID-19 to aid in developing a strong immune response against the disease¹⁸.

About 52.56% of the participants were unaware of vaccination protocols for patients with bleeding disorder and 60.90% responded immuno-compromised patients can get vaccinated followed by
80.77% considering blood sugar control is mandatory before vaccination in diabetic patients. However 55.77% and 80.13% recommended avoiding COVID19 vaccines in pregnancy and in children respectively. Felten, Boekel, Elimat et al 19,20,21 in 2021 suggested that vaccination was significantly associated with the fear, but not with the presence of additional comorbidities or with the immuno-compromised status. However it is believed that COVID-19 vaccines are well tolerated by patients with autoimmune diseases. Pal et. al. observed very mild adverse effects following vaccination and recommended vaccination should be prioritized in patients with type 1 and type 2 diabetes mellitus as they are at a higher risk of poor prognosis with COVID-19 22.

Only 42.31% were aware of components in Vaccine and 64.10% are uncertain about the current covid-19 vaccines resistant to new variants. 89.10% were aware of the false negative results and test positive even after vaccination and 41.03% were unsure about the activities of these vaccines invented from one country be effective for all countries. As several vaccines are launched due to the public health emergency, based on data from clinical trials most of the health care workers are unsure about their efficacy23. Confidence among health care professionals can be intensified through awareness programs, panel discussion, eliciting their concerns, and involving them in vaccine endorsements. Scientific approach along with Self-identity has been associated with beliefs regarding COVID-19 vaccination24. Thus exploring and understanding barriers along with implementation of the infectious diseases epidemiological education and vaccination developments for avoiding the strengthening of vaccine-averse identities within undergraduate dental curriculum for better training of dental students for future outbreaks is necessary.

Conclusion
Most of the dental students were aware of vaccines available for the COVID 19 infection however show very low vaccination rate due to several barriers such as lack of awareness, fear and insufficient data on efficacy and unsure vaccination protocols for patients with systemic diseases. Thus immediate implementation of epidemiology education associated with infectious diseases and vaccination developments should be incorporated by organizing awareness programs along with integrating in the undergraduate dental curriculum.

Conceptualization & Data Curation- Ashika, Elakiya,Malleeswari
Writing Original Article-Dr.K.Sheela & Ashika
Review & Editing – Dr.Ponsekar Abraham, Dr. K. Sheela

Conflict Of Interest: The author has no conflict of interest for this study

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Conflict of Interest: Nil

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Cross Sectional Study on Estimation of Stature from foot Length in Telangana

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Abstract

Height is an important factor for identification. Estimation of height from mutilated bodies has significance in medicolegal investigations. Foot length is equally significant for estimating the stature like other body parts by using linear regression equation. A cross sectional study on correlation of foot length in relation to the height of an individual was conducted in the age group of 18-25 years in Telangana population during the period from 1st January 2019 to 31st December 2019. A total of 150 subjects, among which 75 male and 75 female were included in the study. Measurement of foot length has been taken as length between the heel to the tip of longest toe, measured by using Vernier caliper. All the study subjects were healthy individuals without any abnormalities. The data was statistically analyzed by using SPSS (version 25) software.

The linear regression formula for estimating the height is $y = a + (bx)$, $y =$ dependent variable (height), $a =$ constant, $b =$ independent variable coefficient, $x =$ independent variable i.e length of foot. For total population the right foot formula is $y = 39.25 + (5.18 \times x)$, for left foot the formula is $y = 37.08 +(5.24 \times x)$. The formula for male population for right foot is $y = 64.38 +(4.22 \times x)$ and for left foot is $y = 62.23 + (4.30 \times x)$. The formula for female population, right foot is $y = 52.47 + (4.54x)$ and left foot is $y = 48.18 + (7.95x)$. The separate formula for male and female individual gives more accurate results.

Calculated stature is almost close to the height of the individual; ±5 cm difference was observed in most of the individuals. The stature as per our study is approximately 7 times more than the length of the foot.

Key words: Stature, foot length, linear regression.

Introduction

Human height, weight, foot length are variables that differ among individual to individual, and among various ethnicities and races. Anthropometry is an age old study of systemized measuring techniques that express the dimensions of human body. The study of anthropometry has proven an increased significance in forensic medicine. Relation between different parts of the body with height has been of great importance,

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due to an increase in the number of catastrophic events leading to mass disasters. Identification of human beings for legal or humanitarian reasons are performed by various methods in which height constitutes a vital element particularly in those involving mutilated, decomposed or amputated bodies due to catastrophic events like bomb blast, earthquakes, terror attacks, mass accidents etc.

Vitruvius\(^1\), 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 and 4 cubits make a man’s height. He used these measurements and concluded that man’s outspread arms are equal to his height.

Height is an important factor in identification of the biological profile of an individual. Ruthishauser\(^2\), for the first time showed that the reliability of prediction of height from foot length was as high as that from long bones, as ossification and maturation in foot occurs earlier than the long bones. The foot size along with the other anatomical structures of the body like the head, trunk and lengths of upper and lower limbs is assumed to influence a definitive biological correlation with stature.

Various authors, T.A. Philip 1990\(^3\); H. Ozden 2005\(^4\); ozasalan et. al., 2003\(^5\) turks residing in Istanbul; sanli et. al., 2005\(^6\); grivas et. al., 2008\(^7\) have made use of regression equation, has been found more reliable in estimating stature from foot dimensions.

Several studies conducted in India, O.P. Jasuja, 1987\(^8\); V K Sharma 1978\(^9\); agnihotri et. al. 2007\(^10\); krishan and Sharma 2007\(^11\); estimated foot length is a reliable indicator of stature.

The aim of the present study is to correlate the height of the individual from foot length in the specified population of Telangana. This study would also assist in development of a linear regression equation for the estimation of stature from foot length.

**Materials and Methods:**

A Cross sectional study on correlation of length of the foot in relation to the height of an individual was conducted in Medical students of Telangana region in the age group of 18 to 25 years, during the period of January 2019 to December 2019. A total of 150 individuals, 75 male and 75 female were taken as participants in this study. Foot length of right and left foot 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, foot length was taken as distance between the heel and the longest toe centered by the line and measured by using vernier calipers.

**Figure 1:** Measurement of foot length using vernier caliper.

**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 foot length 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 foot).
Results

A cross-sectional study on estimation of stature from foot length was conducted in Telangana region during the period from 1st January 2019 to 31st December 2019, the following results were found.

### Table 1: Anthropometric measurement data for total population:

<table>
<thead>
<tr>
<th>Anthropometric measurements(mean ± SD)</th>
<th>Men</th>
<th>Female</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ht(mean ± SD)</td>
<td>169.74 ± 7.14</td>
<td>156.80 ± 6.80</td>
<td>163.40 ± 9.51</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight (mean ± SD)</td>
<td>57.05 ± 10.49</td>
<td>50.42 ± 11.45</td>
<td>53.80 ± 11.43</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rt foot length (mean ± SD)</td>
<td>24.93 ± 1.31</td>
<td>22.94 ± 1.25</td>
<td>23.96 ± 1.62</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lt foot length (mean ± SD)</td>
<td>24.95 ± 1.31</td>
<td>22.92 ± 1.22</td>
<td>23.95 ± 1.62</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The mean height of total population in our study group is 163.40 ± 9.51. Length of right foot is 23.96 ± 1.62 and left foot length is 23.95 ± 1.62. For male population the right foot length is 24.93 ± 1.31 and the left foot length is 24.95 ± 1.31. For female population the right foot length is 22.94 ± 1.25 whereas the left foot length is 22.92 ± 1.22. Foot length is approximately 2 cm less in female population compared to male population, the p value less than 0.001 shows significance of the data.

### Table 2: Prediction for total study population:

<table>
<thead>
<tr>
<th>Independent Variables (Length)</th>
<th>Formula ((y = a + bx))</th>
<th>R square</th>
<th>P value</th>
<th>Standard Error</th>
<th>95% Confidence Interval (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right Foot (cm)</td>
<td>(y = 39.25 + 5.18 x)</td>
<td>0.78</td>
<td>&lt;0.001</td>
<td>0.22</td>
<td>4.73 – 5.62</td>
</tr>
<tr>
<td>Left Foot (cm)</td>
<td>(y = 37.08 + 5.24 x)</td>
<td>0.79</td>
<td>&lt;0.001</td>
<td>0.21</td>
<td>4.81 – 5.67</td>
</tr>
</tbody>
</table>

Formula for linear regression: \(y = a + (b x)\) where \(y = \text{Dependent variable (height)}; a = \text{constant}; b = \text{Independent variable coefficient}; x = \text{Independent variable (length of the foot)}\)

P value <0.01 is significant. R Square 0.78 for right foot and 0.79 for left foot, standard error 0.22 shows the data is more significant. 95% confidence interval within the range of 4.73-5.62 for right foot and 4.81 - 5.67 for left foot revealed that the results are almost accurate.

### Table 3: Prediction for Male study population.

<table>
<thead>
<tr>
<th>Independent Variables (Length)</th>
<th>Formula ((y = a + bx))</th>
<th>R square</th>
<th>P value</th>
<th>Standard Error</th>
<th>95% Confidence Interval (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right Foot (cm)</td>
<td>(y = 64.38 + (4.22 x))</td>
<td>0.60</td>
<td>&lt;0.001</td>
<td>0.40</td>
<td>3.42 – 5.02</td>
</tr>
<tr>
<td>Left Foot (cm)</td>
<td>(y = 62.23 +(4.30 x))</td>
<td>0.62</td>
<td>&lt;0.001</td>
<td>0.38</td>
<td>3.53 – 5.08</td>
</tr>
</tbody>
</table>

### Table 4: Prediction for Female study population

<table>
<thead>
<tr>
<th>Independent Variables (Length)</th>
<th>Formula ((y = a + bx))</th>
<th>R square</th>
<th>P value</th>
<th>Standard Error</th>
<th>95% Confidence Interval (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right Foot(cm)</td>
<td>(y = 52.47 + (4.54x))</td>
<td>0.70</td>
<td>&lt;0.001</td>
<td>0.35</td>
<td>3.84 – 5.24</td>
</tr>
<tr>
<td>Left Foot (cm)</td>
<td>(y = 48.18 + (4.73x))</td>
<td>0.72</td>
<td>&lt;0.001</td>
<td>0.34</td>
<td>4.04 – 5.42</td>
</tr>
</tbody>
</table>

### Discussion

A cross-sectional prospective study on estimation of stature from foot length conducted in Telangana region in the year 2019, in the age group of 18 to 25 years. Results are revealed that there is a significant correlation between foot length and stature. The linear regression formula developed from the above data collected in the region of Telangana is more appropriate in estimating the height of the individual.
of this area.

The linear regression formula for entire study population \( y = a + (bx) \). For right foot the formula is \( y = 39.25 + 5.18 \times \) and for left foot the formula is \( y = 37.08 + 5.24 \times \). The formula for male population for right foot is \( y = 64.38 + 4.22 \times \) and for left foot is \( y = 62.23 + 4.30 \times \). The formula for female population for right foot is \( y = 52.47 + 4.54x \) and left foot is \( y = 48.18 + 7.95x \). The separate formula for male and female individual gives more accurate results because the mean height difference for male and female population is about 13 cm and the mean foot length difference of 2 cm was observed in study population. The stature as per our formula is approximately 7 times more than the length of the foot.

Study conducted by Potturi BR\(^2\) in Telangana population in the year 2013 also found similar results.

Study conducted by Nath\(^3\) et. al., in 1999 has derived a multiplication factor for estimation of stature from foot length in Srinagar and Garhwal, UP with reasonable accuracy. These were 6.87 for males and 6.73 for females. These results are almost similar to our study.

A study conducted in north Indian population by Jain\(^4\) et. al., they formulated multiplication factor as 6.59 for reconstructing of stature in the age group of 17-20 years shows different results.

**Conclusion**

The present study has established definite correlation between stature and foot length. Linear regression formula derived from the data gave accurate results for estimating stature. A separate formula for male and female individuals provides more accurate results. Statures calculated with these equations are close to the normal height. ±5 cm difference was observed in the study group. The height of the individual is approximately 7 times the foot length. Several previous studies conducted in various parts of India and our present study establishes a correlation with foot length and height of the individual.

**Conflict of interest:** Nil

**Ethical clearance:** Yes

**Source of funding:** Self

**References**

A Cross Sectional Study of Pattern of Mechanical Trauma Cases at Tertiary Care Institute of Central Gujarat

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Abstract

Introduction: As per current changing trends in India, non communicable diseases and traumatic injuries cases are uprising in compare to infectious diseases. Mechanical trauma cases are increasing worldwide. Among trauma cases, road traffic accident injuries are one of major causes to produce disability & mortality in younger population. Objectives: This study was conducted with aim to study the pattern of mechanical trauma cases at tertiary care institute of central Gujarat, India and to identify the factors related to the trauma.

Materials and Methods: A cross sectional study conducted among mechanical trauma patients visiting at Dhiraj Hospital, SBKSMIRC, SVDU, Vadodara between September to December 2021. Total 50 patients were studied as per inclusion and exclusion criteria. The data was collected in pre-tested semi-structured proforma which included pattern of mechanical trauma cases and the factors related with. The data was compiled and statistically analyzed.

Observations and Results: Out of total 1084 indoor patients at Dhiraj Hospital during the study period, 50 cases were of having various mechanical trauma. Among them, 34 cases were of Road Traffic Accident injuries, 9 cases were of fall from height, 4 cases were of Thermal injuries, 3 cases were of other mechanical trauma. Lower limbs were most commonly involved(42%) body part followed by upper limb (32%). Most common factor for causing the road accident (38%) was wrong side driving or overspeeding. Maximum accident cases were noted in evening times(between 4pm to 8pm). 54% cases had minor injuries while 32 cases had major bodily injuries.

Conclusion: Burden of mechanical trauma cases was 5% of all indoor cases of Surgery and allied departments. Maximum cases (88%) were of hard and blunt type of mechanical injuries and we have not noted anychemical and radiation injuries.In maximum cases, the mode of injury was RTA.Main factor causing road traffic accident was over speeding and wrong side driving. Most of the causative factors for the incident are rectifiable by use of vehicle safety measures and awareness of road traffic regulations among community.

Key words: Mechanical trauma, Injury, Road traffic accident.

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social changes have altered the health scenario. In a developing country like India, infectious diseases were the leading cause of mortality in the past decades, but at present, non communicable diseases and traumatic injuries are on the rise. Globally about 5.8 million people die each year as a result of traumatic injuries\textsuperscript{1}. Mechanical injuries are injuries produced by mechanical force—blunt, sharp or firearms. The severity and extent of mechanical injuries depend on the amount of force delivered to the body— if the weapon deforms or breaks on impact; some energy is spent in deforming or breaking. Lesser energy is thus delivered to the body, and injury is less severe. Legal definition of injury— Any harm, whatever illegally, caused to any person in body, mind, reputation or property\textsuperscript{2}. A road traffic accident (RTA) can be defined as “an event that occurs on a way or street open to public traffic; resulting in one or more persons being injured or killed where at least one moving vehicle is involved”\textsuperscript{3}. Worldwide, everyday about 3400 people die due to road traffic accidents (RTA) and predicted to result in death of around 1.9 Million people annually by 2020\textsuperscript{4}. Among trauma road Traffic accidents are one of the major causes of disability and mortality in the younger population and are projected to be the Fifth leading contribution to global burden\textsuperscript{5}.

**Aims and Objectives**

1. To study the pattern of mechanical trauma cases at tertiary care institute of central Gujarat, India.
2. To identify the factors related to various types of trauma and its causes.

**Materials and Methods:**

Across sectional study was conducted at SBKSMI&RC and Dhiraj hospital, Waghadia, Sumandeep Vidyapeeth, Gujarat in September to December 2021. **Sample size:** The patients visiting Dhiraj Hospital during September to December 2021 were included fulfilling inclusion criteria. Dhiraj Hospital is affiliated to SBKSMI&RC which is a tertiary level medical facility in central Gujarat and receives patient from Vadodara and surrounding districts of central Gujarat as well as adjacent region of Madhya Pradesh.

**Inclusion criteria:** All type of mechanical trauma patients visiting Dhiraj hospital for medical treatment and willing to give consent were included in the study.

**Exclusion criteria:** Patients not giving consent for this study were excluded.

**Study method:** The study subjects enrolled according to the inclusion and exclusion criteria. All participants were provided with a participant information sheet which includes study title, purpose and confidentiality of study, nature of study and Protection for patient and security etc. Written informed consent for the case study were obtained. Data collected as per pre-tested, semi-structured proforma. It includes profile pattern of mechanical trauma and factors affecting it for example type of trauma, manner of injury, part of body involved in injury, If RTA then factors responsible for RTA, most common occupant vehicle, use of safety measure, time of trauma etc. All data were collected and statistically analyzed in MS Office 365.

**Observation and Results**

During the study duration of four months, total 1084 patients were admitted as indoor cases in orthopedic, general surgery, plastic surgery, neuro surgery, pediatrics departments etc. Surgery and allied departments. Among them, 50 cases (approx. 5\%) were of various mechanical trauma.

All the cases we observed were of accidental manner. No cases of suicidal, homicidal or undetermined manner were observed. Out of all 50 cases, RTA cases were 34, fall from height cases were 9 thermal injury cases were 4 and other type trauma cases were 3.

Road traffic accident (RTA) is found to be the major cause of Trauma followed by Fall from height and thermal injuries etc.

Out of total accidental cases studied, 80\% were males and 20\% were females. Most common affected age group was 41-50 years (30\%) followed by 21-30 years (22\%), 31-40 years (20\%), 51-60 years (16\%), and equally affected in <20 years and >61 years i.e 6\%
(Table 1) The occupation status of study participants i.e. 60% participants had salaried job/daily wage workers followed by 14% unemployed/home maker, 12% were professional/private business, 8% were commercial vehicle driver and 6% were students.

Maximum cases (88%) were of hard and blunt type of mechanical injuries. (Figure 1) Lower limb were most commonly affected body part in 42% cases followed by 32% upper limb, 30% head, 6% back, 6% neck and 2% chest. Head is one of the vital body part, so if protected by safety measures like helmet, the injuries could be non-fatal. (Figure 2)

Out of RTA cases, most common responsible factor for causing accident, as per patient’s views, was over speeding wrong side driving in 38% cases followed by 16% cases were due to drug abuse/alcohol intake, 13% were due to stray animal while bad condition vehicle, poor quality road and bad weather accounts for 10% cases and 5% were due to use of mobile phone during driving. (Figure 3)

Out of RTA cases reported, 77.8% cases involved two-wheeler driver followed by 18.5% were two-wheeler passenger and 3.7% were four-wheeler passenger. (Figure 4) Most of cases were using two-wheeler followed by pedestrians, bicycle and heavy vehicle; while most common vehicle of opposite party was heavy vehicle followed by four-wheeler and two-wheeler. Only 2.2% road traffic accident patient observed practiced safety measures, while incidence, like helmet and seat belt which shows lack of use of safety measures while vehicle driving.

Out of thermal injury cases, 50% cases were due to flame burns and 25% were due to moist heat burns and 25% were due to electric shock injuries.

Maximum accidents i.e. 42% were noted at evening time (4-8pm) followed by 28% were early morning (6 am-12pm), 16% were at night (8pm-6am) and 14% were at afternoon (12-4pm).

Out of all accidental cases, 54% had minor injuries at the time of admission and they were stable followed by 32% had major bodily injuries and 14% were thermodynamically critical on admission. Out of all accidental cases reported to the hospital, 74% patients were received primary treatment from either primary healthcentre, private clinic, home remedies or any other hospital before admissions to the hospital while 26% patients were directly admitted to the hospital without receiving any primary treatment else.

Discussion

Road traffic accident (RTA) is found to be the major cause of Trauma followed by Falls from height and burns etc which is in agreement with Rajni S et. al6. In present study 80% were males and 20% were females affected while most commonly affected age group was 41-50 years (30%) followed by 21-30 years (22%). Sharma et. al7. studied total 306 patients of mechanical injury cases reported to the department of emergency. In which approx. 75% cases were male and 25% cases were female. Most of the study participants i.e. 30% belong to the age group of 21-30 years. which is consistent with Kharat R et. al.8 where males were predominantly affected and maximum number of cases were reported in the age group of 21-30 years while most common affected age group in present study was 41-60 years. Malik Y et. al.9, Kishore SK et al.10 and Singh T K et.al.11 reported the maximum number of cases in the age group of 20-40 years.

The leading cause of death was Road Traffic Accidents (83%) and Blunt mixed injury was the most common type of injury noted in Sharma et al study which is consistent with our findings where most common type of injury was due to hard blunt injury. Jagtap et al12 did retrospective study and studied the total 2450 registered “medico-legal” cases in casualty of which approx. 80% cases were males and 20% were females. Most common affected age group was 21-30 years and around 47% cases registered were due to road traffic accidents which are in agreement with present study except age group.

In present study 60% participants had salaried job/daily wage work, 6% were students and 14% unemployed/home maker which is nearly consistent with Jha N et al13 who had studied the RTA cases had approx. 52% participants were laborer/employed in service and 13.6% were housewife/unemployed while approx. 16% were students.

In present study, most common part of body involved in injury was lower limb (42%) followed...
by upper limb (32%) and head (30%) which is in agreement with Shamim M et al\textsuperscript{14} where also lower limb (44.7%) were the commonest body part involved in road traffic accidental injuries. Similarly in Kishore SK et al\textsuperscript{10} where in 52.8 % cases lower limb affected. In around 74 % patients’ primary treatment was given before admission to the hospital in our study while Rajni S et al\textsuperscript{6} reported that pre hospital care received in around 62%.

In present study 38.7% RTA cases were due to over speeding, 16.1% were due to drug intake/ alcohol intake and 9.7% were due to poor quality of roads while in Kumar S et al\textsuperscript{15} approx. 64% cases were due to alcohol intake which was quite high as compared to present study and 40% were due to over speeding which is nearly consistent with our study findings. Agnihotri A et al\textsuperscript{16} also reported that most common cause to be speeding (36.17%), followed by negligence of traffic rules (32.44%) and bad road condition (8.5%). Singh SK et al\textsuperscript{17} reported that Drivers’ fault accounted for 78% of total accidents, 76.5% of total injuries and 73.7% of total fatalities in 2013 in India where within the category of drivers’ fault, accidents caused due to over speeding accounted for a highest share of 55.6%, intake of alcohol and drugs accounted for 5.3% and 6.4%, respectively. As a share of total road accidents and deaths, overloading / overcrowding of vehicles accounted for 19.6% and 22.8%, respectively.

In present study out of RTA cases, 77.8% affected patients were driver (two wheeler) and 22.2% were passenger of either two or four wheeler while Farooqui JM et al\textsuperscript{18} also reported that two wheeler users and pedestrians were most commonly affected during RTA. Shamim M et al\textsuperscript{14} reported 53.5% were drivers and 36.9% were passengers, Menon A et al\textsuperscript{19} also reported the two wheeler occupants were most commonly involved which is also consistent with Agnihotri A et al\textsuperscript{16} and Kishore SK et al\textsuperscript{10}. Most of cases were using two wheeler while most common vehicle of opposite party was heavy vehicle followed by four wheeler and two-threw wheeler which is in agreement with Sharma D et al\textsuperscript{20} where most of the injured were using two wheeler and counterpart of its were having four wheeler followed by two wheeler. In present study, only 2.2% patients of RTA had practiced the safety measures like helmet and seat belt while driving which is less than that reported in Shamim M et al\textsuperscript{14} (27.5%)

Present study reported that maximum accidents occurred at the time of evening 42% followed by morning (28%) which is consistent with Kishore SK et al\textsuperscript{10} reported that most common time of trauma was evening i.e. 4 to 8 pm followed by mid noon and morning. Opposite results were reported by Singh YN et al\textsuperscript{21} where most common time of trauma was in morning (33%) followed by Evening (29%).

<table>
<thead>
<tr>
<th>Age group (In years)</th>
<th>Female f (%)</th>
<th>Male f (%)</th>
<th>Total f (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 20</td>
<td>2 (20.0)</td>
<td>1 (2.5)</td>
<td>3 (6.0)</td>
</tr>
<tr>
<td>21-30</td>
<td>1 (10.0)</td>
<td>10 (25.0)</td>
<td>11 (22.0)</td>
</tr>
<tr>
<td>31-40</td>
<td>0 (0.0)</td>
<td>10 (25.0)</td>
<td>10 (20.0)</td>
</tr>
<tr>
<td>41-50</td>
<td>4 (40.0)</td>
<td>11 (27.5)</td>
<td>15 (30.0)</td>
</tr>
<tr>
<td>51-60</td>
<td>3 (30.0)</td>
<td>5 (12.5)</td>
<td>8 (16.0)</td>
</tr>
<tr>
<td>&gt;61</td>
<td>0 (0.0)</td>
<td>3 (7.5)</td>
<td>3 (6.0)</td>
</tr>
<tr>
<td>Total</td>
<td>10 (20.0)</td>
<td>40 (80.0)</td>
<td>50 (100.0)</td>
</tr>
</tbody>
</table>
Figure 1: Distribution of study participants according to type of mechanical trauma

Figure 2: Distribution of cases according to their body part involved in injuries

Figure 3: Distribution of cases according to the factors responsible for RTAs
Study Limitations & Recommendations:

This study has been conducted as a short term project and data collected in cross sectional manner in limited duration of 4 months. For better accurate results and analysis longer duration of study may be done spanning all seasons in a year or more.

Few chemical trauma cases may be admitted under medicine and allied departments are lacking here. This also may be included to get the wholesome picture.

This study is just an observational type analysis. Further more research may be carried out in-depth like impact of use of safety measures in vehicles in RTA cases, impact of early hospitalized treatment in outcome of serious cases of RTA etc.

There are no homicide or suicide cases noted in this study. Which does not mean that no such cases occurs with mechanical trauma.

Conclusion

Total burden of mechanical trauma cases is 5% of all indoor cases of Surgery and allied departments.

Among total 50 cases of mechanical trauma that we studied in our research, maximum cases 88% were of hard and blunt type of mechanical injuries and we have not noted any chemical and radiation injuries.

In maximum cases mode of injury was RTA and that's why manner of injury was accidental.

Main factor causing road traffic accident was over speeding and wrong side driving.

The factors causing the trauma cases are categorized. Most of the causative factors are rectifiable by use of vehicle safety measures and awareness of road traffic regulations among community.

Ethical Clearance: This study was non-interventional observational study conducted after getting approval from SVIEC (Sumandeep Vidyapeeth Institutional Ethics Committee).

Source of Funding: All cost for this study borne by the investigators.

Conflict of Interest: Nil.

References


Comparative Antimicrobial and Wound Healing Potentiality of Onion, Garlic and Ginger

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Abstract

Objective: The aim of the research is to investigate the antimicrobial and wound healing activities of onion (Allium cepa) bulbs, garlic (Allium sativum) cloves, and ginger (Zingiber officinale) rhizomes.

Method: After extraction of onion, garlic and ginger with ethanol, their antimicrobial activity was observed following disc diffusion method. The creams of the nanoemulsion of the dried extracts were prepared and subjected to evaluate their wound healing potentiality against both the excision and incision models.

Result: The present study showed the significant (*p<0.001) wound healing activity of the onion, garlic, ginger and combination of onion garlic and ginger creams in the excision and incision wound model. In excision (*p<0.001) and incision (*p<0.001) model ginger showed maximum potential. The antibacterial efficacy of onion bulb, garlic clove, and ginger rhizome extracts was tested against gram-negative and gram-positive bacterial strains. The highest inhibitory zone against Escherichia coli appeared to be ginger (17 mm).

Conclusion: The extracts of onion, garlic, and ginger have antibacterial action against bacterial pathogens and can be used to treat microbial infections. According to the findings of this study, all the extracts had a positive effect on the excision and incision models, where ginger was observed to be the most effective among them.

Keywords: wound healing, antimicrobial, onion, garlic, ginger

Introduction

Herbal plants have long been used as a treatment and management option for a number of ailments. Plants are used for a variety of medicinal purposes across the world¹. Since ancient time, spices which are used in food processing, have been utilized in animal health treatment due to their various medicinal properties along with earlier scientific studies². Natural chemicals are in more demand than traditional pharmaceuticals due to untoward effects and their increased cost for many customers. Onion, garlic and ginger have traditionally been used to treat a variety of diseases³. Onion (Allium cepa) is an herbal spice belongs to the Liliaceae family. Onions can help to recover colds, heart...
disease, diabetes, osteoporosis, coughs, and sore throats. Onions are rich in carbohydrates, proteins, flavonoids, quercetin, allicin phosphorus, sodium and potassium. Onion sulphur compounds have also been proven to be anti-inflammatory, blocking both the development of thromboxans and the activity of platelet-activating factor. Antioxidant and antibacterial activities are also found in tannins and allicin. Onion has been shown to have antibacterial, antitumor, antidiabetic, antioxidant, antiallergic, antiparasitic, and antifungal effects. Garlic (Allium sativum), which belongs to the Liliaceae family, has been utilised as a spice in food all across the world. Garlic has developed into an excellent natural medicine for wound infection, the common cold, malaria, cough and lung, hypertension, tuberculosis, sexually transmitted illnesses, mental sickness, renal disease, asthma, liver disease, arthritis, bacterial disease, cancer and diabetes. Garlic can also boost the immune system, allowing it to fight sickness and stay healthy. Ginger (Zingiber officinale), is an herbal plant which belongs to the Zingiberaceae family. Ginger contains gingerol and shogaol and has been used for a wide variety of diseases, such as rheumatoid, fever, indigestion, sore throats, muscle aches and pains, constipation, sprains and viral infection around the world ancient times. It is also beneficial in the treatment of baldness, snakebites, toothaches, and respiratory problems. Keeping in view of all the medicinal importance of these important and most usually available spices, an effort has been taken to investigate the onion, garlic, and ginger, for the overall healing of wounds. The research findings will be useful for future study on these three spices in order to improve medicine.

Material and Method

Bacterial Strains

An antimicrobial activity test was performed on onion, garlic, and ginger extracts against pathogenic strains in the microbiology lab of Noida Institute of Engineering and Technology (Pharmacy Institute), Plot No.19, Knowledge Park-II, Greater Noida, Uttar Pradesh 201306. Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Pseudomonas fluorescens were used to perform the antimicrobial activity of onion, garlic and ginger extracts.

Collection and extraction of plant parts

Onion (Allium cepa), Garlic (Allium sativum) and Ginger (Zingiber officinale) extracts were used for the experimental wound healing activity. The fresh onion bulb, garlic cloves and ginger rhizomes were collected from own farmhouse, and was purchased from local market of Greater Noida, U.P. 200gm onion bulb, 200gm garlic bulb and 200gm ginger rhizomes were washed, peeled, and cut into small pieces, and then dried for 7 days in shade. The plant materials were powdered coarsely and separately which were further subjected to extraction with ethanol (90%) separately using Soxhlet apparatus. The obtained extracts were dried by evaporating the solvent. The dried extracts with yield value 16%, 13%, 15% for onion, ginger and garlic respectively, were used for the targeted pharmacological activities. After this the left extracts were stored below ambient temperature until required.

Phytochemical study

To get better knowledge about the phytochemicals in the obtained extracts, qualitative tests for alkaloids, flavonoids, carbohydrates, glycosides, saponins, tannins, terpenoids, proteins, and anthraquinone were performed following standard chemical tests.

Disk diffusion method for antimicrobial testing

The disc diffusion technique was followed to test spices for antimicrobial activity. The entire experiment was carried out in a sterile environment. The nutrient agar medium was prepared and evenly spread (4.0 depth) on the petri dishes. Then test bacterial strain was inoculated individually on each Muller Hinton agar plate and evenly spread throughout the whole surface. The sterile borer (5 mm /10 mm diameter) was aseptically dipped in 70% alcohol for 1 minute before being seeded with bacterial culture on Muller Hinton agar plates. Using micropipettes, the onion, garlic, and ginger extracts were poured into individual wells created by the borer. The inhibitory zone’s diameter was measured in millimetres.
Preparation of nanoemulsions

The Nanoemulsions were formed using the self-emulsification process. Tween 80 and Span 80, as well as ethanol as a co-surfactant, were used. The oil phase consisted of olive oil, whereas the exterior phase consisted of water.

Test for stability

Accelerated storage testing, which included centrifugation, heating, cooling, and a freeze-thaw cycle test, was used to examine the physical stability of onion, garlic and ginger nanoemulsions. The transparency and phase separation of all the formulations were examined visually.

Nanoemulsion characterization

Particle size analyser (PSA) was used to assess droplets and nanoemulsion size distribution. Physica MCR 300 rheometer was used to measure the viscosity of the modified nanoemulsion without dilution.

Preparation of nanoemulsion cream

An improved nanoemulsions containing 10% (w/w) respective extracts were used to make topical herbal cream by completely mixing with the cream base (1:1), yielding the final samples containing 10% (w/w) extracts of onion, garlic, and ginger respectively. Cetyl alcohol, liquid paraffin, cholesterol, soft paraffin, and beeswax made up the cream base.

Experimental animals

Wistar albino rats (225–235 g) were used in this study to assess the potential of onion, garlic, and ginger to cure wounds. The rats came from the Noida Institute of Engineering and Technology (Pharmacy Institute) facility in Greater Noida. The animals were given a two-week acclimatisation period. They were housed in plastic cages at a constant apparent temperature and relative humidity at which they were fed a conventional pellet diet with free access to drinking water. The institution’s animal ethics committee (CPCSEA Registration No. 1845/PO/Re/S/16/CPCSEA) authorized the protocol (IAEC/NIET/2020/02/13) for the animal experiment.

Wound healing activity

For the purposes of this investigation, the rats were separated into six groups, each with six individuals. Group-I (control group) was not treated with any medication. Group- II (Standard group), III, IV, and V (test groups) were treated with standard drug Betadine 10% w/w, onion (10% w/w) formulation, garlic (10% w/w) formulation, ginger (10% w/w) formulation respectively. Group- VI (test group) was treated with the onion, garlic, and ginger (10% w/w) combination formulation. All the treatments were topically applied once a day until the wounds heals.

Excision wound model

The dorsal portions of the animals were shaved after anaesthetizing with ketamine (30 mg/kg, ip). A standard ring was used to mark an area of about 500 mm² on the back of the rat. The measured portion of the skin was then gently cut with sharp scissors to its maximum thickness. On the same day of wound creation and then on every 5 days interval up to 20th day wounds were traced using 1 mm² graph paper to measure the changes in wound area. The parameters observed for better comparison of the overall wound healing are described below.

Incision wound model

After anaesthesia, 4 cm paravertebral incisions were made 1 cm lateral to the midline of the vertebral column, across the whole thickness of the skin. Surgical suture was used to close wounds. The wound length was recorded on every alternate day on and till 15 days after the creation of incision wound. The tensile strength of anaesthetized rats was measured on the tenth post wounding day by following standard procedures.

Parameters for Wound Healing Evaluation

Wound contraction measurement

On days 5, 10, 15, and 20 in this excision wound model, wound area was measured by tracing the wound with a transparent sheet on millimetre-based graph paper for all groups. The percentage of healing wound area was calculated after measuring...
wound contraction every fifth day until the wound was completely healed\(^\text{16}\). The percentage of wound contraction was calculated using the following formula with a 100% initial wound size:

\[
\text{Percent wound contraction} = \frac{\text{Initial wound size} - \text{Specific day wound size} \times 100}{\text{Initial wound size}}\]

**Tensile strength measurement and epithelialization**

The tensile strength of a healed skin wound is used to determine the degree of wound healing. It indicates how resistant the repaired tissue is to break under strain and may indicate the healing tissue’s quality. On the tenth day, all the animals were anaesthetized, the sutures were removed, and the repaired tissue was excised. A line was drawn 3 mm away from the wound on either side of the incision line. On the line facing each other, two Allis forceps were firmly placed. One forceps was fixed, while the other was attached by a thread to a pulley to a freely hanging lightweight polypropylene graded container. The container was gently and gradually filled with standard weights. The weight was gradually transferred to the incision site, pushing the wound edges apart. The weight was halted and documented as soon as the wound opened up\(^\text{18}\). The epithelialization period was estimated as the number of days it required for dead tissue remains of the wound to fall off without leaving any raw wound\(^\text{19}\).

**Hydroxyproline estimation**

The hydroxyproline level in excised wound tissues from all rats was assessed. Tissue samples were dried at 60°C inside a hot air oven to a constant weight, and hydrolysed in 6 NHCl for 4 hours at 130°C. The hydrolysates were exposed to Chloramine-T oxidation for 20 minutes after becoming normalized to pH 7.0. After that, 0.4 M perchloric acid was added to stop the reaction. The samples were then colour-developed in Ehrlich reagent at 60°C with vigorous stirring before being exposed to an ultraviolet spectrophotometer. Using a pure L-hydroxyproline standard calibration curve, the hydroxyproline level of the samples was calculated\(^\text{20}\).

**Statistical analysis**

The observed data are expressed as a mean value. To determine statistical significance, S.E.M. GraphPad was used to perform a one-way analysis of variance (ANOVA) with Dennett’s test, being very significant.

**Results**

**Phytochemical investigation**

From the preliminary phytochemical study, it was interpreted that the ethanolic extracts of onion and ginger possess phytoconstituents of alkaloids, flavonoids, glycosides, carbohydrates and saponins, whereas all the extracts were deprived of steroids. In case of garlic, there was positive result for the presence of alkaloids, flavonoids, glycosides, carbohydrates and saponins, but absence of tannins.

**Nanoemulsion physicochemical characterization**

The modified nanoemulsions were stable throughout the research work (for over 8 months) at room temperatures without substantial particle size changes. The nanoemulsion formulation passed all the stability and characterization tests were the.

**Antimicrobial activity**

The results revealed that the ethanolic extracts of onion, garlic, and ginger possess antimicrobial activity against the selected microbes like *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas fluorescens*, and *Pseudomonas aeruginosa* by using the disc diffusion method. Onion, garlic, and ginger all inhibited the bacterial strains effectively. But Onion and garlic extracts showed less significant effect as compared to the ginger extract, which is described in Table 1. The antimicrobial activity of onion, garlic and ginger has been depicted in Figure 1.
Figure 1: The inhibition zone by ethanolic extracts of onion, garlic, and ginger against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Pseudomonas fluorescens*, where ginger showed a significant effect in the above picture.

Table 1: Antimicrobial zone of inhibition (mm in diameter) by the ethanol extracts of onion, garlic and ginger.

<table>
<thead>
<tr>
<th>Name of the pathogen/ Microbes</th>
<th>Zone of inhibition (mm in diameter)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanollic extract of onion</td>
</tr>
<tr>
<td></td>
<td>25µg/ml</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>7.2±1.3</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>6.6±1.01</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>6.6±1.4</td>
</tr>
<tr>
<td><em>Pseudomonas fluorescens</em></td>
<td>11.2±1.3</td>
</tr>
</tbody>
</table>

All values were reported as mean standard error of the mean (SEM), with in each group. A one-way ANOVA was used to evaluate the data, followed by a significant Dennett’s test.

Wound Contraction

On examining the excision wounds for the effect of the creams of the nanoemulsions of onion, garlic and ginger as well as combination cream for 20 days, it was observed that all animals were completely healed at 20th day except control group. In an excision wound model, wound area measurements revealed that the test groups’ wound sizes were decreased earlier than the control group. In case of ginger and standard group the wounds were completely recovered with hair growth at 18th day of wound creation. The formulations were observed to recover the wounds effectively as there was hair without
any wound mark. In this study ginger showed a significant effect (*$p<0.001$) as compared to that of other groups. The overall wound healing potentiality of the respective formulations are elaborated in Figure 2 and Figure 3.

![Day 5](image1)

![Day 10](image2)

![Day 15](image3)

![Day 20](image4)

Figure 2: Wound healing by the formulations of onion, garlic, ginger, combination, and standard (betadine) at different time intervals after excision wound model creation.

![Graph](image5)

Figure 3: Graph of wound contraction of rats treated with the formulations of onion, garlic, ginger, combination, and standard (betadine).

All values are reported as mean standard error of the mean (SEM), with $n = 6$ animals in each group. A one-way ANOVA was used to evaluate the data, followed by a significant Dennett’s test (*$p<0.001$).
Tensile strength and epithelisation period

Onion, garlic and ginger creams and combination cream of onion, garlic, and ginger were applied and the tensile strength was measured on 10th day after removal of the suture. All the formulations had exhibited significant effect on recovering the tensile strength which was represented in Table 2. On examining the animals till 15th day the extract formulations were observed to possess complete healing by 15th day. In comparison to the other groups, the cream of the nanoemulsion of the combination of all three extracts showed the highest wound healing potentiality and alone ginger cream was also found to be more effective than garlic and onion formulations (Table 2).

The groups which were treated with the formulation of onion, garlic, ginger, combination formulation were completely healed in shorter time period as compared to the control group animals. The ginger cream showed a significant epithelisation period \((p<0.001)\), but less than that of the combination formulation, which are predicted in Table 2.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Tensile strength</th>
<th>Epithelisation Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>170.3±1.21</td>
<td>19.66±1.03</td>
</tr>
<tr>
<td>Standard</td>
<td>206.3±1.36</td>
<td>8.83±0.75</td>
</tr>
<tr>
<td>Onion</td>
<td>185.8±1.47</td>
<td>11.3±0.81</td>
</tr>
<tr>
<td>Garlic</td>
<td>184.5±1.04</td>
<td>12.3±1.21</td>
</tr>
<tr>
<td>Ginger</td>
<td>205.8±1.72</td>
<td>9.5±1.04</td>
</tr>
<tr>
<td>Combination</td>
<td>207.3±1.21</td>
<td>8.5±1.04</td>
</tr>
</tbody>
</table>

All values were reported as mean standard error of the mean (SEM), with \(n=6\) animals in each group. A one-way ANOVA was used to evaluate the data, followed by a significant Dennett’s test epithelisation period \((p<0.001)\) and tensile strength \((p<0.001)\).

Hydroxyproline Content

The wound healing potentiality of onion, garlic, ginger, and combination formulations topical therapy in both incision and excision wounds was validated by the assessment of hydroxyproline concentration as an indicator for collagen turnover.
The hydroxyproline content represents the wound healing process's increased collagen production\[^{26}\]. In this study, the onion, garlic, ginger, and combination formulations caused a substantial (p<0.001) increase in hydroxyproline content in comparison to control group animals, which reflects that the treated animals showed increased collagen synthesis, which facilitates wound healing (Figure 5).

![Figure 5: Graph of dry weight tissue and hydroxyproline content.](image)

All values are reported as mean standard error of the mean (SEM), with \(n = 6\) animals in each group. A one-way ANOVA was used to evaluate the data, followed by a significant Dennett’s test (*\(p<0.001\)).

**Discussion**

The research as planned from the literature on onion, garlic and ginger, to possess potentiality against infections and their easy availability, wound healing properties of onion, garlic, and ginger extracts were investigated after formulating their nanoemulsion creams. The results of antimicrobial activity of the extracts of onion, garlic, and ginger against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Pseudomonas fluorescens* supported the research work\[^{21}\]. Further the wound healing potentiality of the creams of nanoemulsions of onion, garlic, and ginger, as well as their combination reflected their potentiality to heal different types of wounds as compared to the standard agent betadine. Onion and garlic were shown to have less efficient antimicrobial action than ginger which was also linked to their wound healing activity in the same manner in both incision and excision wound models. The nanoemulsion formulation was fruitfully showed potential effect of the extracts by better application by lesser irritation and facilitating their pharmacokinetics parameters to be better absorbed from the site of application to achieve optimal bioavailability for better effect\[^{22}\].

Granulation tissue is mostly made up of fibroblasts, collagen, edema, and new tiny blood vessels that emerge at the end of the proliferative phase\[^{23}\]. As the present study interpreted that onion, garlic, and ginger formulations, as well as their combination formulations, increased wound contraction, skin breaking strength, epithelialization rate, weight and hydroxyproline level of granulation tissue. Improved proliferation and transformation of fibroblast cells into myofibroblasts may be responsible for the increased tensile strength. Myofibroblasts are thought to contribute significantly to wound contraction by causing stress on the surrounding extracellular matrix and secreting extracellular matrix proteins like collagen to keep the contraction stable. Increased tensile strength in both the test and the standard treatment groups revealed that collagen\[^{24}\] maturation was increased by cross linking. The findings of this study clearly showed that the onion, garlic, and ginger formulations, as well as their combination formulation, had a definite pro healing effect in wound healing activity. Myofibroblasts are thought to contribute significantly to wound contraction by causing stress on the surrounding extracellular matrix and secreting extracellular matrix proteins like collagen to keep the contraction stable.
stable. Increased tensile strength in both the test and the standard treatment groups revealed that collagen maturation was increased by cross-linking. Onion, garlic, and ginger were shown to have a larger proportion of wound contraction and a higher tensile strength of healing tissue. The findings of this study also revealed that the presence of alkaloids, saponins, terpenoids and flavonoids in the extracts of onion, garlic, and ginger may be the responsible secondary metabolites for their antimicrobial and wound healing activity. Literature also supported that onion and garlic contain allicin which facilitates in the killing of acne-causing bacteria and helps in wound healing. It also benefits in the reduction of edema, as well as the improvement of blood circulation. Ginger contains the anti-oxidant and anti-inflammatory compounds like gingerol and shogaol, which have been shown to promote the formation of new blood vessels and further supported its wound healing potentiality.

**Conclusion**

This comparative research examined and interpreted the antimicrobial and wound healing properties of onion, garlic, and ginger spices and concluded that ginger is more effective in wound healing than that of onion and garlic though all three have antimicrobial and wound-healing properties which were well comparable to that of the standard drug betadine. The potentiality of ginger for its antimicrobial activity may be the supportive reason behind its potent wound healing potentiality as compared to onion and garlic. The study concluded that the alkaloid, saponins, tannin and flavonoids along with other important secondary metabolites of onion, garlic, and ginger are thought to be responsible for their antimicrobial and wound healing potentiality. The nanoemulsion cream as the planned formulation was also observed to be successful in getting best effect of the extracts with non-irritant application and increased bioavailability. The nanoemulsion formulation of the combination of onion, garlic, and ginger is concluded to possess most potent antimicrobial and wound healing effect due to the content of all above mentioned chemical compositions. The study will be beneficial in providing the effective antimicrobial and wound healing formulation with less cost and more potency.

**Conflict of Interest:** The writers confirm that there are no conflicts of interest among them because this is a collaborative project.

**Funding:** There is no funding available to support this research project. It is entirely self-financed.

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Effect of Smoking on D-dimer Level at COVID-19 Patients

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Abstract

Background: COVID-19 are a type of virus. There are many different kinds, and some cause disease. COVID-19 identified in 2019, SARS-CoV-2. It attaches to cells there, begins to multiply and moves into lung tissue. D-dimer is one of the protein fragments produced when a blood clot gets dissolved in the body. It is normally undetectable or detectable at a very low level unless the body is forming and breaking down blood clots.

Objective of the Study: It’s show effect of smoking on D-dimer level in COVID-19 patients.

Materials and Methods: The current study done in Al-Yarmook Teaching Hospital, was included 20 cases of smoking COVID-19 patients and 20 cases of non smoking COVID-19 patients, all of subject’s age were 40-60 years. This study depend on collect of plasma sample used sodium citrate tubes by Elisa method to measure D-dimer level in all cases.

Results: This study shows significant elevation of D-dimer in smoking COVID-19 group compare with non smoking COVID-19 group.

Conclusion: The current study demonstrate effect of smoking on D-dimer level in COVID-19 patients.

Keywords: COVID-19, Nicotine, D-dimer and smoking

Introduction

Corona viruses (COVID-19) are a family of viruses that can cause respiratory illness in humans. They get their name, “corona” from the many crown-like spikes on the surface of the virus. Severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS) and the common cold are examples of corona viruses that cause illness in humans. COVID-19 enters human body through the mouth, nose or eyes (directly from the airborne droplets or from transfer of the virus from hands to face)¹. The virus travels to the back of your nasal passages and mucous membrane in the back of your throat. It attaches to cells there, begins to multiply and moves into lung tissue. From there, the virus can spread to other body tissues. The symptoms and signs of Covid-19 are fever or chills, cough, shortness of breath or difficulty breathing, headaches and others. There are many factor consider as risk for contracting COVID-19 are:

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When COVID-19 infection the body’s blood-clotting response doesn’t work right. Abnormal clots form, which can lead to internal bleeding or organ failure. The recent studies found that nearly a third of people COVID-19 who enter intensive care unit (ICU) had blood clots in patients’ legs (deep vein thrombosis (DVT)), lungs (pulmonary embolism (PE)) or arteries. When a blood vessel or tissue is injured and begins to bleed, a process called hemostasis is initiated by the body to create a blood clot to limit and eventually stop the bleeding. This process produces threads of a protein called fibrin, which crosslink together to form a fibrin net. That net, together with platelets, helps hold the forming blood clot in place at the site of the injury until it heals. Once the area has had time to heal and the clot is no longer needed, the body uses an enzyme called plasmin to break the clot (thrombus) into small pieces so that it can be removed. The fragments of the disintegrating fibrin in the clot are called fibrin degradation products (FDP), which consist of variously sized pieces of cross-linked fibrin. One of the final fibrin degradation products produced is D-dimer, which can be measured in a blood sample when present. The level of D-dimer in the blood can significantly rise when there is significant formation and breakdown of fibrin clots in the body.

D-dimer is one of the protein fragments produced when a blood clot gets dissolved in the body. It is normally undetectable or detectable at a very low level unless the body is forming and breaking down blood clots. Then, its level in the blood can significantly rise. This test detects D-dimer in the blood. There are several factors and conditions associated with inappropriate blood clot formation. One of the most common is deep vein thrombosis (DVT), which involves clot formation in veins deep within the body, most frequently in the lower legs.

Tobacco which contains nicotine is usually smoked in cigarettes. It is also smoked in cigars and pipes. There are numerous forms of smokeless tobacco including chewing tobacco, and wet and dry. Tobacco which contains nicotine is usually smoked in cigarettes. It is also smoked in cigars and pipes. There are numerous forms of smokeless tobacco including chewing tobacco, and wet and dry.

Figure 1: The cigarettes

The Nicotine is a stimulant drug (chemical formula: \(C_{10}H_{14}N_2\)) that speeds up the messages travelling between the brain and body. Figure 2.

Figure 2: The chemical structure of nicotine

It is the main psychoactive ingredient in tobacco products. Tar and carbon monoxide (a toxic gas) are also released when tobacco is burned, such as when it’s smoked. The smoking have well documented negative effects on health such as stroke, blindness, cataracts (eye diseases), pneumonia, various respiratory diseases (shortness of breath, asthma, coughing fits) and others.

Material and Methods

The current study depended on collection tow groups of 20 smoker cases with COVID-19 infection (SCC-19I) and 20 non smoker cases with COVID-19 infection (NSCC-19I). All these groups not have any other diseases and the age were 40-60 years of both genders, smoking duration was 20 years and all cases classified as Moderate infection. This study measured done in Al- Yarmook technical hospital – Iraq. This study measured plasma level of D-dimer.
after drawing blood and treated with sodium citrate. This parameters were measured by ELISA technique.

Statistic analysis in current study was use the t-test method to show different in D-dimer level between SCC-19I group and NSCC-19I group. The t-test method used mean ± standard deviation (SD) and P-value (>0.05 significant value).

**Results**

This study compare between SCC-19I group and NSCC-19I group by used plasma D-dimer level. The study results shown significant deference between two groups according to plasma D-dimer level. This study result presented elevated of plasma D-dimer level at SCC-19I group when compare with NSCC-19I group. Shown Table 1.

Table 1: Comparison of plasma D-Dimer level between SCC-19I and NSCC-19I groups according to Mean +deviation Standard (SD) by T-test statistic method.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SCC-19I (No.20 cases)</th>
<th>NSCC-19I (No.20 cases)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-dimer (ng/ml)</td>
<td>623 ± 3.1</td>
<td>547 ± 2.6</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

**Discussion**

COVID-19 are a type of virus. There are many different kinds, and some cause disease. COVID-19 identified in 2019, SARS-CoV-2. The virus travels to the back of your nasal passages and mucous membrane in the back of your throat. It attaches to cells there, begins to multiply and moves into lung tissue. COVID-19 has caused a pandemic of respiratory illness that can lead to many complication, commonly is coagulation defect to product various blood clots. The blood clotting is a rapid process, during which a protein called fibrinogen converts into fibrin. Fibrin forms a network that functions as the backbone of the clot, providing mechanical strength called cross linkage. Fibrinogen is the major plasma protein coagulation factor. Low plasma fibrinogen concentrations are associated with an increased risk of bleeding due to impaired primary and secondary hemostasis.

D-dimer is a fibrin degradation product, a small protein fragment present in the blood after a blood clot is degraded by fibrinolysis. It is so named because it contains two D fragments of the fibrin protein joined by a cross-link. D-dimer concentration may be determined by a blood test to help diagnose thrombosis.

This result study demonstrate elevate of plasma D-dimer level in SCC-19I group when compare with NSCC19I group, this elevation due to smoking. The smoking individual already has high level of nicotine that cause elevate of fibrinogen, fibrinogen convert to fibrin then form fibrin cross linkage that cause blood clots. D-dimer act as parameter to evaluation clotting state in patients. This study agree with Açık DY and et. al. 2020, that showed effect of smoking on fibrinogen.

This study also agree with MENEKSE E and et. al. 2021, that explain elevated of D-dimer in COVID-19 infection.

**Conclusion**

The present study conclude shown effect smoking on blood clotting by measured plasma D-dimer level as indicate in COVID-19 patients.

**Conflict of interest:** No

**Source of Funding:** self or other source: self

**Ethical clearance:** Yes

**References**


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Zygosity Test of Twin Pairs Using 26 STR Loci in the Indonesian Population

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Abstract

Background: Twin research can play an important role in understanding the interplay between genes and environment. Classifying twins as monozygotic (identical) or dizygotic (fraternal) is an essential first step in conducting research that will yield valid and replicable findings. Identical twins (theoretically) share 100\% of their genes, while fraternal twins share, on average, 50\% of their genes.\textsuperscript{1} This study aims to determine zygosity in 12 twins with Non-Syndromic Cleft Lip and Palate (NSCLP) discordant, concordant, and non-NSCLP.

Material and Methods: Blood samples from 12 twin pairs, which include of 8 twins Non-syndromic Cleft Lip and Palate (NSCLP) discordant, 2 twins NSCLP concordant, and 2 twins non-cleft (normal). DNA extraction was using Promega Kit and quantified was using Nano drops. DNA amplification was by PCR with 26 loci, which was packaged in Powerplex Fusion 6C Kit. PCR products were sequenced using ABI3500 and the visualization can be seen using Genemapper TM ID-X 1.6 software.

Results: The results show that each pair of 11 twins have a similar alleles in 26 STR loci, which means they are monozygotic twins. Only 1 twin has a different alleles in 10 STR loci, which means they are dizygote.

Conclusion: Zygosity tests on MZ twins using STR loci has high similarity and sensitivity. This test would be more accurate by analyzing a higher number of STR loci.

Keywords: Monozygote; twins; STR; Loci.

Introduction

Zygosity test is usually used to identify the etiology of congenital disease. Monozygotic twins (MZ) or identical twins generated from a single egg cells, fertilized by one sperm cell, while dizygotic twins (DZ) generated from two different egg cells
and fertilized by two different sperm cells. Dizygotic twins come from two zygotes and usually share half of their genome, while MZ share 100% of their genomic information, since they came from the same zygote. However, additional genetic component, such as epigenetic factors and somatic post-zygotic mutations, can explain their different expression in MZ. Another proof of MZ genetic differences has been reported in specific and clinical development. Twin study showed consistent genetic component in Non-Syndromic Cleft Lip and Palate (NSCLP) etiology, with higher compatibility in MZ (25-50%) compared to DZ (3-6%). Molecular analysis of MZ discordance has been tried to identify NSCLP genetic factors. De novo nonsense mutation in IRF6 was detected in twins that affected from discordance twins with clefting Van der Woude syndrome. However, another research using different technical approaches did not succeed in identifying genetic distinction in NSCLP discordance twin’s pair. This twin could become the source of phenotype expression variation, epigenetic, and postzygotic mutagenesis, which can be an alternative method to identify gene of a congenital abnormality. There was a hypothesis that de novo postzygotic mutation could cause discordance MZ twins with NSCLP, which in contrast genetically identical. To test this hypothesis, a research on two pairs of MZ twins with high density of SNP genotypes has been done which was in accordance with the analysis of de novo postzygotic copy number variation (CNV). Identification of molecular genetic distinction between cleft MZ twins indicated that the usage of twins studies could be improved through heritability study to gen discovery.

Besides observing a congenital abnormality and mutation, zygosity test has a function as a forensic identification process zygosity test using STR has very high discrimination. Thirteen STR loci have been found to 1998 and determined by the Global Forensic Group in collaboration with FBI (Federal Bureau of Investigation). To date, STR loci have been improved, which means the examined areas in the chromosome are higher, thus increasing the accuracy during identification process.

Materials and Methods

Study participants

This zygosity test study has been approved by Health research ethics committee, Universitas Airlangga School of Medicine No. 290/EC/KEPK/FKUA/2020. The total sample were 12 Indonesian twin pairs, which consist of 8 twins Non-syndromic Cleft Lip and Palate (NSCLP) discordant, 2 twins NSCLP concordant, and 2 twins non-cleft (normal).

Specimens Collection

The sample was whole blood which was taken into EDTA tube and kept at 4°C.

DNA extraction and quantification.

Promega Maxwell™ 16 purification Kit (promega) was used for DNA extraction according to the manual guide. The concentration and purity were checked by using Nanodrop at 260/280 absorbance. The extracted DNA was kept at -20°C.

DNA Amplification

DNA was amplified using PCR with the powerplex fusion 6C kit according to the manufacturer protocol (Promega, 2018). The kit contained 26 STR loci including D3S1358, D1S1656, D2S441, D10S1248, D13S317, PENTA E, D16S539, D18S51, D2S1338, CSF1P0, PENTA D, TH01, vWA, D21S11, D7S820, D5S818, TPOX, D8S1179, D12S391, D19S433, SE33, D22S1045, DYS391, FGA, DYS576, DYS570.

Sequence Process

Sequencing was done using ABI3500 machine and the result was analyzed using Genemapper™ID-x 1.6 software.

Result

The result of 11 twins indicated that each pair has a similar allele on the 26 STR locuss. Figures 1 to 5 showed the allele from ZT7 samples using Genemapper™ID-x 1.6 software. Figures 6 to 10 displayed ZT8 sample which was the twins’ pair of ZT7. One pair has a different alleles in 10 loci, which means they dizygotic twin.


Discussion

The previous study revealed that >80% of progeny from one of the twins would carry at least one germline mutation which would be detected in their father sperm. Researchers suggested to perform paternity test on the MZ twins with whole genome sequencing. Furthermore, the research conducted by Bruder et. al. differed twins genetically but not specifically for forensic tests. To improve zygosity test accuracy, it is suggested to analyze DNA from Y chromosome to observe the distinction between closely related males.

The usage of STR in zygosity test is so far the gold standard due to the high sensitivity and the wide range of loci detection. The zygosity test of umbilical cord from MZ twins examining 15 STR loci had high sensitivity results. The study conducted by Dziennik et. found that the zygosity test can be performed using cell-free DNA (cfDNA) samples taken from mother’s plasma. However, this is different from one of the studies which reported that the method to differentiate an individual MZ twins cannot be observed by using STR, but employing mitochondrial DNA and finding a heterogenic SNP. Another factor influencing MZ twin’s distinction epigenetically was the source of DNA samples and biological characters. In addition, DNA methylation could also performed to observe the CpG island difference on MZ twins sample.

Conclusion

Based on the results of this research, this supports that the zygosity tests on MZ twins using STR loci
has high similarity and sensitivity. This test would be more accurate by analyzing the higher number of STR loci.

**Declaration of Interest:** All authors declared no potential conflict of interest.

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**Ethical Clearance:** Taken from Health Research Ethics Committee Universitas Airlangga, School of Medicine, Description of Ethical Exemption No. 290/EC/KEPK/FKUA/2020

**References**


Surgical versus Non-Surgical Management in Fibroid Uterus: A Prospective Observational Study

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Abstract

Background and Aim: Uterine fibroid is the benign condition, known to occur more during reproductive period of women. It is mainly caused due to increased level of estrogen & progesterone & peptide growth. Current management strategies mainly involve surgical interventions, but choice of treatment is guided by patient’s age and desire to preserve fertility or avoid ‘radical’ surgery such as hysterectomy

Material and Methods: It is the prospective observational study conducted in patients of department of Obstetrics and Gynecology at tertiary care hospital for the duration of one and half year. The treatment prognosis and outcome was measured in the symptomatic relief in patients, increase in quality of life, decrease in size of fibroid present, requirement of blood transfusion, definitive length of stay in the hospital and successful pregnancy after the line of management.

Results: Of the total 60 patients diagnosed with fibroid uterus, there were 20 patients with fibroid size less than 8 cm and in 40 patients the size of fibroid was more than or equal to 8 cm. Among the 32 patients treated with medical line of management, 10 patients were treated with tablet ulipristal acetate 5 mg od for 3 months. Amongst the 28 patients treated surgically, in 7 patients myomectomy was done, 2 patients underwent vaginal hysterectomy, in 2 patient’s laparoscopic assisted vaginal hysterectomy done (LAVH) and 17 patients underwent total abdominal hysterectomy (TAH).

Conclusion: The choice of treatment must be individualized to the women’s need and her clinical presentation. Medical line of management is best for patients in younger age group, small size fibroid, desire for future fertility. In medical line of management, ulipristal and mifepristone have better outcomes.

Key Words: Laparoscopic assisted Vaginal Hysterectomy, Quality of Life, Total Abdominal Hysterectomy, Uterine fibroid

Introduction

Uterine fibroids are the commonest benign tumours of the uterus, also known as fibromyoma or leiomyoma. The exact etiology is not known, but probably the tumour is of muscle tissue origin. The muscle tissue of origin may be the mature muscle
cells of the uterine wall or the muscle fibres in the blood vessel walls. There is substantial evidence that estrogen place an important role in the growth of myomas.\textsuperscript{1,2}

Various psychological factors like mental shock, grief, mortification, vexation and the stress and strain of modern life could be the root cause of hormonal imbalance through psycho-nuero-hormonal pathway leading ultimately to pathological changes in the uterus. There are three basic types of uterine fibroids which are identified by their location in the uterus, namely intramural, subserous and submucous.\textsuperscript{3,4}

In majority of the patient there are no obvious symptoms. The symptoms usually occur depending on the size and location of the fibroids. Generally the presenting symptoms are menorrhagia, metrorrhagia and gradual swelling of abdomen with dull aching pain.\textsuperscript{5} Moreover pressure symptoms include increased frequency of micturation, retention of urine, hydronephrosis, pedal oedema and in rare cases constipation. About 30% of women with uterine fibroids present with infertility.\textsuperscript{6}

Uterine fibroid is the benign condition, known to occur more during reproductive period of women. It is mainly caused due to increased level of estrogen & progesterone & peptide growth.\textsuperscript{7} Abnormality of chromosome 12 suggests a genetic role in pathogenesis of these tumours. These are more common in nulliparous. These tumours are formed of a mixture of muscle tissue containing fibrous tissue.\textsuperscript{8}

Magnetic resonance imaging (MRI) provides information on number of fibroids, their size and location, vascularization, relationship with endometrial cavity and serosal surface and boundaries with normal myometrium.\textsuperscript{1} Pharmacological management of fibroid includes oral contraceptives, progestins, progesterone receptor antagonist mifepristone, selective progesterone receptor modulator (SPRM) ulipristal, which has mixed progesterone agonist/antagonist and GnRH agonist and antagonist.\textsuperscript{9,10}

Surgical treatment includes hysterectomy (abdominal, vaginal and laparoscopic) and myomectomy (laparotomy, laparoscopic and hysteroscopic). New approaches, such as myolysis, focused ultrasound, transvaginal cryomyolysis and uterine artery embolization (UAE), laparoscopic uterine artery occlusion (LUAO), doppler guided uterine artery occlusion (D-UAO).\textsuperscript{11}

Current management strategies mainly involve surgical interventions, but choice of treatment is guided by patient’s age and desire to preserve fertility or avoid ‘radical’ surgery such as hysterectomy.

Materials and Methods

The present study was undertaken at Medical College and Hospital. The cases were taken from the OPD’s and rural OPD’s during the period of two years. Sixty cases of uterine fibroid were selected on the basis of simple random technique sampling procedure. Sixty uterine fibroid patients belonging to age group 30 - 45 were included in the study. All the pregnant ladies and Fibroid showing changes of leiomyosarcoma are excluded from study.

The choice of the type of treatment depends on the age of the patient, severity of symptoms, desire to become pregnant in future and importance of preservation of uterus. Medical treatment was preferred for young female patients, those with less severe symptoms and for those who have esire for future fertility planning.

The first medical line of management includes GnRH agoists such as leuprolide, Ulipristal acetate, mifepristone (Progesterone receptor antagonist and levonorgestrel IUDs. The drug leuprolide is given as injection, 3.75 mg is given monthly or 3 months. Tab mifepristone 25 mg is given OD daily or 3 months. Tab Ulipristal acetate id given 5 mg daily OD for 3 months. The surgical treatment included the procedure like myomectomy and hysterectomy (Abdominal, laparoscopic and vaginal).
The treatment prognosis and outcome was measured in the symptomatic relief in patients, increase in quality of life, decrease in size of fibroid present, requirement of blood transfusion, definitive length of stay in the hospital and successful pregnancy after the line of management. Outcomes of surgical management measured in terms of quality of life, requirement of blood transfusion, length of stay in hospital and successful pregnancy after the line of management. Outcomes of surgical management measured in terms of quality of life, requirement of blood transfusion, length of stay in hospital and successful pregnancy after the line of management.

Results

A total of sixty patients diagnosed with fibroid uterus were admitted in the hospital and included in the study. Thirty patients were given the medical line of management and thirty patients underwent the surgical procedure. The characteristic features such as age, parity, size of fibroid and desire for future fertility were studied. The 10 patients after medical line of management have undergone surgery.

The age range of the included patients was from 25 to 60 years. The maximum number of 42 patients did belong to the age group of 35 to 55 years. There were 13 patients that belong to the age below 35 years and there were 5 patients with age more than 55 years.

Of the total sixty patients diagnosed with fibroid uterus, 18 patients were nullipara, 12 patients were primipara and the remaining 30 patients were designed as multipara. The nullipara and primipara designated patients were considered for medical line of management. The rest multipara patients were considered for surgical line of treatment.

Of the total 60 patients diagnosed with fibroid uterus, there were 20 patients with fibroid size less than 8 cm and in 40 patients the size of fibroid was more than or equal to 8 cm. Among the 40 patients, the medical line of treatment was given to 12 patients and only 2 patients responded while remaining 10 patients landed up in surgery.

Among the 32 patients treated with medical line of management, 10 patients were treated with tablet ulipristal acetate 5 mg od for 3 months. After 3 months, 07 patients had relief of symptoms and quality of life improved while, 03 patients had no relief of symptoms and required surgery subsequently. Among the 10 patients treated with ulipristal, size of fibroid was decreased in 9 patients.

The 12 patients were given injection leuprolide 3.7mg monthly for 3 months, 6 patients showed relief of symptoms and improved quality of life, remaining 6 patients underwent surgery. The size of fibroid decreased in 10 patients.

The 10 patients treated with tablet mifepristone 25 mg OD daily for 3months, among these 8 patients showed relief of symptoms and improved quality of life, 2 patients needed subsequent surgery. In 6 patients treated with mifepristone, size of fibroid decreased. In 4 patients, LNG was inserted and all these patients had improved quality of life, and size of fibroid decreased in only 1 patient.

Amongst the 28 patients treated surgically, in 7 patients myomectomy was done, 2 patients underwent vaginal hysterectomy, in 2 patient’s laparoscopic assisted vaginal hysterectomy done (LAVH) and 17 patients underwent total abdominal hysterectomy (TAH). All patients treated surgically had relief of symptoms, had improved quality of life.

Among the 32 patients treated medically, 2 patients needed blood transfusion. In 28 patients treated surgically, 9 patients needed blood transfusion. Length of stay in hospital is more with surgical line of management. Among the surgeries length of stay is higher in total abdominal hysterectomy and is less in myomectomy patients.

Table 1: Medical management of the uterine fibroids patients

<table>
<thead>
<tr>
<th>Medicine</th>
<th>Relief of symptoms</th>
<th>Requiring surgery</th>
<th>Decrease in size</th>
</tr>
</thead>
<tbody>
<tr>
<td>ulipristal acetate</td>
<td>7</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>leuprolide</td>
<td>6</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>mifepristone</td>
<td>8</td>
<td>2</td>
<td>6</td>
</tr>
</tbody>
</table>

Discussion

The present study was designed to demonstrate the best choice of treatment for patients with diagnosis of fibroids in uterus. The factors that were taken in consideration were size of fibroids, desire for future fertility and age of patients. Patient with small size fibroid (size <5 cm) and/or desire for future fertility should be considered for medical line of management.
Our study demonstrates the best choice of treatment for patients with primary diagnosis of uterine fibroids, considering the factors such as size of fibroid, age of the patient, desire for future fertility. Patient with small size fibroid (size <5 cm) and/or desire for future fertility should be considered for medical line of management.

For younger patients, medical line of management is best and among the medical line of management, ulipristal and mifepristone have come up with best results, in terms of improvement in lifestyle, relief of symptoms, successful future pregnancies and decrease in size of fibroid.

Donnez et al\textsuperscript{12} study concluded that ulipristal effectively control bleeding and shrink fibroids in patients with symptomatic fibroids. Levens et. al. study found ulipristal effective for reducing the size of individual fibroids and the overall fibroid burden as measured by total fibroid and uterine volume. A single course of 5 or 10 mg reduced fibroids size by 17 to 38\%. Carbonell et al\textsuperscript{13} studies stated that 68 to 100 percent of women in their trials reported pelvic pain. By three months of treatment, this was reduced to a range of 9 to 28 percent with those in lower dose groups having lower/equivalent prevalence of pelvic pain. LNG just reduces menorrhagia in fibroid uterus, it has nothing to do with the size of fibroid. Tosun et. al. suggested that LNG- IUD can improve bleeding even among women whose fibroid symptoms were considered appropriate for surgical intervention. However, quality of study was poor thus evidence to guide care is inadequate.\textsuperscript{14}

Hysterectomy is preferred by the patients with low socioeconomic status as patients need to get treated in one setting, patient cannot afford medical line of management. Surgical morbidity/mortality such as blood loss, bowel injury, bladder/urethral injury, infection, postoperative pain and death have all been reported with hysterectomy.\textsuperscript{15}

**Conclusion**

The choice of treatment must be individualized to the women’s need and her clinical presentation. Medical line of management is best for patients in younger age group, small size fibroid, desire for future fertility. In medical line of management, ulipristal and mifepristone have better outcomes.

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

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**Conflict of Interest:** None declared

**References**


Trichloroethylene-induced Cellular Damage was Associated with Significant changes in the Concentrations of Caspase-3 and Glutathione in human lymphocytes

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Abstract

Trichloroethylene (TCE) is a volatile organic compound that is widely used in industries and potential source of environmental contamination. The aims of this study were focused on the effects of TCE on cellular viability and evaluation of critical markers like caspase-3 and glutathione (GSH) in human lymphocytes. The experiments were studied in thirty volunteers. The TCE concentrations of 0.002, 0.004, 0.008, 0.016 and 0.032 mM/L were treated in human lymphocytes for 48 h. Lymphocytes were cultured and effect of TCE on viability of lymphocytes was studied by MTT assay. The expression of caspase-3 in TCE treated lymphocytes was studied by ELISA and the concentration of glutathione in TCE treated lymphocytes was studied by colorimetric technique. The results showed that, in the experimental groups at various TCE concentrations had lower cellular viability than the control group (p<0.05). The caspase-3 concentration was increased when TCE concentration increased (p<0.05). The glutathione concentration was decreased when TCE concentration increased (p<0.05). It is indicated that TCE effected on cellular viability. The expression of caspase-3 enzyme and glutathione concentrations were changed by TCE toxicity in lymphocytes.

Keywords: Trichloroethylene, Lymphocyte, Cellular viability, Caspase-3 enzyme, Glutathione.

Introduction

Trichloroethylene (TCE) is a volatile and colorless liquid that is miscible with many non-polar organic solvents. It has been found in underground water and many surface waters as a result of the manufacture, use, and disposal of the chemical. It is used mainly as a degreaser for metal parts. It is also an ingredient in adhesives, paint removers, typewriter correction fluids. Trichloroethylene is readily absorbed in
the gastrointestinal tract and the lungs. In humans and animals, initial uptake following inhalation is rapid. The major metabolic route is oxidation (by cytochrome P-450 mixed-function oxygenase) giving trichloroethanol, trichloroethanol-glucuronide and trichloroacetic acid as the major metabolites (identified in both humans and animals). Chloral hydrate is an intermediate in this oxidative biotransformation and its formation is probably preceded by the conversion of trichloroethylene to its epoxide. Several minor metabolites of trichloroethylene have also been identified, including the mercapturic acid N-acetyl-S-(dichlorovinyl)-L-cysteine (DCVC), which is formed in the kidneys from the glutathione conjugate of trichloroethylene. Occupational exposure to trichloroethylene is associated with excess incidences of liver cancer, kidney cancer and non-Hodgkin lymphoma.¹²³

Caspases are a family of cysteiny1 aspartate-specific proteases that are highly conserved in multicellular organisms and function as central regulators of apoptosis. Caspase-3 is one of the key mediators in apoptosis. This protein cleaves and activates caspases 6 and 7 and the protein itself is processed and activated by caspases 8, 9, and 10. It is the predominant caspase involved in the cleavage of amyloid-beta 4A precursor protein, which is associated with neuronal death in Alzheimer’s disease. Alternative splicing of this gene results in two transcript variants that encode the same protein. Caspase-3 is activated in the apoptotic cell both by extrinsic (death ligand) and intrinsic (mitochondrial) pathways. Activated Caspase-3 is responsible for the cleavage of poly ADP-ribose polymerase, actin and sterol regulatory element binding protein, which are associated with apoptosis. The zymogen feature of caspase-3 is necessary because if unregulated, caspase activity would kill cells indiscriminately.⁴ Caspase-3 has been found to be necessary for normal brain development as well as its typical role in apoptosis, where it is responsible for chromatin condensation and DNA fragmentation. It is now being shown that caspase-3 may play a role in embryonic and hematopoietic stem cell differentiation.⁵

Glutathione (GSH) is a tripeptide formed by glutamic acid, cysteine, and glycine. The glutamic acid forms a particular gamma-peptic bond with cysteine by its gamma glutamyl group. Two forms of GSH are possible: the reduced form (GSH) which represents the majority of GSH, reaching millimolar concentration in the intracellular compartment, and the oxidized form (GSSG) that is estimated to be less than 1% of the total GSH. Intracellularly, the majority of GSH is found in the cytosol (about 90%), while mitochondria contain nearly 10% and the endoplasmic reticulum contains a very small percentage conjugates with GSH, either spontaneously or enzymatically, in reactions catalysed by GSH-S-transferases (GST). Human GSTs are divided into two distinct family members: the membrane-bound microsomal and cytosolic family members. The conjugates formed are usually excreted in the bile. It can undergo modification to mercapturic acid. Another important GSH function is the maintenance of the intracellular redox balance and the essential thiol status of proteins. Glutathione plays important roles in antioxidant defence nutrient metabolism, and regulation of cellular events (including gene expression, DNA and protein synthesis, cell proliferation and apoptosis, signal transduction, cytokine production and immune response, and protein glutathionylation).⁶ Glutathione deficiency contributes to oxidative stress, which plays a key role in aging and the pathogenesis of many diseases (including Alzheimer’s disease, Parkinson’s disease, liver disease, cystic fibrosis, sickle cell anemia, HIV, AIDS, cancer, heart attack, stroke, and diabetes).⁷

A major function of glutathione (GSH) is the detoxification of xenobiotics and some endogenous compounds. Caspase-3 is one of the key mediator in apoptosis. TCE can produce oxidative stress in human. Oxidative stress can disturb GSH homeostasis and affect apoptosis mechanism. Cellular damage and its potential role in TCE-mediated pathogenesis is not clearly understood. The relationship between the depletion of cellular glutathione, generation of ROS, and activation of caspase-3 is not known. In this work, we aim to understand the relationship between these events as they relate to oxidative stress. Therefore, to assess the effect of TCE exposure on lymphocyte was studied. This study aims to investigate the effects of TCE on the cellular viability, the concentration changes of caspase-3 enzyme and glutathione in
human lymphocytes. Glutathione and caspase-3 concentration changes due to trichloroethylene toxicity may be associated with oxidative stress and lead to increase the progression of cancer.

Materials and Methods

Chemicals

Trichloroethylene (CAS No. 79-01-6) was purchased from Lobachemie (Mumbai, India). MTT (3-[4.5-dimethy-thiazol-2-yl]-2,5-diphenyltetrazolium bromide) was procured from Bio Basic Canada (Ontario, Canada). Dimethylsulphoxide (DMSO) was purchased from Sigma-Aldrich (St. Louis, USA.). RPMI medium and chemical for cell culture were purchased from Thermo Fisher Scientific (Massachusetts, USA).

Specimen collection

Heparinized blood was collected from 30 healthy volunteers (age 20-25 years and no history of hematologic diseases and genetic disorders) after getting their informed consent. Approximately 5 mL of blood was collected from each healthy volunteer. The peripheral blood mononuclear cell (PBMC) isolation was done following Debey’s study. Lymphocytes were cultured for a study on the effects of TCE on lymphocyte viability, the concentration changes of caspase-3 enzyme and glutathione in human lymphocytes.

Study on the effect of TCE on lymphocyte viability

Human lymphocyte cultures from the same subject were separated to be both control (n = 30) and experimental (n = 30) groups. The study about lymphocyte viability was performed by added TCE conc. 0.002, 0.004, 0.008, 0.016 and 0.032 mM/L to the experimental groups, respectively. The 1x10^5 cells/mL of lymphocytes were added in RPMI 1640 medium (containing fetal bovine serum, antibiotics and phytohemagglutinin M) and incubated at 37 °C for 24 h. After 24 h, the concentrations of TCE 0.002, 0.004, 0.008, 0.016 mM/L and 0.032 mM/L were added in lymphocyte cultures and incubated for 48 h. After 48 h incubation time, TCE treated lymphocytes were studied about the concentration change of caspase-3 by Human Caspase-3 Instant ELISA Kit (Invitrogen, USA).

Study on the effect of TCE on the glutathione

The 1x10^5 cells/mL of lymphocytes were added in RPMI 1640 medium (containing fetal bovine serum, antibiotics and phytohemagglutinin M) and incubated at 37 °C for 24 h. After 24 h, the concentrations of TCE 0.002, 0.004, 0.008, 0.016 mM/L and 0.032 mM/L were added in lymphocyte cultures and incubated for 48 h. After 48 h incubation time, TCE treated lymphocytes were studied about the concentration change of glutathione by Glutathione Colorimetric Assay Kit (Biovision, USA).

Statistics analysis

The toxicity of TCE on lymphocyte viability was tested by ANOVA. The effects of TCE on the concentration changes of caspase-3 and glutathione were analysed by ANOVA and p values of less than 0.05 were considered statistically significant.

Results

Effect of TCE on viability of lymphocytes

The study about TCE-induced cytotoxicity at concentrations of 0.002, 0.004, 0.008, 0.016 and 0.032 mM/L for 48 h. were done. The results showed that the percentage of viable lymphocyte was decreased when the concentrations of TCE increased (Figure 1) (p<0.05). The 50% inhibitory concentration (IC_{50}) was 0.029 mM/L which effected growth or depletion of human lymphocytes.
Effect of TCE on the caspase-3 and glutathione

The results showed that the expression of caspase-3 reduced progressively in a dose dependent manner in relation to treatment of lymphocytes with TCE ($p<0.05$) (Figure 2). The results showed that the concentration of glutathione (reduced form) was changed when lymphocytes exposed with TCE as shown in Table 1. TCE reduced the concentration of glutathione (reduced form) in a dose dependent manner ($p<0.05$).

Table 1. The concentrations of glutathione in TCE exposed lymphocytes at the various concentrations of TCE.

<table>
<thead>
<tr>
<th>TCE concentration (mM/L)</th>
<th>Reduced glutathione concentration (μg/ml)±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.126±0.08</td>
</tr>
<tr>
<td>0.002</td>
<td>0.094±0.04*</td>
</tr>
<tr>
<td>0.004</td>
<td>0.067±0.01*</td>
</tr>
<tr>
<td>0.008</td>
<td>0.063±0.01*</td>
</tr>
<tr>
<td>0.016</td>
<td>0.040±0.02*</td>
</tr>
<tr>
<td>0.032</td>
<td>0.030±0.01*</td>
</tr>
</tbody>
</table>

*TCE significantly reduced reduced glutathione concentration at 48 h incubation time compared with the control group ($p<0.05$).

Figure 1. The percentage means of lymphocyte viability at the various concentrations of TCE.

*TCE significantly reduced lymphocyte viability at 48 h incubation time compared with the control group ($p<0.05$).

Figure 2. The percentage means of lymphocyte viability at the various concentrations of TCE.

*TCE significantly reduced lymphocyte viability at 48 h incubation time compared with the control group ($p<0.05$).
Discussion

Many studies have established that the direct treatment of cells with oxidants like hydrogen peroxide induces apoptosis and several non-oxidant apoptogenic agents such as tumor necrosis factor and cycloheximide also elicit oxidative stress. It is indicated that Reactive Oxygen Species (ROS) generation may be a conserved apoptotic event. Several studies suggest that ROS generation and antioxidant depletion mediate the events leading to apoptosis. However, the presence of an oxidative mechanism in apoptosis is unclear.9

TCE metabolism plays a critical role in eliciting its mutagenicity, carcinogenicity and other adverse health effects. Trichloroethanol, trichloroethanol-glucuronide and trichloroactic acid are the major metabolites and the mercapturic acid N-acetyl-S-(dichlorovinyl)-L-cysteine (DCVC) is a minor biotransformation product of TCE. TCE or its metabolite DCVC can stimulate the increasing ROS generation, increasing pro-inflammatory response, increasing apoptosis, and decreasing supply of energy metabolites. TCE can induce oxidative stress in cells.10 Huang et al, 2015 observed that TCE was associated with increased variability of DNA methylation. DNA methylation may play a role in the pathogenesis of TCE exposure-related diseases and lymphocytes decreased with increased exposure to TCE.11 Our study observed that TCE could decrease lymphocyte viability.

Apoptosis involves the activation of caspases. Caspases (cysteinyl aspartate proteases) are expressed as zymogens and become activated by an apoptotic signal. Once a caspase is activated, it may initiate an amplified apoptotic pathway by activating other caspases, leading to rapid cell death. 14 mammalian caspases have been identified and categorized into initiator, effector, and inflammatory caspases. Caspase-3 is the major effector caspase activated by initiator caspases in the execution phase of apoptosis. Once activated, the protease cleaves proteins involved in DNA and cytoskeleton structure, leading to irreversible self-destruction. The activation of caspase-3 may depend on redox status. Activated caspase-3 is responsible for the cleavage of poly ADP-ribose polymerase, actin and sterol regulatory element binding protein, which are associated with apoptosis. TCE-induced DNA damage was associated with significant activation of PARP-1 and increases in caspase-3, cleaved caspase-8 and -9 in the liver cells.12 The effects of TCE toxicity were studied on the normal human liver cells (LO2 cells) and hepatocytes with CYP2E1 gene overexpression. It showed that caspase-3, caspase-8 and caspase-9 mRNA expression increased by 30% - 600% in CYP2E1-overexpressing cells by TCE.13 Oral administration of TCE in rats induced DNA strand breaks and induced the expression of caspase-3,-7 and -9.14 TCE could induce oxidative stress, reduced cellular viability in lymphocytes and it also increased caspase-3 activation in this study. The concentration of caspase-3 was increased when TCE concentration increased.

Glutathione (GSH) is an important regulator of the cellular redox condition. Glutathione exists in both the cytosol and the mitochondria. It is the most abundant, non-protein physiological antioxidant and involved in regulation of the cell cycle. The sulfhydryl group (−SH) of the cysteine is involved in reduction and conjugation reactions that are usually considered as the most important functions of GSH. Sources of oxidants GSH plays a major role in removal of many reactive species. Glutathione regulates the action of glutathione-peroxidases and glutathione-transferases.6 A decrease in the cellular antioxidant concentration is associated with the generation of an excess of ROS. Xenobiotics and some endogenous compounds are electrophiles and form conjugates with GSH, either spontaneously or enzymatically, in reactions catalysed by GSH-S-transferases (GST). Human GSTs are divided into two distinct family members: the membrane-bound microsomal and cytosolic family members. The conjugates formed are usually excreted in the bile, but can also undergo modification to mercapturic acid. Another important GSH function is the maintenance of the intracellular redox balance and the essential thiol status of proteins. Glutathione exists in reduced (GSH) and oxidized (GSSG) states. The ratio of reduced glutathione to oxidized glutathione within cells is a measure of cellular oxidative stress where increased GSSG-to-GSH ratio is indicative of greater oxidative stress. In healthy cells and tissue, more than 90% of the total...
The glutathione pool is in the reduced form (GSH), with the remainder in the disulfide form (GSSG). In the reduced state, the thiol group of cysteinyl residue is a source of one reducing equivalent. Glutathione disulfide (GSSG) is thereby generated. The oxidized state is converted to the reduced state by NADPH. This conversion is catalysed by glutathione reductase. TCE could alter glutathione redox homeostasis and glutathione precursors. GSH plays a vital role in the protection of TCE-induced oxidative stress and apoptosis, which may be mediated through a p53-dependent pathway in human lung cancer cells. TCE induced deactivation of cytochrome P-450 and loss of liver glutathione in male rat. Its depletion could induce apoptosis.

Experimental animal and human data indicate that TCE metabolism occurs through two major pathways: cytochrome P-450 (CYP)-dependent oxidation and glutathione (GSH) conjugation catalyzed by GSH S-transferases (GSTs). GSH depletion can induce apoptosis. The disturbances in GSH homeostasis are involved in the etiology and progression of many human diseases including cancer. While GSH deficiency, or a decrease in the GSH/glutathione disulfide (GSSG) ratio, leads to an increased susceptibility to oxidative stress implicated in the progression of cancer, elevated GSH levels increase the antioxidant capacity and the resistance to oxidative stress as observed in many cancer cells.

TCE-induced DNA damage was associated with increases in the levels of caspase-3 in liver cells and glutathione plays a vital role in the protection of TCE-induced oxidative stress and apoptosis, which may be mediated through a p53-dependent pathway. The concurrent depletion of glutathione and generation of ROS may be required for the activation of caspase-3. Our study observed that TCE treated lymphocytes reduced glutathione concentration in cells. The concentration of glutathione was decreased when TCE concentration increased. It is concluded that TCE caused oxidative stress in cells. Oxidative stress caused by TCE treated lymphocyte could reduce lymphocyte viability and it changed the concentrations of caspase-3 and glutathione in human lymphocytes.

Conclusion

TCE could reduce viability of lymphocytes and significantly changed the expression of caspase-3 and glutathione concentrations in human lymphocytes.

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Ethical Clearance: This study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by Thammasat University ethical committee. The project identification code was EC 175/2020 and date of approval was Jan 20, 2021.

Conflict of Interest: The authors declare no conflicts of interest.

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Fungal Hallucinogens: Chemistry, Synthesis, Behavior, Toxicity and Detection Methods in Forensic Perspective

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Abstract

Certain species of the mushrooms contain the Indole Alkaloids such as Psilocybin and Psilocin which exhibit the psychedelic property. These types of mushrooms can be identified by their unique property known as the blue bruising. These alkaloid compounds may cause the hallucinations and other symptoms. The psilocybin and Psilocin are synthesized from the amino acid Tryptophan and resemble the structure of the hormone Serotonin. They also act as an agonist for the 5-HT₂A receptors. Intoxication of these compounds can lead to excitation of the nerves which can eventually cause the hallucination, tachycardia, dilated pupils and dysuria in certain cases which may lead to adverse effects. The metabolic product of the Psilocin is psilocin-o-glucuronide which is excreted in the urine within 6-8 hours of ingestion. Psilocybin and psilocin can be analyzed by preliminary color tests with Ehrlich reagent, Marquis reagent and Mandelin’s reagent. In this review, brief reports are presented about the chromatographic analysis of these compounds. This article also discusses the overview of the psychoactive alkaloids present in certain species of mushrooms.

Key words: Psilocin, Psilocybin, Serotonin, 5 HT 2A receptors, agonist, detection limits.

Introduction

Psilocybin and its active metabolite Psilocin is the prime psychedelic compound in the psychoactive mushrooms such as Psilocybe, Conocybes, Inocybes etc.¹ The mushroom producing the Psilocybin and the Psilocin has gained an attention from ancient period. Upon studies Guzzman framed a classification depending on the nature of the compound present in it². The unique characteristic of the mushrooms that produces psilocybin and its active metabolite Psilocin is generally called as the “blue bruising” which may due to their metabolism of action. Because of this unique property, the mushrooms can be easily identified³. This bruising property is generally adephosphorylation and an oxidation process, where the dephosphorylation is catalyzed by the PsiP enzyme and oxidation of the Psilocin under the catalysis of the enzyme PsiL present in the mushrooms.
The mushrooms producing these compounds were later noticed by the scientists and then studied. These compounds were first isolated and identified in 1958 and they were first synthesized in 1959 by Albert Hoffman. Upon studies, the contents vary in range from 0.2% - 1% in dry weight. The psilocybin is the major ingested form which is converted into the psilocin by the enzymes present in the body which dephosphorylates the Psilocybin into the active metabolite Psilocin. These compounds present in the mushrooms affect the nervous system and causes neurological effects such as hallucination, auditory hallucination and mental alterations. This is due to their structure which resembles the Serotonin hormone. They differ from each other by functional group, where the Psilocybin contains the phosphoryl group and its active metabolite has a hydroxyl group. Due to their structural similarity, they have higher affinity towards the 5-HT receptors. Their adverse effects are seen within an hour of ingestion. The compounds were metabolized by the body and they are excreted as conjugated compounds. They are eliminated gradually in the urine.

The aim of this review article is to bring an updated knowledge on Psilocybin and its active metabolite Psilocin with the historical perspective and their mechanism and metabolism. This also includes the clinical responses to their intake which is staged into three phases. The toxicity of these compounds is also discussed in this article.

Components of Group I Psychoactive Molecules - Psilocybin and Psilocin

Psilocybin and Psilocin are psychoactive molecules that are commonly found in the genus *Psilocybe* predominantly and in other associated species. The ingested form is found to be the Psilocybin which then converted into psilocin by dephosphorylation reaction in the body. This conversion is catalyzed by the enzymes that are generally alkaline phosphatase. These compounds can act very similar to the LSD (Lysergsäurediethylamid) and Mescaline causing the neurological effects.

The milestone about the psilocybin and its active metabolite Psilocin was studied by Albert Hoffmann. The Swiss chemist Albert Hoffman initially started to study about the LSD by ingesting it himself during 1930s and for which he was called as Father of LSD. In 1958 Albert Hoffman and his colleagues isolated Psilocybin and trace amounts of Psilocin for the *Psilocybe Mexicana*, which created a mark on psychoactive drugs. This was the first natural phosphorylated compound detected. With references to the study, there was a great development in the understanding of the compound which leads to Psilocybin assisted therapy. Upon the studies, it was found that they resemble the structure and their properties are detailed.

Structure of Psilocybin and its active metabolite Psilocin

The Psilocybin and its active metabolite Psilocin are Tryptamine derived Indole alkaloids isolated from the mushrooms exhibiting the hallucinogenic properties. The psilocybin is also called as O-phosphoryl-4-hydroxy-N,N-dimethyltryptamine and the psilocin is called as 4-hydroxy-N,N-dimethyltryptamine. The Psilocybin and its active metabolite Psilocin have the backbone structure of Tryptophan. Hence they resemble the structure of Serotonin hormone and being an agonist of it. From the Figure, the structural difference of the compounds can be noted by the presence of Phosphoryl functional group in Psilocybin and a hydroxyl group at the functional site of Psilocin. The psilocybin when ingested it rapidly dephosphorylated to Psilocin.

Physico-chemical Characteristics

The Psilocybin usually tastes like ammonia. The Psilocybin is found in the form of colorless crystals in boiling water and it is sensitive to temperature. Its melting point is found to be 224.0°C. It is fairly soluble in boiling methanol, water and difficultly soluble in ethanol and insoluble in chloroform and benzene. Its molecular weight is found to be 284.27 g/mol. They appear as green crystals when extracted. Psilocin is lipid soluble and hence it is diluted by acidified aqueous solution and very slightly soluble in water. Its melting point is found to be 173-176°C. Its molecular weight is to be 204.3g/mol.

Biosynthesis of Psilocybin and its active metabolite Psilocin

The biosynthesized Psilocybin and its active metabolite Psilocin synthesized from the mushrooms
were studied by using radio isotopes. Later, these compounds have been synthesized using dibenzylphosphoryl chloride by Hoffmann which was replaced by Tetrabenzyl pyrophosphate. Later Kargbo initiated direct phosphorylation of Psilocin by using Phosphoryl chloride. The first published synthetic event which included the labelling of radiotracer has explained the derivation of Psilocybin and psilocin by Agurell and Nilsson in 1968 by $^{14}\text{C}$ and $^3\text{H}$. Later Fricke et al. made a refined biosynthetic pathway by sequencing the genomes of \textit{Psilocybecubensis} and \textit{Psilocybecyanescens}. They used the 4-hydroxy-\textit{L}-tryptophan as the substrate and identified enzymes that are involved in the biosynthesis. The enzymes were named as PsiD, a decarboxylase enzyme and PsiH a monoxygenase, Psik a kinase that transfer the phosphoryl group and a PsiM an S-Adenosyl dependent Methyl Transferase which mainly catalyzes the terminal Methylation step.

In this synthesis pathway the \textit{L}-tryptophan is decarboxylated as in the Agurell description leading to the formation 4-hydroxy Tryptamine. The 4-hydroxy tryptaine is further phosphorylated to Norbaeocystin by the action of PsiK enzyme. This Norbaeocystin is then converted to Baecystin with PsiM enzyme upon methylation, upon further methylation the psilocybin is formed in the presence of PsiM enzyme. Further the dephosphorylation converts the psilocybin to psilocin. The genome and the loci identified by Fricke and the loci is seen in figure.1. The psiR represents the Putative Transcriptional Regulator. The psiT1 and psiT2 are the major facilitator type transporter. The psiK has Kinase enzyme activity. The psiH is a P 450 monoxygenase enzyme locus. The psiM is for the encodation of Methyl transferase enzyme. The psiD is responsible for the decarboxylase enzyme.

![Figure 1: Map of loci of the Psilocybin and its active, metabolite Psilocin biosynthesis](image)

**Mechanism of action**

The psilocin, active metabolite targets at the $5\text{HT}_{2\text{A}}$ receptors. These receptors are adversely expressed in the apical dendrites of the cortical pyramidal cells. In humans, the active compound psilocin is present in a significant amount in the plasma within 20-40 minutes after ingestion. The maximum concentrations were found to reach in 80-100 minutes. The effects are completely eliminated within 4-6 hours. The elimination half-life of Psilocybin in the plasma is found to be 160 minutes and the active metabolite Psilocin half-life is found to be 50 minutes. The pharmacokinetic and forensic studies on the compounds revealed that psilocin is mostly (80%) eliminated as psilocin-O-glucuronide as a result of conjugation process. The conjugated compound further undergoes enzymatic hydrolysis extends their detectability in the urine samples. The figure 2 describes the mechanism of action of Psilocin.

The mechanism of action is by activation of prefrontal network and glutamate release by the intoxication of compound. The compound may increase the extracellular glutamate levels by prefrontal cortex through the stimulation of post synaptic serotonin especially $5\text{HT}_{2\text{A}}$ receptors that are located on the large glutamatergic pyramidal cells which are seen in the cortical layer V and layer VI layer of cortical cells, where layer V projects to the neurons.

When the glutamate is released it leads to the activation of AMPA ($\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolle propionic acid) and NMDA (N-methyl-D-aspartate) receptors. The psilocin can also directly activate the 5 HT $^{2\text{A}}$ receptors. This activation is thought to ultimately lead to increase of brain derived neurotrophic factor (BDNF).
The mechanism of action is very closely related with other compounds such as dopamine, epinephrine and other bio amines. The psilocybin increases the 5-HT$_{2A}$ receptors in the post synaptic membranes and stimulates neural excitatory effects. The dissociation constant ($K_i$) of psilocybin is $>10,000\text{nM}$ whereas the psilocin has ($K_i$) $107.2\text{nM}$ which means the psilocin can bind more strongly to the receptor. The pharmacokinetics of Psilocybin and Psilocin is well established. After ingestion, the rapid dephosphorylation of Psilocybin occurs under acidic environment of the stomach or by alkaline phosphatase (and other nonspecific esterase) in intestine and kidney. This may cause to generate the Psilocin and the Psilocin in turn which can easily cross the blood brain barrier. The inhibition of the enzyme alkaline phosphatase can cause competitive substrates such as β-glycerophosphate which prevents the symptoms of intoxication.

Upon further metabolism, the Psilocin is demethylated and deaminated oxidatively by the liver. This process is catalyzed by the Monoamine Oxidase enzyme (MAO) or by an alcohol dehydrogenase. The intermediate formed was 4-hydroxyindole-3-acetaldehyde which may yield 4-hydroxy-indole-3-acetic acid, 4-hydroxy-indole-3-acetaldehyde and 4-hydroxytryptophol. The Psilocin may cause the competitive inhibition of the MAO. It also may metabolize the Serotonin which in turn elevates their levels and simultaneously decreases the 5-HIAA. It can also undergo minor oxidative pathway which leads to the formation of the O-quinone or Immunoquinone compounds. They are oxidized either enzymatically with hydroxyindol oxidase enzymes such as ceruloplasmin etc., or non-enzymatically with Fe$^{3+}$.

In the analysis of the serum sample obtained after 5 hours of intoxication, it showed that 80% of the Psilocin in the conjugated form of Psilocin-O-glucuronide and it is eliminated in the urine. This glucuronidation of the hydroxyl group is the important detoxification step. The extensive glucuronidation is achieved by the UDP Glucuronosyl Transferase (UGT)1A10 in the small intestine. The UGT1A9 is the main contributor to the glucuronidation when it is absorbed in the circulation.

Toxicity and LD$_{50}$

As discussed in the clinical response, the Psilocin binds to the 5-HT$_{2A}$ receptors and causes the neuronal excitation and exhibits the symptoms. In the case of the toxic effects, the hyper activation of the receptor causes the symptoms such as drowsiness, increases in mood changes, altered state of consciousness, weakness, dizziness, nausea and vomiting in humans. When the dose exceeding 15mg may create severe
effects such as acute agitation, shift in emotions, sensory modalities and visual perceptions. Upon the study of the lethality of these compounds, the LD$_{50}$ for animals has been reported in Table 1.$^{32}$

Table 1: Toxicity level of Psilocybin

<table>
<thead>
<tr>
<th>Animal Model</th>
<th>Route of administration</th>
<th>LD$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Intraperitoneal</td>
<td>196 mg/kg</td>
</tr>
<tr>
<td></td>
<td>Intravenous</td>
<td>74 mg/kg</td>
</tr>
<tr>
<td>Rat</td>
<td>Intravenous</td>
<td>75 mg/kg</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Intravenous</td>
<td>7 mg/kg</td>
</tr>
</tbody>
</table>

Forensic analysis from bodily fluids and hair

In the analysis of the bodily fluids, generally the active metabolite Psilocin is present rather than Psilocybin. During the analysis the blood samples, the samples were centrifuged and the plasma is collected for the analysis. The proteins present in the plasma were precipitated by the addition of methanol or by adding 20% of PEG 6000 on ice. In some cases, to prevent the oxidation of Psilocin, ascorbic acid may be used and this is essential to pre column in the HPLC separation of the compounds.$^{36}$

Once after the separation of precipitated proteins at a slight acidic condition, the solid phase extraction is performed. In the cation exchange sorbent separation, the precipitated proteins were adjusted to the pH 6.8 with lithium acetate where it aids in protonating the compound which is eluted with methanol$^{37}$. In the reverse phase, Bond Elut C18 cartridges were used. The blood and urine samples up to 2-1 ml can be treated with magnesium carbonate buffer and at a pH of 9.3. Then, the product is eluted with methanol and acetic acid$^{38}$. The enzymatic reaction with glucuronidase will yield a relatively high concentration of psilocin from plasma, serum and urine samples$^{39}$.

In detecting the Psilocin and Psilocybin in the bodily fluids, the capillary electrophoresis is a common method in qualitative and quantitative analysis. Their limit of detection is found to be a 13 ng/ml of urine (direct measurement) and 7 ng/ml of urine (with a system of preliminary Sequential Injection -SPE)$^{40}$. When it comes to the Gas chromatography along with spectrometry generally electron ionization mode is applied in the detection of the Psilocin and Psilocybin$^{41}$. Among these techniques, it is believed that the more prominent detection of Psilocin and Psilocybin is achieved by Chemiluminescence methods$^{41}$.

In the cases of forensic evaluation, the DNA study plays a major role. Generally, the Psilocin and the Psilocybin were determined using the PCR (polymerase chain reaction) techniques specifically by the Amplified Fragment Length Polymorphism. One of the prominent screenings is found to be the Internal Transcribed Spacer region analysis which leads a great analysis method by reaching the relationships between the genius and species. In a recent study, there was a method developed for the detection of Psilocin from hair by using LC-MS/MS method. Initially 10 mg of cut hair is taken in 1 ml of acid methanol in the presence of Psilocin-d$_{20}$ for 2 hours at 4$^\circ$C. The chromatographic separation was performed with reverse phase column HSS C18 with a gradient elution of 8 mins. The estimated limit of detection was 0.4 pg/mg. When a person is exposed to these compounds repetitively, then the concentration would be 2.5 pg/mg for segment of 0-1 cm, 4.4 pg/mg for segment 1-2 cm and 5.4 pg/mg for segment 2-3 cm$^{42}$.

Detection techniques

There are preliminary test that helps to detect the presence of the psilocybin and the psilocin. The test includes the reagents such as the Ehrlich reagent and the Marquis reagent which are commonly used in identifying the Indole compounds. This color test is only to detect the presence of compounds further for confirming the compounds, chromatography is required.

Ehrlich test

In the Ehrlich test, p-dimethylaminobenzaldehyde along with methanol under acidic condition with the help of concentrated orthophosphoric acid leads to the electrophilic substitution reaction producing grey violet – violet color$^{43}$.

Marquis test

In the Marquis test, 40% formaldehyde with glacial acetic acid under acidic condition maintained
by concentrated sulfuric acid causes the green-brown color which indicates the possible presence of Psilocin$^{44}$.

*Mandelin’s test*

The reagent is composed with 1% ammonium vanadate in sulfuric acid which determines a wide range of colors where reacting with psilocybin produces a green color.

*Thin Layer Chromatography (TLC)*

In the TLC, the solvent system consists of n-Butanol: acetic acid: water is used. For visualization, the UV and spray agents can be used. In the UV visualization, the compounds can be visualized at 254 nm and 365 nm and appears as dark blue spots. There are two spray reagents such as Ehrlich Reagent and Paradimethylaminocinnamaldehyde. The paradimethylaminocinnamaldehyde produces blue and Ehrlich reagents produce violet – grey violet color. The detection limit of paradimethylaminocinnamaldehyde is 20ng of Psilocybin and 10ng of Psilocin. The retention factor ($R_f$) is found to be 34 for Psilocybin and 59 for Psilocin$^{43}$.

*Detection limits*

In the detection of Psilocybin and Psilocin, there are various techniques involved and each of the techniques has its own detection limits. Table 2 explores the overall techniques used and their detection limits.

**Table 2: Overall techniques and their Detection limits$^{45}$**

<table>
<thead>
<tr>
<th>TECHNIQUES</th>
<th>DETECTION LIMITS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PSILOCIN</td>
</tr>
<tr>
<td>Ion mobility spectrometry</td>
<td>-</td>
</tr>
<tr>
<td>HPLC</td>
<td>UV Absorption</td>
</tr>
<tr>
<td></td>
<td>Fluorescence</td>
</tr>
<tr>
<td></td>
<td>Electrochemical</td>
</tr>
<tr>
<td></td>
<td>ESI-MS</td>
</tr>
<tr>
<td>GC-MS</td>
<td>4.9 x 10^{-4}</td>
</tr>
<tr>
<td>TLC</td>
<td>1.5 x 10^{-4}</td>
</tr>
<tr>
<td>Capillary Electrophoresis</td>
<td>-</td>
</tr>
<tr>
<td>Chemiluminescence</td>
<td>9.0 x 10^{-10}</td>
</tr>
</tbody>
</table>

**Conclusion**

The psychedelic compounds present in magic mushrooms may cause neurological discomforts in humans because of their oxidizing behaviour. The fungal hallucinogenic compounds, such as, Psilocybin and Psilocin can play a major role in the therapeutic applications of the neuronal disorders. These compounds seemed to be efficient in the therapeutic studies and they also have an effective response in treatments. Furthermore, they also possess psychedelic effects because of which they can also cause toxic effects. Upon the action, the molecules increase the 5HT 2A receptors at the synaptic membranes. This leads to neuronal excitation and leads to hallucination in severe cases. The metabolic fate of the psilocin is eliminated as psilocin-O-glucuronide. Due to the higher stability, they may extend the time of detection in the urine samples. These can be detected using various techniques such as capillary electrophoresis, gaschromatography, HPLC and also with chemiluminescence technique. Biosensors can be used in the detection of the mycotoxins and ergot alkaloids. Future studies focusing in these perspectives may help in better detection of these compounds in the human samples and also in the forensic analysis.

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Conflict of Interest: None declared.

References


A Comparative Study to the Persistence Post COVID Syndrome and the Infection Period between Vaccinated and Non-vaccinated Patients in Al Basrah Province

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Abstract

Introduction: The base stones in COVID-19 management were symptoms resolution and the mortality avoidance. Consequently, there were a focus on the early recognition and the appropriate treatment however the assumption of that COVID-19 patients suffer ends with the end of infection were not completely write. Persistent post-COVID syndrome (PCS) also called long COVID is a pathologic state, which involves the persistent of a physical and medical abnormal conditions after three weeks of COVID-19 infection diagnosis.

Objective: A study was conducted to compare the persistence of COVID syndrome and the infection period between vaccinated and non-vaccinated patients.

Methodology: Data of a total of 756 volunteers were analyzed. and divided in to two main groups of Covid-19 infected people: 1- vaccinated and 2-non-vaccinated patients each group were subdivided in to two other subgroups: a-patients with infection period less than 14 days and b-patients with infection period more than 14 days, performing the research in Al-Basra province. A 12 questions form was established and filled by making direct interviews with the volunteers.

Results: 28% of individuals reported unvaccinated and 71.95% report vaccinated. vaccinated participants in the study (71.95%±12.56 SD) have an infection period of (1-14) days (78.62%±10.58 SD), whereas (21.25%±4.76 SD) have an infection period of more than 14 days. There were differences in the symptom spectrum between the groups. Long-term persistent symptoms such as cough, hair loss, impotence, loss of taste and/or smell, poor memory, and dyspnea were significantly associated with vaccinated participants when compared to the unvaccinated participants, who had the same duration of illness (1-14) days.

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Conclusion: People have to be more aware about COVID-19 and should get vaccinated, another topic is how longer residual PCS last and whether they have a long-term impact on quality of life.

Keywords: Post COVID syndrome, COVID-19, Symptoms, Vaccine.

Introduction

Since December 2019, coronavirus pandemic has cross the many parts of world creating millions of infected people and hundreds of thousands of dead, the base stones in COVID-19 management were symptoms resolution and the mortality avoidance. Consequently, there were a focus on the early recognition and the appropriate treatment however the assumption of that COVID-19 patients suffer ends with the end of infection were not completely write.

Sever acute respiratory syndrome- corona virus 2 (SARS-CoV-2) which affect both lower and upper respiratory tract producing symptoms starting from sore throat, common cold, fatigue, fever, dry cough, nasal congestion, and sometimes diarrhea reaching to severe pneumonia, difficulty in breathing and ends with death beside the disrupted flavor and taste sensation earlier before respiratory involvement, some of these symptoms persist even after recovery.

Persistent post-COVID syndrome (PCS) also called long COVID is a pathologic state, which involves the persistent of a physical and medical abnormal conditions after three weeks of COVID-19 infection diagnosis. This pathological state ranges from cough, breathlessness, poor memory, impotence, hair loss and loss of smell or taste reaching to pulmonary and cardiac fibrosis.

The PCS cause of happening is multifactorial and more than one mechanism may be involved, mainly the immune response after trauma or sever infection which is a systemic inflammatory response a long-lasting counterbalance anti-inflammatory response will occur. And despite the vaccine development with different production strategies and with different protection margin, still there was a small percentage of people who received the food and drug administration (FDA) recommended vaccine doses still acquiring symptomatic or asymptomatic SARS-CoV-2 infection, this called a vaccine breakthrough infection which defined as the detection of SARS-CoV-2 RNA or antigen in a respiratory specimen collected from fully vaccinated person after 14 days or more from the last dose of COVID-19 vaccine. This lead to the question of does the infected vaccinated people also suffer the PCS and dose vaccination strengthen the humans’ immunity so they could compete the disease in shorter duration in this study These questions will be investigated and discussed.

Materials and Method

Data of a total of 756 volunteers were analyzed. and divided in to two main groups of Covid-19 infected people: 1- vaccinated and 2-non-vaccinated patients each group were subdivided in to two other subgroups: a-patients with infection period less than 14 days and b-patients with infection period more than 14 days, performing the research in Al-Basra province. A 12 questions form was established and filled by making direct interviews with the volunteers, this form was covered by a massage explain the research purpose and background. Volunteers were asked if they have PCS like cough, breathlessness, poor memory, impotence, hair loss and loss of smell or taste. The answers considered to be a yes or no questions represented in a binary feature either 1 for having the specific PCS or 0 for not. Other information was asked from each volunteer including age, sex, province, chronic diseases, type of vaccine and the method of COVID-19 infection diagnosis. The Inclusion criteria included age above 18, with no risk factors or chronic diseases and belonging to Al-Basrah city, any volunteer below the age 18, with risk factors or chronic diseases and/or doesn’t settled in Al-Basrah were not included, the interviews started from 4th of October to 25th of November, 2021. after data collection were finished, data analysis by using t- test were done to calculate significance. A comparative measure between the four subdivisions were made to determine the percentage of the persistence of PCS and the duration of infection.

The Symptom form’s composition

To avoid terminology misunderstanding, the duration of the condition was measured from the
time it was first diagnosed with COVID-19, with the premise that at least two months should elapse following healing.

The following systems were used to categorize patients’ symptoms: general (tiredness, fever, and rhinorrhea), respiratory (abdominal pain, breathlessness, and cough), cardiovascular (increased blood pressure, ischemia, and MI), diabetic, dermatology (hair loss and rashes), and GIT (gastrointestinal infection) (abdominal pain, loss of smell and taste, vomiting and diarrhea). A special form was used to assess each symptom.

Statistical analysis

When appropriate, percentages, frequencies, and mean SD were utilized. A p value of less than 0.05 was considered statistically significant. Version 19.0 of Predictive Analytics Software was used. In parametric situations, the t-test was employed to compare continuous variables for distributed data and categorical variables. Multivariate logistic regression was used to identify independent factors of symptoms.

Pre-Processing

The main steps in pre-processing are to remove all of the samples that had missing data in all categories. There are no missing values for the symptoms features since, as binary features, they refer to “1” when a participant indicates they have a symptom and “0” otherwise. Following pre-processing, a sample of 756 participants (494 with symptoms after recovering and 262 with no symptoms after recovering) who meet the criteria is obtained. With this number of samples, it is possible to assume a confidence level of 100% when evaluating the region mentioned above.

Results

According to the findings of this study, 756 people who had recovered from COVID-19 were asked whether they wanted to participate in the study, and they all accepted. We received 756 forma replies in 168 hours, from 4th of October to 25th of November 2021. The overall mean age of the participants was 34.5 years (SD ±2.91). The total sample for the validation study was 756 people that filled out the form (317 recorded men and 439 recorded females). 28% of individuals reporting unvaccinated and 71.95% reporting vaccinated. Figures 1 and 2 show the sociodemographic features of the study population.

For those who have recorded, all of whom are healthy individuals (not have risk factor or any chronic disease). The most prevalent answers for the diagnosis of COVID 19 infection by Pcr, Ct scan, and others are (64.3%), (16.6%), and (19.1%), respectively. More than half of those who have participated have received the Pfizer vaccine (70.77%) and about (17.64%) have received the Sino pharm vaccine while the remainder takes AstraZeneca (11.58%).

Furthermore, our findings clearly demonstrated that non-vaccinated people in study (28% ±7.43 SD), (68.09% ±3.37 SD) of them had an infection period 1-14 days, whereas (31.51%± 2.74 SD) of them had an infection period more than 14 days.

Vaccinated participants in the study (71.95%±12.56 SD) have an infection period of (1-14) days (78.62%±10.58 SD), whereas (21.25%±4.76 SD) have an infection period of more than 14 days.

Table 1, 2 shows the characteristics of participants who answer the form about symptoms that persist after infection recovery.

There was a non-significant difference (p<0.05) in the ages of the participants in the study, with 34.5% having an age of (18-25), 35.45% having an age of (26-35), and 30% having an age of (>35), p=0.052.

Health Status:

In this study, roughly (48.15 %) of the participants who were recorded as vaccinated had no apparent symptoms, while about (30.47 %) exhibited obvious symptoms.

Discussion

The study’s findings will help raise public awareness about the COVID-19 epidemic’s implications. The current study used a form to collect the majority of its data, found a clear difference in PCS between vaccinated and unvaccinated people. During the interview asks participants a variety of questions, the most essential of which are PCS, whether they were vaccinated or not, as well as the types of vaccines they received and the number of
days they were infected\textsuperscript{19}.

Regarding to the study (Table 1, 2), the strongest indications of PCS infection were cough, loss of smell and taste, hair loss, loss of concentration, impotence, and shortness of breath.

**Table 1: Characteristic of non-vaccinated participants about symptoms that appears after recovery (n=212).**

<table>
<thead>
<tr>
<th>Symptom that appears after recovery</th>
<th>Infection duration ranges from 1 to 14 days No.</th>
<th>Percentage %</th>
<th>Infection duration more than 14 days No.</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough</td>
<td>28</td>
<td>13.2</td>
<td>12</td>
<td>5.66</td>
</tr>
<tr>
<td>Breathlessness</td>
<td>19</td>
<td>8.96</td>
<td>8</td>
<td>3.77</td>
</tr>
<tr>
<td>Poor memory</td>
<td>32</td>
<td>15</td>
<td>11</td>
<td>5.18</td>
</tr>
<tr>
<td>Impotence</td>
<td>13</td>
<td>6.13</td>
<td>2</td>
<td>0.94</td>
</tr>
<tr>
<td>Hair loss</td>
<td>23</td>
<td>10.8</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>loss of smell or taste</td>
<td>30</td>
<td>14</td>
<td>19</td>
<td>8.96</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>145</strong></td>
<td><strong>68.0%</strong></td>
<td><strong>67</strong></td>
<td><strong>31.51 %</strong></td>
</tr>
</tbody>
</table>

**Table 2: Characteristic of vaccinated participants about symptoms that appears after recovery (n=544).**

<table>
<thead>
<tr>
<th>Symptom that appears after recovery</th>
<th>Infection duration ranges from 1 to 14 days No.</th>
<th>Percentage %</th>
<th>Infection duration more than 14 days No.</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough</td>
<td>50</td>
<td>9.19</td>
<td>10</td>
<td>1.8</td>
</tr>
<tr>
<td>Breathlessness</td>
<td>20</td>
<td>3.67</td>
<td>4</td>
<td>0.73</td>
</tr>
<tr>
<td>Poor memory</td>
<td>39</td>
<td>7.16</td>
<td>8</td>
<td>1.47</td>
</tr>
<tr>
<td>Impotence</td>
<td>20</td>
<td>3.67</td>
<td>3</td>
<td>0.55</td>
</tr>
<tr>
<td>Hair loss</td>
<td>58</td>
<td>10.66</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>loss of smell or taste</td>
<td>54</td>
<td>9.9</td>
<td>5</td>
<td>0.919</td>
</tr>
<tr>
<td>There are no visible signs.</td>
<td>187</td>
<td>34.37</td>
<td>75</td>
<td>13.78</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>428</strong></td>
<td><strong>78.62 %</strong></td>
<td><strong>116</strong></td>
<td><strong>21.249 %</strong></td>
</tr>
</tbody>
</table>

According to our findings, 262 people in the vaccinated group (n=544) exhibit no PCS.

In this study, when comparing the data between healthy groups (vaccinated and unvaccinated), there was a significant difference (p<0.05) between vaccinated people and unvaccinated people in the duration of illness (1-14) days through the symptoms following recovery from infection, p=0.017.

There was an insignificant difference (p<0.05) between (vaccinated people and unvaccinated people) in the duration of illness lasting more than 14 days through the symptoms following infection recovery p=0.085, Figure 1,2 represents the value difference in the PCS.
Negative PCR is not the end of patient monitoring for individuals recovered from COVID-19; continued and long-term monitoring of the patients is required for evaluation of post-COVID-19 manifestations and early intervention with the important indicators. Furthermore, continuing counseling with the subjects is critical not only for keeping strong adherence to the drugs, but also for detecting early warning indications of developing significant symptoms. Medication adherence has a positive impact on patient outcomes\textsuperscript{20}.

There are a number of benefits to our research. First, the study had the largest number of participants, with 756 people in Al-Basrah. Second, this study examines PCS using a one-to-one question. Third, depending on the vaccination and the length of illness, the study included a sufficient number of people from each category. Fourth, in our study, we enrolled a comparison group, which allowed us to compare a persistent symptom between persistence post-COVID vaccinated and other non-vaccinated, and to demonstrate that long-term symptoms were more persistent in unvaccinated COVID-19 people.
Limitations

Our study had several limitations, the most significant of which being the survey’s timing, which runs from 4th of October to 25th of November, 2021. The basis of this investigation is the participants’ self. There could be differences in how patients evaluate, express, and treat their symptoms.

Conclusion

People have to be more aware about COVID-19 and should get vaccinated, according to our findings. This research also concludes that 34.65% of vaccinated people had no PCS, but that the symptoms of the same group of vaccinated people are milder than the symptoms of unvaccinated people. There is misgiving that people’s awareness about immunizations is lacking. As a result, in order to activate their immune systems and protect themselves against the pandemic, individuals must be fully vaccinated.

To summary, there are various unknowns that need to be investigated. Some of these unknowns involve this investigation, such as whether there are persistent symptoms, that is, if they are a warning sign of the body, indicating that damage is still occurring, or if they are a result of the weakening of the systems caused by the COVID 19 infection. Another topic is how longer residual PCS last and whether they have a long-term impact on quality of life. For this, it is necessary to understand the short, medium, and long-term scope of potential physical and psychological consequences following COVID, including in these questions, if the times set for social isolation are adequate.

Without a doubt, one must continue to query in order to get knowledge that adds to understanding how this disease grows, the consequences it might cause, and the best method to deal with it while experiencing the fewest side effects.

Ethics:

The Ethics Committee of the University of Basra/Medical College approved this study design. The initiative received enthusiastic approval from the committee.

Conflict of interest: The authors declared no conflict of interest in the manuscript.

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Reference


The Development and Standardization of a Proctological Agent Based on Black Cumin (Nigella Sativa L.) Extract

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Abstract

Annotation. Nigella sativa L. (Ranunculaceae) is one of the promising plants that has a wide spectrum of pharmacological activity. The black cumin is known to have been used for the treatment of cataracts, bronchial asthma, cholelithiasis and urolithiasis, helminthic invasions, diabetes, hemorrhoids, and many other diseases.

The aim of this study is to develop and standardize a drug based on a dry extract of the black seed– black cumin (Nigella sativa L.).

The technology of preparing suppositories of N. sativa dry extract by fusion molding was proposed, and its antiulcer activity was studied. The quality of suppositories was assessed according to the following indicators: characteristics, average weight of suppositories, melting point, identification, quantification. The study of the antiulcer activity of the drug was carried out on the model of phenolic proctitis. It was found that the tested drug significantly reduces the area of necrosis, hyperemia, and also significantly reduces the edema and the length of the damaged section of the intestine relative to the entire length of the rectum, on the model of phenolic proctitis. The data obtained indicate the presence of reliable antiulcer activity in the tested drug, which gives reason to recommend it for proctitis, hemorrhoids and in a number of other proctological diseases with concomitant inflammation, edema, hyperemia and ulceration.

Key words: dry extract of Nigella sativa, suppositories, thymoquinone, anti-inflammatory activity.

Introduction

Black cumin (Nigella sativa L) is a well-known annual herb of the buttercup family (Ranunculaceae). It is cultivated in the Mediterranean, North Africa, Central Asia, India, the Middle East and Russia³. It has a wide therapeutic effect², and information about its medicinal properties can be found in the works of Abu Ali ibn Sina (Avicenna)³.

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According to literary sources, \textit{N. sativa} seeds are used as an antitussive\textsuperscript{4}, gastroprotective\textsuperscript{5}, sedative\textsuperscript{6}, antifulcer\textsuperscript{7}, anti-inflammatory, immunomodulatory and antitumor drug \cite{8-11}, hepatoprotective agent\textsuperscript{12}, healing gastric ulcer\textsuperscript{13}, and as an agent for treating male infertility\textsuperscript{14}. There is evidence of their use in cardiovascular disorders\textsuperscript{15}, improving memory\textsuperscript{16}, and as an antioxidant\textsuperscript{17}.

The component composition of \textit{N. sativa} seeds is represented by the content of fatty acids, essential oils, vitamins, phenolic compounds, alkaloids, saponins, sterols, minerals, amino acids, proteins and carbohydrates\textsuperscript{18}. Thymoquinone (TQ) is one of the most active biological compounds of \textit{N. sativa}\textsuperscript{19}. Thymoquinone also has a wide pharmaco-biological spectrum, in particular, antioxidant, antimicrobial, anti-inflammatory, antiparasitic, antitumor, hypoglycemic, hypotensive, hepatoprotective and anti-asthmatic, as well as neuroprotective effects\textsuperscript{20,21,22}.

To date, the pure TQ is not used independently. However, \textit{N. sativa} seeds and oil, being the raw materials for its extracting, are mainly used in France, Germany, Italy, Great Britain, and the United States to prepare pharmaceutical products. A dry extract is also obtained from it.

Based on the above, the development of an advantageous drug in the form of suppositories based on dry extract of \textit{N. sativa} for introduction into medical practice in areas such as genitourinary, proctology, oncology, and endocrinology is promising and seems to be reasonable.

\textbf{Aim of the research}: The development and standardization of medicinal product based on dry extract of \textit{N. sativa}.

\textbf{Materials and Methods}

The study object was dry extract of black cumin, suppository bases widely used in factory production: Witepsol H 15 (IOI Oleo GmbH, Germany), Witepsol W 35 (IOI Oleo GmbH, Germany) and locally-made solid confectionery fat, as well as cocoa butter as a classic suppository base for comparison.

The quality of the prepared suppositories was assessed by the appearance, average weight of the suppositories, aqueous solution PH, the TQ identification and quantitative content, the time of complete deformation and the melting point in accordance with the procedures developed by us and the standard ones given in the State Pharmacopoeia of the Republic of Uzbekistan (SP RUz) 1\textsuperscript{st} ed., Vol. 1 (2020).

To determine the rate and completeness of the TQ release from suppositories, we conducted the “Dissolution” test using the “rotating basket” apparatus. 500 ml purified water served as the dissolution medium at 37±10\textdegree C. The basket rotation speed was 100 rpm. At regular intervals, samples were taken from the dissolution medium with its replenishment. The samples were filtered and the TQ quantitative content was determined therein by the spectrophotometric method in the UV region.

The identification and quantitative analysis were conducted using a Shimadzu UV-1800 spectrophotometer.

\textbf{Results and Discussion}

To conduct a study aimed at choosing the best suppository base, the following lipophilic bases widely used in factory production were used: Witepsol H 15, Witepsol W 35, and locally-madesolid confectionery fat, as well as cocoa butter, as a classic suppository base for comparison. For the listed bases, the physicochemical and structural-mechanical indicators recommended by the State Pharmacopoeia of the Republic of Uzbekistan, 1\textsuperscript{st} edition, Vol. 1, 2020 (“melting point” 2.2.16, p.38; “freezing point” 2.2.18, p.40) were determined.

One of the important requirements for fatty suppository bases is stability under conditions of long-term storage. The main indicators reflecting the intensity of oxidative processes in fats during storage are acid, iodine, peroxide indices, which were determined on the samples.

The dosing accuracy in case of fusion molding depends on the size of the mold deepening, the density of the base and the APIs included in the suppositories, on the uniformity of mixing the base with the active agent.

The substitution factor is variable and varies with the base viscosity, drug particle size, API density and
amount, and air volume included. The substitution factor for the dry extract of *Nigella sativa* (NSDE) was determined as follows: 30 suppositories were prepared from the suppository base by fusion molding without NSDE and weighed on an analytical balance. 80% of the base by weight of 30 suppositories was melted in a porcelain dish in water bath. The same procedure was used to prepare NSDE containing suppositories. In doing so, a weighed amount of NSDE was added to the molten base, dispersed and evenly poured into the same mold, after which the cavities were filled with the remaining base from the weight of 30 suppositories. The molds were cooled at −4°C for 15 minutes.

The substitution factor $F$ was calculated by the formula:

$$P = \text{the weight of 30 suppositories without NSDE, g; } O = \text{the weight of 30 suppositories with NSDE, g; }$$

$$A = \text{the total weight of the NSDE in 30 suppositories, g; } F = \text{the substitution factor.}$$

The amount of the base, taking into account the substitution factor, was calculated by the formula:

$$J = (P - F*A)*n,$$

where, $P$ is the average weight of the suppository without NSDE, g; $A$ is the amount of NSDE in grams spent on the preparation of 1 suppository; $F$ is the substitution factor; $n$ is the number of suppositories for which the calculation is performed. The loading quantities of various bases with NSDE, taking into account the calculated substitution factors, are shown in Table 1.

### Table 1: The substitution factors and the number of bases required for the preparation of NSDE-containing suppositories

<table>
<thead>
<tr>
<th>Type of base</th>
<th>Weight of supp. without NSDE(placebo), g (P)</th>
<th>Weight of supp. with NSDE, g (O)</th>
<th>Substitution factor (F)</th>
<th>Number of supp. for which calculation is performed (n)</th>
<th>Calculation of bases taking into account the displacement factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocoa butter</td>
<td>21.5954</td>
<td>22.9432</td>
<td>0.6533</td>
<td>20</td>
<td>19.056</td>
</tr>
<tr>
<td>Witepsol W 35</td>
<td>21.5970</td>
<td>22.9452</td>
<td>0.6713</td>
<td>20</td>
<td>19.085</td>
</tr>
<tr>
<td>Witepsol H 15</td>
<td>21.5969</td>
<td>22.9451</td>
<td>0.6712</td>
<td>20</td>
<td>19.084</td>
</tr>
<tr>
<td>Solid confectionery fat</td>
<td>21.686</td>
<td>23.7106</td>
<td>0.6696</td>
<td>20</td>
<td>18.918</td>
</tr>
</tbody>
</table>

As the results show, the NSDE substitution factors for the bases used are approximately the same, and their values are in the range of 0.6712 – 0.6696.

Suppositories were prepared by fusion molding with the presence of a certain amount of NSDE, which was injected directly into suppository bases. To make NSDE-containing suppositories by fusion molding, plastic and metal molds were used, which made it possible to obtain suppositories weighing 0.95-1.05 g. The mold cavities were preliminarily lubricated with a hydrophilic lubricant in order to facilitate the removal of congealed suppositories.

To prepare a certain number of suppositories, the amount of base and NSDE was calculated. Witepsol-based suppositories were prepared without the use of an emulsifier, since the bases themselves have emulsifying properties. When molding suppositories based on cocoa butter and solid confectionery fat, distilled monoglycerides were used as an emulsifier, i.e. emulsifier No.1 and emulsifier No.2.
The suppository base was placed in an evaporation dish and melted in water bath. The NSDE was added to the molten base in parts, with constant stirring. The resulting suppository mass was stirred until the temperature of the mixture became close to the freezing point of the suppositories and quickly poured into pre-cooled molds. The resulting suppositories were inspected for quality. Suppositories had a smooth surface, the same torpedo shape, a homogeneous mass without inclusions. The uniformity of suppositories was determined visually by the absence of spangles, inclusions and pieces of the base on a longitudinal section. The average weight was determined according to the procedure given in the SPRUz, 1st ed., Vol. 1, 2.9.5. “Uniformity of the mass of dosed drugs” (p. 412), weighing 20 suppositories with an accuracy of 0.001 g.

The results obtained indicate the compliance of the prepared suppositories with the requirements of regulatory documents in terms of the average weight variation. The average weight variations do not exceed ±5%.

Further, for NSDE-containing suppositories, the iodine index, peroxide index and acid index were determined according to the above procedure. The study results have shown that the addition of NSDE does not affect the indices of lipophilic bases.

The adding of a dry extract does not significantly affect the physicochemical and structural and mechanical properties of suppository bases.

The rate and completeness of the TQ release from the suppositories was determined according to the above procedure (“Dissolution” test).

Sampling in a volume of 5 ml was performed after 15, 30, 60, 120, 180, 240 minutes with replenishment of the dialysis medium. 5 ml of dialysate was placed in a 25 ml volumetric flask, adjusted to the mark with solvent. The samples were filtered through filter paper and the optical density of the resulting dialysate was measured on a spectrophotometer at a wavelength of 255 nm in a cuvette with a layer thickness of 1.0 cm relative to the solvent.

The percentage of TQ release \(O(X, \text{mg})\) from suppositories in dialysates was calculated by the formula:

\[
\frac{D_1 	imes m_0 	imes b 	imes W 	imes 20}{D_0 	imes m_1 	imes V 	imes 100} \times \frac{1}{1000} = X
\]

where, \(D_1\) and \(D_0\) the optical density of the test solution and that of the TQ reference sample solution (RSS), respectively;

\(m_1\) and \(m_0\) is a weighed amount of the suppository taken for analysis and that of TQ used to prepare the TQ RSS, g, respectively;

\(P\) is the average weight of suppositories, g;

\(V\) the volume of samples used for analysis, ml;

\(W\) is the TQ content in its reference sample (98%).

Preparation of the TQ reference sample solution (RSS). About 0.0195 g (accurately weighed amount) of TQ (Sigma-Aldrich) should be placed in a 50 ml volumetric flask, dissolved in 30 ml of 70% ethyl alcohol, the volume of the solution is brought to the mark with the same solvent and stirred. 0.5 ml of the resulting solution is placed in a 50 ml volumetric flask, the volume of the solution is brought to the mark with 70% ethyl alcohol. The solution is used freshly prepared. The results are shown in Table 2.

**Table 2: Results of the TQ release from suppositories**

<table>
<thead>
<tr>
<th>Type of suppository bases</th>
<th>Thermostating time, min</th>
<th>NSDE content in the sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>X, g</td>
</tr>
<tr>
<td>Cocoa butter</td>
<td>15</td>
<td>0.000069</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.000156</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.000351</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>0.000618</td>
</tr>
</tbody>
</table>
The results obtained indicate that the nature of the base affects the TQ release from suppositories. The most complete and fastest release of TQ occurs from Witepsol H 15 and Witepsol W 35.

Based on the studies carried out, the following composition of NSDE-containing suppositories was proposed.

**Composition:** NSDE– 0.15g

Witepsol H 15 or W 35, a sufficient amount to prepare suppositories weighing 1.03 - 0.999.

The evaluation of the quality of suppositories was carried out in accordance with the requirements for this dosage form.

Appearance: suppositories of dark cinnamon color, torpedo-shaped, of the same size, with a smooth surface, of sufficient hardness to ensure ease of administration. The absence of inclusions, determined visually on the section, indicates the homogeneity of the suppositories. In appearance, the suppositories met the requirements of the SP RUZ, p 5.11, p.1099.

The average weight of the NSDE-based suppositories was 1.03±0.05 g, and the weight variation was within ±5%.

The pH value of 10% water extraction.

The melting point for the NSDE-based suppositories was 36.4±0.5°C, which meets the requirements of regulatory documents.

For the identification and quantification analysis of suppositories, we used a previously developed technique using a spectrophotometric method of analysis in the UV spectrum by TQ, which is the main active ingredient of the dry extract of *Nigella sativa*. Previously, it was found that the suppository base does not absorb in this region of the spectrum and does not affect the results of the quantitative determination of active substances.

1.0g (accurately weighed amount) of crushed suppositories was placed in a 50 ml flask, 5 ml of 70%
ethyl alcohol was added and heated in water bath until completely melted. The flask with the contents was stirred for 3 minutes, cooled and filtered. The extraction was repeated with another 5 ml portion of the solvent and combined. TQ was identified by the position of the maximum at 255±2 nm in the 220–350 nm region in the UV absorption spectrum in an alcohol solution prepared for quantitative determination (Fig. 1).

Figure 1: UV spectrum of an alcohol solution of suppositories

To confirm the assignment of these maxima to TQ, the absorption spectrum of reliable TQ RSS was recorded under the same conditions. It was revealed that both spectral curves have the same character (Fig. 2).

In addition, it was proposed to identify TQ in suppositories by TLC on TLC Plates, Silica gel on Aluminum, 20 X 25x25 cm (Sigma-Aldrich) plates, followed by visualization of light brown adsorption zones by irradiation in UV light with a wavelength of 365 nm. For this, an alcohol extract of suppositories, prepared for quantitative analysis, and a 0.01% solution of the TQ RSS in 95% ethyl alcohol were applied by 0.005 ml (5 μg) per chromatographic plate. The plate with the applied samples was dried in air for 1-2 min, then placed in a chromatographic chamber saturated with a solvent system (o-xylene: cyclohexane in a ratio of 8: 2). Chromatography was performed in an ascending manner. When the front of solvents reached the end of the plate (finish), it was taken out and dried in air for 2-3 minutes. Then it was heated for 5 minutes in a drying chamber at a temperature of 100°C and viewed under a UV lamp. TQ appeared as brown spots on a white background. The chromatogram showed 2 spots with Rf 0.37 and at the start, respectively.

In a quantitative analysis, the TQ content in one suppository in milligrams (X, mg) in terms of the average weight of the suppository was calculated by the formula:

\[ X = \frac{D_1 \times m_1 \times b \times W \times 20}{D_0 \times m_0 \times V} \]

where, \( D_1 \) and \( D_0 \) are the optical density of the test solution and the TQ RSS, respectively;

\( m_1 \) and \( m_0 \) is a weighed amount of the suppository taken for analysis and that of TQ used to prepare the TQ RSS, g, respectively;

\( P \) is the average weight of suppositories, g;

\( V \) is the volume of samples used for analysis, ml;

\( W \) is the TQ content in its standard sample (98%).

The content of TQ (C₈H₉NO₂) in one suppository should be from 14.4 mg to 17.6 mg.

The results of the quantitative determination of TQ in suppositories and the metrological characteristics of the analysis method are shown in Table 3.
Table 3: Results of quantitative determination of TQ in suppositories by spectrophotometry

<table>
<thead>
<tr>
<th>TQ found (X, mg)</th>
<th>Metrological characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.99</td>
<td>X_{av} = 16.62</td>
</tr>
<tr>
<td>16.34</td>
<td>S^2 = 0.11905</td>
</tr>
<tr>
<td>16.74</td>
<td>S = 0.345</td>
</tr>
<tr>
<td>16.85</td>
<td>ΔX = 0.9591</td>
</tr>
<tr>
<td>16.18</td>
<td>ε = 5.77%</td>
</tr>
</tbody>
</table>

As can be seen from the table, the TQ content is 16.62 mg, and the error of the analysis method does not exceed ± 5.77%.

Microbiological purity and pharmacological tests were conducted on the basis of the laboratory of microbiology and pharmacology of the Testing Center at “Med Standard” LLC Scientific Center.

Microbiological control was carried out in accordance with the SP RUz 1<sup>st</sup> ed., Vol. 1, 2.6.11. “Microbiological purity”<sup>p.245</sup>. The tests were carried out immediately after the manufacture of the suppositories, after a year and finally after two years. To test suppositories for microbiological purity, a 10 g sample of suppositories under aseptic conditions was emulsified in 100 ml of phosphate buffer solution pH 7.0 (PBS) using glass beads and a minimum amount of emulsifier Tween-80; in this case, mechanical shaking was used, and heating to a temperature not exceeding 45°C. After obtaining a homogeneous emulsion, the sample was diluted in 10 ml of sterile PBS to 1:100 and 1:1000, then 1 ml of each dilution was introduced into sterile Petri dishes, followed by pouring onto medium No. 1 (nutritious meat-peptone agar or soybean-casein agar ) and No. 2 (Sabouraud medium with glucose). The Petri dishes with the solidified medium were inverted and incubated at 32.4°C and 22.5°C. After 48 hours and finally after 5 days, the number of grown colonies of aerobic bacteria and fungi was counted. The final number of bacterial colonies was counted from two dishes, the average value was found and multiplied by the corresponding dilution rate, and the CFU number was calculated in grams or ml.

When testing for Escherichia coli, 10 ml or an amount corresponding to 1 g or 1 ml, for inoculation in a suitable amount of casein soybean broth, should be mixed and incubate at a temperature from 300°C to 350°C for 18-24 hours.

The container is shaken, transferring 1 ml of the inoculated casein soybean broth to 100 ml of MacConkey broth, incubated at a temperature of 42°C to 44°C for 24-18 hours. Then, it is subcultures on plates with MacConkey agar at a temperature of 30°C to 35°C for 18-24 hours.

As a result of the studies, no colonies were found, which indicated the compliance of the drug with the requirements for microbiological purity of the State Pharmacopoeia RUz,<sup>1</sup>ed. (2020).

One of the most important criteria for assessing the quality of any dosage form is its stability during storage. The stability study was carried out on 5 series of suppositories during storage in a refrigerator (4±1°C). The suppositories were stored in a polyvinyl chloride (PVC) cell strip. The quality of the suppositories was checked on the day of preparation, as well as after 3, 6, 12, 18, 24 months. Quality control of the developed dosage form during storage was carried out using standard indicators in accordance with the SP RUz: in appearance, determination of the average weight, melting temperature, pH of the aqueous medium, time of complete deformation, identification and quantitative determination. When determining the stability indicators of the Witepsol-based NSDE-containing suppositories, it was found that in the process of natural storage, the appearance, physicochemical indicators of identification and the quantitative content of the medicinal substance met the requirements of the regulatory documentation for this dosage form (Table 4).
Table 4: Quality indicators of the NSDE-containing suppositories during natural storage at a temperature of 4±1°C

<table>
<thead>
<tr>
<th>Quality indicators</th>
<th>Standardized requirements</th>
<th>Shelf life, months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Appearance</td>
<td>Opaque brown torpedo-shaped suppositories</td>
<td>same</td>
</tr>
<tr>
<td>Average weight, g</td>
<td>should be in the range of 0.95 g to 1.05 g,</td>
<td>in the range of 0.95 g to 1.05 g,</td>
</tr>
<tr>
<td>pH of the aqueous extract</td>
<td>6.1-6.7</td>
<td>6.3±0.2</td>
</tr>
<tr>
<td>Melting point, °C</td>
<td>37°C max.</td>
<td>37°C</td>
</tr>
<tr>
<td>Time of complete deformation, min.</td>
<td>15 min max.</td>
<td>10.0±1.0</td>
</tr>
<tr>
<td>Identification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-maximum absorption in the region of 220-350 nm of alcohol solution</td>
<td>255±2 nm</td>
<td>255±2 nm</td>
</tr>
<tr>
<td>-value in UV light at a wavelength of 365 nm</td>
<td>2 spots with Rf, respectively 0.37 and at the start</td>
<td>conforms</td>
</tr>
<tr>
<td>Content, mg/g</td>
<td>14.4-17.6</td>
<td>16.8</td>
</tr>
</tbody>
</table>

As follows from the data presented in Table 4, the prototypes of NSDE-containing suppositories had quality indicators that meet the regulatory requirements during the observed period of 2 years, therefore, the shelf life is 2 years.

The antiulcer activity of the suppository was preliminary studied. The experiments were carried out on a phenolic proctitis model. The criterion for assessing the pharmacological activity of the drug was: a decrease in the area of necrosis (in mm²) and the intensity of inflammation of the mucous membrane (in points), as well as the length of the damaged section of the intestine relative to the entire length of the rectum (in percent), compared with the control. The results obtained were processed by the method of variation statistics according to the Student’s criterion at p = 0.05. As a result of studying the antiulcer activity of the suppository, it was found that the test drug at a dose of 90.3 mg/kg has the highest effect. The data obtained indicate the presence of reliable antiulcer activity in the tested drug, which gives reason to recommend it for proctitis, hemorrhoids and in a number of other proctological diseases concomitant with inflammation, edema, hyperemia, and ulceration.

Conclusions

1. The effect of suppository bases on the physicochemical and structural-mechanical properties of suppositories has been studied. It was found that the selected bases provide the necessary quality parameters for the developed suppositories.
2. The indicator of the substitution factor for the NSDE has been established, its value is in the range of 0.6712 to 0.6696.
3. The best results were obtained based on Witepsol H-15 and W-35. The proposed composition of NSDE-containing suppositories: NSDE – 0.345 g; Witepsol H-15 and W-35 are sufficient to prepare suppositories weighing 1.03 - 0.999. Based on the results of the studies, a technological scheme for the preparation of NSDE-containing suppositories was proposed.
4. The quality of suppositories containing a dry extract of N. sativa was evaluated according to indicators: appearance, average weight, pH of the aqueous extract, melting point, time of complete deformation, identification, quantitative content of thymoquinone, microbiological purity. The stability of suppositories has been studied and the shelf life has been established when stored in a refrigerator at 8°C, namely 2 years.
5. The obtained preliminary data on the study of the pharmacological activity of suppositories indicate the presence of reliable antiulcer activity in the tested drug, which gives reason to recommend it for proctitis, hemorrhoids and in a number of other proctological diseases concomitant with inflammation, edema, hyperemia, and ulceration.
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**References**


Risk Factors for Diabetic Ketoacidosis in Children with Type 1 Diabetes Mellitus: A Single-center, Cross-sectional Study in Indonesia

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Abstract

Background: Diabetic ketoacidosis (DKA), the leading cause of mortality in childhood with diabetes mellitus (DM). Identification of risk factors for DKA in children with type 1 DM (T1DM), can aid in reducing the incidence of DKA.

Objective: To determine risk factors for DKA in children with T1DM.

Methods: This was a single-centre, cross-sectional study of patients aged 0–16 years diagnosed with T1DM who were hospitalised in or visited Dr. Soetomo General Hospital in Surabaya between January 2016 and June 2021. Data on sex, age, parental educational background, parental occupation, parental age, family history of DM and DKA frequency were collected, and statistical analyses using the SPSS software.

Results: The risk of DKA was higher in children with working mothers (OR 9.3, 95%; CI 2.168–40.182, p = 0.003). Significant correlation between maternal age younger than 40 years and DKA frequency (OR 1.73, 95%; CI 8.684–3.478, p = 0.000). The highest frequency of DKA was observed in children with low parental educational level, whereas there was no correlation between age at diagnosis and parental educational level with DKA.

Conclusion: Young maternal age and working mothers might be associated with increased frequency of DKA in children with T1DM.

Keywords: diabetic ketoacidosis; type 1 diabetes mellitus; risk factor; parental age; occupation

Introduction

Approximately 26 per 100,000 children are diagnosed with type 1 diabetes mellitus (T1DM) every year, with the rate continually increasing approximately 4% per year. Despite the increased life expectancy associated with the improvements

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in diabetes care, the mortality rate remains higher in children with T1DM than in the general population and the leading cause of death is diabetic ketoacidosis (DKA). Globally, the incidence of DKA at presentation varies, ranging from 13% to 80%. Children presenting with DKA are at higher risk for life-threatening complications including cerebral oedema, cerebral ischaemia and hypoxic brain injury. These patients are also more likely to require admission to an intensive care unit, which contributes to greater healthcare spending.

It is unclear why only some children present with DKA. Furthermore, whether the development of DKA is a consequence of delayed diagnosis and treatment or a reflection of a particularly aggressive form of diabetes remains unknown. Therefore, identification of factors associated with DKA at diagnosis and understanding the relative importance of delayed diagnosis and treatment are important for the advancement of our understanding of DKA and for the development of patient-, professional- and population-based interventions to reduce the rate of children presenting with DKA.

The factors associated with DKA in children can be categorised into individual factors (age, sex, ethnicity, family history of diabetes, body mass index and parental consanguinity), family factors (parental education, family structure, health insurance status, rural versus urban residence, family income, parental employment and social status), physician-related factors (delayed diagnosis, diagnostic error, number of medical consultations before diagnosis, delayed treatment and presence of a structured diabetes care team) and disease-related factors (duration of symptoms, pattern and frequency of symptoms and preceding infection or febrile illness). In a study examining the association of parental educational level with DKA prevalence, Sadauskaite-Kuehne et. al. found that the DKA prevalence was higher in Lithuanian children than in Swedish children (21.3% vs. 7.3%, \( P < 0.0001 \)) and that younger age at the time of diagnosis and low maternal educational level influenced DKA in Lithuanian children.

**Materials and Methods**

This was a cross-sectional study including children diagnosed with T1DM based on the International Society for Pediatric and Adolescent Diabetes guidelines. Patients with T1DM aged 0–16 years who were hospitalised in or visited the Pediatric Endocrinology Outpatient Clinic at Dr. Soetomo General Hospital in Surabaya between January 1, 2016 and June 30, 2021 were included, and patients with incomplete medical records during recruitment were excluded. A total 50 patients signed informed consent and joined the study. Data for the study were obtained from the medical records of the Pediatric Endocrinology Outpatient Clinic and from interviews with the parents of inpatients.

**Data Analysis**

All statistical analyses were performed using SPSS version 21. Univariate analyses were performed to determine frequency of variables, and chi-squared test (Fisher’s exact test) was performed to analyse the association between independent and dependent variables. Multivariate logistic regression analysis was performed to analyse risk factors that affect in DKA in children. The results were reported as odd ratios (ORs) with 95% confidence intervals (CIs). A \( p \) value of <0.05 was considered to indicate statistical significance.

**Results and Discussion**

The baseline characteristics of 50 patients with T1DM included in the study are summarised in Table 1. Briefly, the male/female ratio was 1:1.5, 32 patients experienced at least one DKA episode, and 6 patients experienced DKA more than once.

**Table 1: Baseline characteristics of the patients with T1DM included in the study**

<table>
<thead>
<tr>
<th>Variable</th>
<th>N = 50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>30</td>
</tr>
<tr>
<td>Male</td>
<td>20</td>
</tr>
<tr>
<td>Family history of DM</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>4</td>
</tr>
<tr>
<td>No</td>
<td>46</td>
</tr>
</tbody>
</table>
Bivariate analysis revealed that there was an association between working mothers and DKA (OR 9.33, 95% CI 2.168– to 40.182, \( p = 0.003 \)), indicating an increased DKA risk among children with working mothers.

Multivariate analysis revealed significant correlation between maternal age below 40 years and DKA frequency (OR 1.73, 95% CI 8.684–3.478, \( p = 0.000 \)). The highest DKA frequency was among the patients with low parental educational level. However, there was no statistically significant correlation between parental educational level and DKA.

The present study, which aimed to provide updated information on potential factors associated with the development of DKA, revealed a number of key findings: parental occupation, sex, family history of DM, DKA frequency, socioeconomic status and parental educational level.

First, we found that parental occupation was association with the risk of DKA in children with T1DM. Specifically, children with working mothers were at higher risk of DKA. The signs and symptoms suggesting DKA might have been recognised earlier in the children with nonworking mothers included in the study. Two studies examined the effect of parental employment on the frequency of DKA. A study in Sweden found that the risk of DKA was higher in children with nonworking mothers (OR 4.8, 95% CI 1.8–13.1), whereas another study in Lithuania reported that the paternal employment status did not have an effect on the rate of DKA (OR 1.17, 95% CI 0.53–2.57).4

In the present study, the educational level was senior high school in majority of the fathers and mothers of the children who experienced DKA (52% and 44%, respectively). Several studies reported that higher parental educational level was a protective factor in DKA.6,7,8 As suggested in other studies, better parental educational level may aid in the improvement of metabolic regulation in children with T1DM.9

The current study cohort was predominantly composed of female children with T1DM, whereas DKA developed predominantly in male children,
with a male/female ratio of 1.3:1. A systematic review of 21 studies revealed that sex did not have an effect on the frequency of DKA in 20 studies and that only one study including 2121 subjects reported a small, albeit statistically significant, increase in the frequency of DKA in female patients (OR 1.30, 95% CI 1.07–1.58, \( p = 0.0079 \)).

In the current study, only four patients had a family history of type 2 DM and none of the patients had a family history of T1DM. Azab et al. stated that a positive family history of T1DM was associated with increased T1DM risk among relatives. These results are in contrast with the study by Roche et al. who described the abrupt onset of T1DM and the absence of family history.

Numerous studies demonstrated that younger age was associated with increased DKA risk at onset. In some studies, most of the patients with newly diagnosed T1DM and DKA were younger than two years of age, which may be due to the lower efficacy to control metabolic deterioration and/or low speech ability in expressing issues in younger children. In contrast, only 6 (12%) patients with T1DM who were 5 years of age or younger experienced DKA.

One major study limitation of the present study is the small sample size because of the lower rates of T1DM in Asian populations compared with Caucasian populations; therefore, the current study findings might not be representative of other populations.

**Conclusion**

The present single-center, cross-sectional study in Indonesia has identified several factors associated with DKA in children with T1DM, which might be considered as important targets to reduce the incidence of DKA at diagnosis in Indonesia, especially East Java. The risk of DKA was influenced by local culture. Young and poor children were disproportionately affected, although DKA occurred even in children from affluent and well-educated families.

Increased public awareness and greater medical alertness concerning the symptoms and signs of DKA are warranted. Additionally, improved access to care may additionally reduce the severity and cost of initial T1DM treatment in children.

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**Ethical clearance:** We sought approval of this research from the Clinical Research Unit of Dr. Soetomo General Hospital Surabaya, Indonesia, as our Ethical Committee Review Board. The ethical clearance was issued by the Clinical Research Unit of Dr. Soetomo General Hospital Surabaya (number 1023/KEPK/III/ 2019).

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**Conflict of Interest:** The author reports no conflict of interest in this work

**References**


Nephrotoxicity Related to Iodinated-Based Contrast Media: From Pathophysiology to Management

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Abstract

Contrast Induced Nephropathy (CIN) or Contrast Induced Acute Kidney Injury (CI-AKI) is defined as sudden deterioration of renal function which is caused by administration of contrast media. Iodinated Contrast Media (ICM) is frequently used to enhance anatomical structure in medical imaging, for both diagnostic and therapeutic procedure, such as angiography (vascular visualization of internal organ in computed tomography), urography (imaging of the urinary tract), and coronary intervention. The pathophysiology of nephrotoxicity related to ICM has not been completely understood. Hemodynamic changes, direct tubular cell toxicity and oxidative stress may contribute to pathophysiology of CI-AKI. Screening patient’s risk factors, selecting contrast and avoiding nephrotoxic drug are several ways to be performed prior to administration of ICM to avoid CI-AKI. In addition to fluid administration and hemodialysis, medications such as N-acetylcysteine, statins, furosemide and nebivolol can be used to manage CI-AKI.

Keywords: Iodinated Contrast Media, Contrast Induced Acute Kidney Injury, Nephrotoxicity

Introduction

Iodinated Contrast Media (ICM) is frequently used to enhance anatomical structure in medical imaging, for both diagnostic and therapeutic procedure, such as angiography (vascular visualization of internal organ in computed tomography), urography (imaging of the urinary tract), and coronary intervention. However, like any other pharmaceutical in clinical practice, ICM also possess its beneficial and adverse side effect which should be aware of nephrotoxicity effect following intravascular administration of ICM.¹

Contrast Induced Nephropathy (CIN) or Contrast Induced Acute Kidney Injury (CI-AKI) is defined as sudden deterioration of renal function which is caused by administration of contrast media, that cannot be attributable by other sources.²

Recent metadata in 2017, by collecting 5.9 million registry data, found 5.5% patient was developed AKI after receiving ICM at any type and route.³ From the Prevention of Serious Adverse Events Following Angiography (PRESERVE) trial, which studied the overcome at patients with CKD undergoing cardiac angiography, CI-AKI is occurred in 9% of all

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participants. However, other retrospective study of 6902 patients with Chronic Kidney Disease (CKD) showed no difference to Acute Kidney Injury (AKI) in patient who received intravenous CM on CT scan compared with patient who underwent non-contrast CT. Both group had an average creatinine rise of 0.3 – 0.4 mg/dL after contrast administration.

Although CI-AKI remained controversial, this adverse side effect is faced by practitioner. However, as a physician should be responsible to the administration of CM to the patient. The incidence of CI-AKI may decrease as more physician recognize the risk and prevention. Therefore, the pathophysiology, clinical presentation, risk assessment, and management of CI-AKI should be understood to reduce the severity of this nephrotoxicity effect.

**Iodinated Contrast Media (ICM)**

Table 1: Types of Iodinated Contrast Media (ICM)

<table>
<thead>
<tr>
<th>Class of Contrast Agent</th>
<th>Type of Contrast Agents</th>
<th>Iodine/Particle Ratio</th>
<th>Osmolality (mOsm/kg(H_2O))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ionic High-Osmolar Contrast Media (HOCM) monomer</td>
<td>Diatrizoate (Renografin)</td>
<td>1.5</td>
<td>1870</td>
</tr>
<tr>
<td></td>
<td>Ioxithalamate (Telebrix)</td>
<td>1.5</td>
<td>2130</td>
</tr>
<tr>
<td>Non-ionic Low-Osmolar Contrast Media (LOCM) monomer</td>
<td>Iohexol (Omnipaque)</td>
<td>3</td>
<td>780</td>
</tr>
<tr>
<td></td>
<td>Lopamidole (Isovue)</td>
<td>3</td>
<td>790</td>
</tr>
<tr>
<td></td>
<td>Lomeprol (Iomeron)</td>
<td>3</td>
<td>620</td>
</tr>
<tr>
<td></td>
<td>Leversol (Optipray)</td>
<td>3</td>
<td>790</td>
</tr>
<tr>
<td></td>
<td>Lopromide (Ultravist)</td>
<td>3</td>
<td>770</td>
</tr>
<tr>
<td></td>
<td>Iopentol (Imagopaque)</td>
<td>3</td>
<td>810</td>
</tr>
<tr>
<td>Non-ionic Iso-Osmolar Contrast Media (IOCM) dimers</td>
<td>Iodixanol (Visipaque)</td>
<td>6</td>
<td>290</td>
</tr>
<tr>
<td></td>
<td>Iotrolan (Isovist)</td>
<td>6</td>
<td>290</td>
</tr>
</tbody>
</table>

These contrast media mainly used intravascularly for angiography and computed tomography, but since they are mainly excreted by the kidney, it also used for urography. Iodinated water soluble contrast media is preferred to water insoluble contrast media to outline cavities. These are examples of the usage of ICM to outline anatomical structure of cavities in clinical practice:

- Cholesystography requires an ICM that is excreted mainly by the liver, iodipamide and ioglycamide have been long preferred, iodoxamate and iotroxate are more efficiently excreted by this route.
- In gastrointestinal tract, insoluble barium sulfate is still mainly used, but water soluble ICM may be considerable in special circumstances.
- For Retrograde urography, many different water soluble ICM can be used, since they are diluted sufficiently.
• Hysterosalpingography is mostly performed with LOCM.  

Pathophysiology

The mechanism of nephrotoxicity related to ICM has not been fully explained, however there are several factors may contribute to this event.

Hemodynamic Changes

The IV injection of ICM changes the vascular dynamic. After the administration of contrast, the renal bloodflow is initially increase followed by prolonged decrease in bloodflow or constriction of renal vessels. Contrary, the extrarenal bloodflow initially decrease followed by prolonged increase in bloodflow or dilatation of extrarenal vessels. These opposing conditions cause the elevation of renal vascular resistance, decreasing of renal blood flow, and decreasing of glomerular filtration rate (GFR).  

The most affecting part in renal, due to decreasing of renal blood flow is the medulla. In normal conditions, the medullary oxygen tension is 30 to 40 mm Hg lower than in the cortex. If this hemodynamic change occured, medullary oxygen tension may lower than normal and lead to medulla ischaemia.

Oxidative Stress

The ascending loop of Henle has increased energy consumption due to contrast agents because these are osmotic diuresis inducers. The administration of contrast agents also decreases medullary oxygenation and increases medullary oxygen consumption, it has highly consequence to hypoxia injury. Medullary hypoxia result in the formation of ROS (Reactive Oxygen Species) which is lead to direct tubular and endhotelial injury. This can cause endothelial dysfunction and dysregulation of tubular transport. The superoxide anions react with NO (Nitric Oxide) to produce peroxinitrate anion which is more noxious to endothelial cells. Nitric Oxide is known to be vasodilator, the reaction of ROS with NO will cause the depletion of NO itself.

Direct Tubular Cell Toxicity

ICM also has direct cytotoxic effect to tubular and endothelial cell which is lead to apoptosis and cell death. The direct taken up of ICM to tubular cell result in apoptosis to tubular cell and stasis of contrast in the tubule. The high concentration of ICM in the tubules brings the persistent toxicity of surrounding tubular cells.

Diagnosis of CI-AKI

The diagnosis of AKI is made by following KDIGO (Kidney Disease Improving Global Outcome) criteria, if one of the criteria below is appeared on the patient within 48 hours after ICM administration.

1) Absolute serum creatinine increase ≥ 0.3 mg/dL (> 26.4 µmol/L).
2) A percentage increase in serum creatinine ≥ 50 % (≥ 1.5-fold above baseline).
3) Urine output reduced to ≤ 0.5 mL/kg/hour for at least 6 hours.

Figure 1: Pathophysiology of Renal Toxicity Related to The Administration of Iodinated Contrast Media (ICM)
For CI-AKI the sign and symptom which is found above should not be attributable to any other identifiable cause of kidney failure. After the elevation of SCr level, there is typically a gradual decline at 3 to 7 days. For almost all patients with CI-AKI, the increase in SCr is within the first 24 hours; in patients with serious renal impairment, virtually all will have an increase in SCr within 24 hours. Any delay in SCr elevation results in lower detection rates of CI-AKI.\textsuperscript{16}

**Prevention**

To avoid CI-AKI for the patient, doing an administration of ICM only when clinically necessary. Thus, there are several ways should be done by physician before administer ICM for both diagnostic or therapeutic procedure.

**Table 2: Mehran Risk Score for CI-AKI**

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypotension (SBP &lt; 80mmHg)</td>
<td>5</td>
</tr>
<tr>
<td>Intra-aortic Balloon pump</td>
<td>5</td>
</tr>
<tr>
<td>Congestive heart failure (NYHA III or IV)</td>
<td>5</td>
</tr>
<tr>
<td>Age &gt; 75 years</td>
<td>5</td>
</tr>
<tr>
<td>Anemia (Ht &lt; 39% in man ; &lt; 36% in woman)</td>
<td>5</td>
</tr>
<tr>
<td>Diabetes</td>
<td>5</td>
</tr>
<tr>
<td>Volume of injected contrast media</td>
<td>1 for each 100 ml</td>
</tr>
<tr>
<td>Serum creatinine concentration &gt; 1.5 mg/dl or eGFR &lt; 60 ml/min/1.73m\textsuperscript{2}</td>
<td>4</td>
</tr>
<tr>
<td>or eGFR &lt; 60 ml/min/1.73m\textsuperscript{2}</td>
<td></td>
</tr>
<tr>
<td>Risk Score Risk of Contrast induced Nephropathy Risk of Dialysis</td>
<td></td>
</tr>
<tr>
<td>Low                            ≤ 5</td>
<td>7.5 %</td>
</tr>
<tr>
<td>Moderate                       6-10</td>
<td>14 %</td>
</tr>
<tr>
<td>High                           11-15</td>
<td>26.1 %</td>
</tr>
<tr>
<td>Very High                      ≥ 16</td>
<td>57.3 %</td>
</tr>
</tbody>
</table>

**Patient Selection**

Screening the risk factors of CI-AKI for the patient is important. For those with risk factors, creatinin serum can be easily measured to evaluate renal function. Creatinin serum can be used to calculate eGFR. The risks for CI-AKI is clinically important below an eGFR 60 ml/min, so it is often used as cutoff for when physicians must be consulted. Newer evidence suggest that if the eGFR below 45 ml/min, physicians must consider the necessity of ICM administration and its possible alternatives.\textsuperscript{17} Multiple risk assessment has been developed to determine the administration of contrast media. Mehran et. al., created risk score to predict CI-AKI after coronary intervention. The higher the risk score, the greater possibility of CI-AKI to the patient.\textsuperscript{18}

**Contrast Selection**

Choosing the correct contrast agents is also important to avoid CI-AKI. There few consideration to determine the correct contrast agent for patient. (1) Type: Type of ICM should be chosen based on osmolality and ionicity of contrast media. Osmolality of ICM compared to plasma is crucial to the risk of nephrotoxicity. Contrast media usually greater viscosity and osmolality (molecules per kilogram of water) than plasma. Ionicity is characteristic of molecule to disperse into cation and anion, leading to
more molecule per kilogram of water and increasing osmolality. Nonionic contrast media is preferred due to less osmolar than ionic contrast media. Iodine atoms of ICM influence the osmotoxic effect of contrast media: the higher the ratio to dissolve particle, the better the attenuation of x-ray. Few studies demonstrated that adverse side effect to contrast media range from 5 – 12 % for HOCM and 1 – 3 % for LOCM. Thus, LOCM seems to be more beneficial to the prevention of CI-AKI than HOCM. Recent study of meta-analysis have found no significant difference in the rates of AKI between IOM and LOCM.

(2) Dosage: The dose of contrast agent also affects the risk of CI-AKI since it is dose-dependent to contrast media, thus the lowest possible dose decrease the risk of nephrotoxicity.

* Cigarroa’s formula: 5 mL of contrast per kg b.w./SCr (mg/dL) with maximum acceptable dose of 300 mL for diagnostic coronary arteriography.

* Laskey’s formula: volume of contrast to calculated creatinine clearance ratio with a cut-off point of the ratio at 3.7 for PCI; a ratio > 3.7 would be associated, following contrast use, with a decrease in CrCl. Recently Gurm et. al. have suggested a cutoff point at 2.0: below a ratio of 2.0 AKI would be a rare complication of PCI, but it would increase dramatically at a ratio of 3.0.

* A new formula seems to be superior and consists of a ratio of grams of iodine to the eGFR; a ratio of 1.42, or even better a ratio of 1.0, would prevent contrast-induced AKI.

(3) Route of administration : Intravenous administration has known to be less risky than intra-arterial contrast media. Few studies demonstrated that intra-arterial ICM administration is more nephrotoxic because of the higher acute intrarenal concentration. In aortography procedure, the closer ICM injection to renal arteries, the higher risk of CI-AKI.

Avoidance of Other Potential Nephrotoxic Drugs

Several drugs may also nephrotoxic to the patient such as aminoglycosides, vancomycin, amphotericin B, metformin, and NSAID. These drugs should be discontinued for patients who obtain contrast agents. Metformin (as an oral antihyperglycemic drug) has to be known that stimulates intestinal production of lactic acid, which may cause severe lactic acidosis in AKI condition. Metformin should be discontinued 12 hours before the administration of contrast agents and can be given 36 hours after the procedure.

ICM are pharmaceutical agents that used for diagnostic and therapeutic procedure. Avoidance of using other nephrotoxic drug may decrease the risk of CI-AKI. Following drugs should be discontinued to prevent renal toxicity effect: aminoglycosides (have a direct nephrotoxic effect), ciclosporin A (direct cellular toxin that may disrupt lysosome function in tubules and stimulate tubulo-interstitial alterations), amphotericin (causes dystal tubule dysfunction), NSAID (reduce the synthesis of endogenous vasodilator prostaglandin, which will increase the renal toxicity of ICM).

Management

Fluid Management

Administration of fluids is key to prevent the risk of CI-AKI. IV infusion of 0.9 % saline at an infusion rate of 1 mL/kg BW/hour should begin 6 – 12 hours before the injection of the contrast drug and continue for up to 12 – 24 hours after the injection. The rationale for hydration causes expansion of intravascular volume, then depress the renin-angiotensin cascade and reducing renal vasoconstriction and hypoperfusion; the result is an increase of diuresis, thus limiting the duration of contrast material contact with renal tubules and its toxicity on tubular epithelium.

Other studies have found better results with the use of sodium bicarbonate rather than sodium chloride. A procedure would be administration of a bolus of 3 mL/kg BW/ hour for 1 hour of a solution of sodium bicarbonate 154 mEq/L before the injection of contrast drug, followed by 1 mL/kg/hour for 6 hours. However, others have disagreed, since no better benefit with sodium bicarbonate rather than sodium chloride.
Medication

1. N-Acetylsistein

Important role for ROS has been involved in renal toxicity caused by contrast agents. Thus, antioxidant is suggested to be useful for CI-AKI. NAC has characteristic to free radical-scavanger and vasodilating effect of nitric oxide. NAC may be given as an oral dose of 600 mg twice daily (day before and day of the procedure) or an IV dose of 150 mg/kg half an hour before the procedure or 50 mg/kg administered for 4 hours.30

2. Statin

Patti et al. demonstrated that a short-term high dose of atorvastatin (80 mg, 12 hours before intervention followed by a further 40 mg preprocedure dose) decreased the incidence of CI-AKI in patients undergoing percutaneous coronary interventions. Mechanism that can explain this trials is statins decrease the vasoconstricting response to angiotensin and the synthesis of endothelin, thereby preventing renal hypoperfusion and medullary hypoxia. Furthermore, contrast agents have to be known in increasing the incidence of inflammation, with formation of ROS and proinflammatory cytokines, and complement activation, which is lead to tubular obstruction. Statins have pleiotropic effects: antioxidative, anti-inflammatory, antithrombotic and reduce endothelin secretion.31

3. Furosemide

Furosemide has an effect of reducing active tubular reabsorption and increasing urine output, in order to decrease the duration of contrast agent’s nephrotoxic effect with tubular epithelium. To prevent salt depletion, adequate fluid replacement should be given.32 However, Solomon et al. reported, there was an exacerbation of renal dysfunction when the loop diuretic furosemide was used in addition to IV saline solution. Therefore, the usage of furosemide as prevention of nephrotoxic effect remains controversy.33

4. Nebivolol

A β1-adrenergic receptor antagonist, nebivolol (5 mg/day for 1 week or 5 mg every 24 hours for 4 days) has been proven in patients with renal dysfunction undergoing coronary angiography to protect against CI-AKI, possibly acting via its antioxidant properties and NO-mediated vasodilating action.34

Hemodialysis

Different types of hemodialysis may remove contrast drugs from the blood in patient with chronic kidney disease. High-flux hemodialysis and hemodiafiltration remove contrast more effectively than low-flux hemodialysis or hemofiltration.35 However, prophylactic hemodialysis in patients with reduced renal function does not diminish the incidence of CI-AKI.36

Conclusion

CIN or CI-AKI is defined as sudden deterioration of renal function which is caused by administration of contrast media, that cannot be attributable by other sources. Contrast agent cause the elevation of renal vascular resistance, decreasing of renal blood flow, and decreasing of GFR. Contrast agent has direct cytotoxic effect to tubular and endothelial cell which is lead to apoptosis and cell death. Contrast agent also decreases medullary oxygenation and increases medullary oxygen consumption, it has highly consequence to medullary hypoxia. Medullary hypoxia result in the formation of ROS (Reactive Oxygen Species) which is lead to direct tubular and endotelial injury. Selecting the right contrast type, dose and route of administration, screening patient’s risk factor using Mehran risk score and avoiding nephrotoxic drug are important to prevent CI-AKI. Medications such as N-acetylcysteine, statins, furosemide and nebivolol can be used to manage CI-AKI in addition to fluid administration and hemodialysis.

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References


Moringa oleifera Ameliorates Chlorpyrifos Induced Oxidative Stress in Male Wistar Rats

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Abstract

Background: Chlorpyrifos is a widely used organophosphate pesticide. It exerts its toxic effect by inhibiting of cholinesterases and induction of oxidative stress.

Methods: In the present study, Soya oil, Chlorpyrifos, Moringa oleifera, Chlorpyrifos + Moringa oleifera, were given to male rats through gavage for 2 weeks. The influence of chlorpyrifos on brain cholinesterase activity and indicators of oxidative stress malondialdehydecatalase, glutathione, superoxide dismutase and also histopathological changes in the brain of Wistar rats was investigated compared to control group.

Results: There was significant increase in the brain malondialdehyde of the chlorpyrifos group compared to the control and chlorpyrifos + Moringa oleifera group. Also there were significant decreases in the superoxide dismutase, catalase, glutathione and acetylcholinesterase concentrations in the chlorpyrifos group compared to the control and chlorpyrifos + Moringa oleifera group. The rats showed significant changes in body, liver, kidneys and heart weights. No significant haematological changes were seen following treatment. While some histopathological changes were detected in brain tissues in the chlorpyrifos treated group, less histopathological changes were observed in chlorpyrifos + Moringa oleifera treated groups at the end of the 2 weeks’ study.

Conclusion: Overall, these results support the hypothesis that chlorpyrifos causes oxidative stress and Moringa oleifera extract can help reduce the changes induced by chlorpyrifos.

Keywords: chlorpyrifos, oxidative stress, Moringa oleifera, relative organ weight.
Introduction

The use of pesticides in developing countries to improve and preserve farm yields exposes farmers and consumers alike to direct and/or indirect pesticide intoxication(1). Although government regulatory agencies try to limit the market growth of pesticides, its use it still widespread. The most common pesticide used in developing countries is chlopyrifos (CPF)(2). Chlorpyrifos (O, O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate) is a broad spectrum crystalline organophosphate insecticide with agricultural and household applications(3). Exposure to CPF could either be via respiratory, oral and dermal routes (3). Exposure to CPF has been associated with developmental, neurological and reproductive effects (4,5,6). CPF produces toxic effects mainly via the inhibition of acetylcholinesterase and other non-acetylcholinesterase dependent mechanisms such as induction of apoptosis, mitochondrial dysfunction and free radical generation in cells(7). However, the latter is independent on the former. Increased free radical generation overwhelms the natural antioxidant defence of the body resulting in cell damage (8). Boosting the antioxidant status via administration of extracts of plants rich in antioxidants may prevent cell damage. Moringa oleifera also known as drum stick or horse radish tree belongs to the plant family Moringaceae(9,10). Mature Moringa oleifera leaves contain high amounts of antioxidants. (9). Therefore, the present study explores the hematotoxic, brain histological and biochemical toxicity induced by CPF and the possible protective effects of M. oleifera extract.

Materials and Methods

Pesticides

Commercial grade CPF (20% EC, Termicot®, Sabero Organics, Gujarat limited, India), was bought from Main market, Sokoto. It was prepared by reconstituting in soya oil (Grand Cereals and Oil Mills Ltd., Jos, Nigeria). The pesticide mixture solutions were prepared as follows. The CPF + soya oil solution used in this work protected from light and stored at room temperature.

Plant Material, authentication and Preparation of Extract

Fresh Moringa oleifera leaves was bought in bulk from local Market; KofanGawo, Sokoto state Nigeria. The leaves were authenticated, and identification number obtained. Then, a specimen was kept in the herbarium of Department of Botany, Faculty of Biological Sciences, UsmanuDanfodiyo University, Sokoto where a voucher number UDUH/ANS/0225 was deposited.

The leaves were air dried in the laboratory at room temperature and ground into powder with the help of an electrical grinder. The powder was subjected to soxhlet extraction with dehydrated alcohol. The extract obtained was filtered through Whatman filter paper and vacuum dried at 40-50°C to get a blackish green semisolid mass. From the yield obtained, 1 gram of the extract was dissolved in 10 ml of normal saline and mixed finely to make a solution. From this solution 200 mg/kg dose was calculated prior to daily use.

Experimental animals

A total of 24 adult male adult Wistar rats were divided into 4 groups of 6 animals each: Group A: Soya oil, Group B: chlorpyrifos-treated (chlorpyrifos only), Group C: Moringa oleifera-treated and Group D Moringa oleifera + chlorpyrifos-treated Control group which received soya oil at a dose of 2 ml/kg was administered via gavage once per day for 2 weeks. Chlorpyrifos-treated group received chlorpyrifos dissolved in soya oil at a dose of 52.6 mg/kg once daily via gavage. Moringa oleifera-treated group was given Moringa oleifera dissolved in normal saline at a dose of 200 mg/kg once per day via gavage. Moringa oleifera + Chlorpyrifos-treated group received Moringa oleifera dissolved in normal saline (200 mg/kg) via gavage, then 30 minutes later, chlorpyrifos 52.6 mg/kg was administered, also via gavage. The treatments were administered in the morning (between 07:00 and 9:00 h). On the 14th day of treatment, the rats in each group were sacrificed and dissected. The rats were euthanized with ketamine and xylazine combinations. Blood samples were collected from the heart, placed into sterile tubes, and centrifuged at 3500 rpm for 20 min to separate the serum. Blood samples were taken for the haematological tests. Brain
samples were taken to assess histological changes via light microscope examination. For the antioxidant enzymes and malondialdehyde concentrations, a known weight of the brain sample from each animal was homogenized in a known volume of ice-cold phosphate buffer to obtain a 10% homogenate. This was centrifuged at 3000 × g for 10 min to obtain the supernatant. The supernatant was then used to determine levels of MDA, SOD, GSH, CAT and AChE in the brain sample by measuring the absorbance of the samples in a UV spectrophotometer (Model 721A, Jeiferson Ltd, USA).

**Determination of brain Malondialdehyde (MDA) and antioxidant enzyme activities**

Malondialdehyde in brain tissue supernatant was separated and determined as conjugate with TBA as described by(11). MDA reacts with TBA to form MDA-TBA complex. The absorbance was measured at 534 nm to determine the MDA concentration.

SOD activity was determined according to the method described by (12) by assaying the autooxidation and illumination of pyrogallol at 440 nm for 3 min. The SOD activity was expressed was U/mg of protein. Acetylcholinesterase activity was assayed by using the kinetic colorimetric method (13) and enzyme activity was expressed as nmol/mg protein.

CAT activity was measured according to the method described by (14). This was measured by assaying the hydrolysis of H$_2$O$_2$ and the resulting decrease in absorbance at 240 nm over a 3 min period at 25 °C. Results were expressed as mmol/mg protein.

GPx activity was measured using H$_2$O$_2$ as substrate according to the method described by (12).

**Haematological parameters**

Blood samples with anti-coagulant EDTA were analyzed for hematological parameters [red blood cell (RBC) counts, hemoglobin, hematocrit, white blood cell (WBC) counts, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and platelets (PLT)] using autohematology analyser.

**Histopathology**

The brain harvested from the rats were fixed in Bouin Fluid and the areas of the cerebral cortex and cerebellum were dissected out. 7-10 mm semi-serial cuts slice tissues were dehydrated through ascending grades of ethanol (70%, 90% and 95% v/v), cleaned in xylene, and embedded in paraffin wax. The slides were stained with haematoxylin and eosin for light microscopic examination.

**Statistics**

The data were analyzed using graphpad prism version 8. The significance of differences was calculated using one-way analysis of variance (ANOVA) followed by Tukey’s procedure for multiple comparisons. P<0.05 was considered statistically significant.

**Results**

The brain MDA concentration in the CPF treated group was significantly higher (P < 0.001) compared to those obtained in the soya oil, MOL, and CPF + MOL groups, respectively. The MDA concentration in CPF + MOL group was significantly higher compared to those from recorded in the S/oil group and MOL group (P < 0.038 and P < 0.001, respectively) (Table 1).

**Table 1: Effect of exposure to soya oil (S/oil), chlorpyrifos (CPF), M. oleifera and chlorpyrifos + M.oleifera on rat brain malonaldehyde, superoxide dismuthase, catalase, gluthathione and acetylcholinesterase concentration.**

<table>
<thead>
<tr>
<th>Oxidative Stress Markers</th>
<th>S/OIL</th>
<th>CPF</th>
<th>MOL</th>
<th>CPF + MOL</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA conc. (nMol/mg protein)</td>
<td>274.7 ± 9.57</td>
<td>345.0 ± 13.1**</td>
<td>177.8 ± 14.6***</td>
<td>294.5 ± 9.3*</td>
</tr>
<tr>
<td>SOD conc. (U/mg protein)</td>
<td>32.30 ± 1.87</td>
<td>26.80 ± 0.95***</td>
<td>34.5 ± 1.29*</td>
<td>29.3 ± 2.5**</td>
</tr>
<tr>
<td>CAT conc. (mmol/mg protein)</td>
<td>171.5 ± 2.5</td>
<td>126.5 ± 13.3***</td>
<td>245.6 ± 9.67”</td>
<td>169.8 ± 6.5</td>
</tr>
<tr>
<td>GSH conc. (µmol/mg protein)</td>
<td>29.0 ± 0.8</td>
<td>21 ± 1.2”</td>
<td>33.8 ± 4.53”</td>
<td>25.0 ± 2.30*</td>
</tr>
<tr>
<td>AChE conc. (IU/mg protein)</td>
<td>4463.3 ± 190.9</td>
<td>4457.4 ± 106.7</td>
<td>4287.3 ± 22.5</td>
<td>4205.2 ± 70.1*</td>
</tr>
</tbody>
</table>

* Significant differences from the control group at p < 0.05. ** p < 0.01. *** p < 0.001.
The brain SOD concentration in the CPF treated group was significantly lower ($P < 0.001$) compared to those obtained in the soya oil, MOL, and CPF + MOL groups, respectively. The SOD concentration in CPF + MOL group was significantly lower compared to those from recorded in the S/oil group and MOL group ($P < 0.01$ and $P < 0.085$, respectively) (Table 1).

The brain catalase concentration in the CPF treated group was significantly lower ($P < 0.001$) compared to those obtained in the soya oil, MOL, and CPF + MOL groups, respectively. There was no significant difference between the catalase concentration in CPF + MOL and S/oil group. However, the catalase concentration in the MOL group was significantly higher compared to those recorded in the S/oil group and CPF + MOL groups ($P < 0.01$, respectively) (Table 1).

The brain gluthathione concentration in the CPF treated group was significantly lower ($P < 0.001$) compared to those obtained in the soya oil, MOL, and CPF + MOL groups, respectively. The GSH concentration in MOL group was significantly higher compared to those from recorded in the S/oil group ($P < 0.01$, respectively) (Table 1).

The brain acetylcholinesterase concentration in the CPF + MOL treated group was significantly lower ($P < 0.05$) compared to those obtained in the soya oil, MOL, and CPF, respectively. There was no significant difference in the AChE concentration in S/oil, MOL and CPF treated groups (Table 1).

Week one after the administration of chlorpyrifos, body weights of rats in the CPF group decreased significantly compared to those in the s/oil group ($p < 0.05$). While those in the MOL and MOL + CPF increased compared to their weights at week 0. By week 2, the rats in the CPF group differed significantly ($p < 0.05$) compared to those in the S/oil group. There was no significance between the weights of rats in S/oil, MOL and MOL + CPF groups (Table 2).

**Table 2: Effects of Chlopyrifos and treatment with M. oleifera on body weights of rats administered CPF and treated with M. oleifera (Mean ± S.E., $n = 6$ group).**

<table>
<thead>
<tr>
<th>Weeks</th>
<th>S/OIL</th>
<th>CPF</th>
<th>MOL</th>
<th>CPF + MOL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>230.9 ± 12.54</td>
<td>191.1 ± 6.59</td>
<td>164.0 ± 7.80</td>
<td>207.9 ± 4.53</td>
</tr>
<tr>
<td>1</td>
<td>256.0 ± 25.71</td>
<td>170.3 ± 83.71*</td>
<td>178.6 ± 15.32*</td>
<td>220.0 ± 6.91</td>
</tr>
<tr>
<td>2</td>
<td>258.7 ± 27.41</td>
<td>178.1 ± 34.18*</td>
<td>186.0 ± 35.70</td>
<td>231.0 ± 14.89</td>
</tr>
</tbody>
</table>

* Significant differences from the control group at $p < 0.05$.

**Changes in haematological parameters**

There were no statistically significant changes in the haematological parameters when control group was compared to CPF, MOL and CPF + MOL groups at the end of the 14th day dosing period (Table 3).

**Table 3: Haematological profiles of rats exposed to chlopyrifos and treated with M. oleifera (Mean ± S.E., $n = 6$ group)**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>S/Oil</th>
<th>CPF</th>
<th>MOL</th>
<th>CPF + MOL</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC ($\times 10^6 /\mu l$)</td>
<td>8.52±1.09</td>
<td>7.30±0.30</td>
<td>8.42±0.61</td>
<td>7.70±0.41</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>18.34±1.06</td>
<td>15.40±0.61</td>
<td>15.67±0.35</td>
<td>16.98±1.09</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>47.06±6.06</td>
<td>38.18±1.38</td>
<td>45.77±3.74</td>
<td>42.28±2.28</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>55.20±0.65</td>
<td>52.30±1.15</td>
<td>54.23±1.11</td>
<td>54.85±0.42</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>43.94±10.29</td>
<td>40.28±1.19</td>
<td>35.40±3.05</td>
<td>40.02±0.79</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>24.40±5.95</td>
<td>21.08±0.48</td>
<td>19.08±1.49</td>
<td>21.93±0.33</td>
</tr>
<tr>
<td>WBC ($\times 10^3 /\mu l$)</td>
<td>12.98±2.33</td>
<td>19.28±2.37</td>
<td>24.77±4.28</td>
<td>13.53±3.01</td>
</tr>
<tr>
<td>Granulocytes (%)</td>
<td>31.36±9.87</td>
<td>24.14±1.77</td>
<td>26.03±2.81</td>
<td>29.43±8.53</td>
</tr>
<tr>
<td>Band Neutrophils ($\times 10^3 /\mu l$)</td>
<td>3.50±0.32</td>
<td>4.66±0.67</td>
<td>6.77±1.59</td>
<td>3.48±0.70</td>
</tr>
<tr>
<td>Parameters</td>
<td>S/Oil</td>
<td>CPF</td>
<td>MOL</td>
<td>CPF + MOL</td>
</tr>
<tr>
<td>------------------</td>
<td>--------------</td>
<td>--------------</td>
<td>--------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>60.62±9.87</td>
<td>67.86±1.84</td>
<td>66.53±3.12</td>
<td>62.53±8.59</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>8.02±0.29</td>
<td>7.20±1.29</td>
<td>7.43±0.34</td>
<td>8.033±1.07</td>
</tr>
<tr>
<td>Platelets (x 10^3 /μl)</td>
<td>454.80±83.22</td>
<td>514.80±58.64</td>
<td>618.00±120.00</td>
<td>409.00±65.35</td>
</tr>
</tbody>
</table>

Relative organ weight

The relative organ weights (organ/body weight ratios) are shown in Figure 1. The relative liver, heart and kidney weight of rats treated with CPF increase significantly (p < 0.01, 0.001 and 0.018, respectively) compared to those in the S/OIL, MOL and CPF + MOL groups. The relative weights of spleen were significantly decreased (P < 0.05) in the CPF group compared to those in the S/oil, MOL and CPF + MOL groups. However, there was no significant difference in the relative weight of seminal vesicle in all groups, although that of CPF treated group was lower compared to the other groups.

Figure 1: Relative organ weights of rats following treatment with S/oil, CPF, MOL and CPF + MOL. The results represent Means ± S.E. *Significant differences from the control group at *p < 0.05, **p < 0.01).

Figure 2: Photomicrograph of cerebral cortex of rat in the CPF group (H & E X 400). Showing Severe vacoulations(black arrow), Marginal clumping of Chromatin of Nuclei of Most Neurons (white arrow), Congested Blood Vessel surrounded by perivascular edema (yellow arrow)

Figure 3: Cerebral cortex of MOL treated rats showing normal tissue (H & E x 400)
Discussion

Experiments by previous researchers have reported various toxicological effects of CPF this is because CPF is lipophilic and easily cross the cell membrane to the cytoplasm \(^{(2)}\). This study investigated acute CPF induced toxicity and the ameliorative effect of MOL in male Wistar rats. CPF-treated animals also exhibited significantly higher MDA and decreased SOD, CAT and GSH activities than the control group. These may be as a result of peroxidation of membrane lipids and injury to cellular components as earlier reported by \(^{(5,7,15)}\). Oxidative stress occurs when the intracellular antioxidant defence system is overwhelmed by the generation of ROS, leading to increases in toxic molecules \(^{(5)}\). However, the amelioration of the lipoperoxidation in the group treated with CPF + MOL may be due to reduction in lipoperoxidative damage to the brain by MOL as demonstrated by its low MDA concentration in the present study. MOL extract is a rich source of antioxidants containing major bioactive compounds like quercetin, kaempferol, vitamin A and ascorbic acid, which are responsible for its antioxidant activities \(^{(9,10)}\). Irreversible inhibition of acetylcholinesterase in the peripheral and central nervous systems contributes to the cholinergic syndrome induced by an acute exposure to organophosphate insecticides \(^{(16)}\). In the present study, CPF caused a significant reduction in brain acetylcholinesterase concentration which was ameliorated with MOL treatment.

Body and relative organ weights are pointers of some general toxicity in animals after exposure to toxicants \(^{(1)}\). In this study, statistically significant decreases were seen in rats treated with CPF at week 1 and 2, respectively. However, there was an increase in body weight seen in rats in the CPF + MOL group. The increase in body weight in the CPF + MOL group may be because of the vitamins and minerals present in MOL which restores appetite and It was observed that the relative weights of the liver, kidneys and adrenals were significantly increased in CPF group as compared to those rats in the SO, MOL and CPF + MOL groups the controls. Also, there was a significant decrease in the spleen weight of rats in the CPF group. The decrease may be as a result of damage to internal organs caused by pesticides \(^{(17)}\).

Histopathology is a widely used biomarker for the investigation of pesticide toxicity \(^{(18,19)}\). The result of the brain antioxidant markers tests also corroborated the histopathological lesions of severe vacuolations in the cerebral cortex, marginal clumping of chromatin of nuclei of neurons and congested blood vessel surrounded by perivascular oedema seen in the CPF treated group in this study (Figure 2). CPF is known to induce various histopathological changes in the organs of rats as those seen in the present study \(^{(20)}\). Therefore, the results of this study provide insights that *Moringa oleifera* supplementation may mitigate oxidative stress in individuals who are at risk of acute CPF exposure.

Conclusion

Overall, it could be concluded that chlorpyrifos induces oxidative stress in male Wistar rats. The analysis revealed higher MDA and decreased SOD, CAT and GSH activities in the CPF treated group than the control group. The oxidative stress may be attributed to peroxidation of membrane lipids and injury to cellular components. *Moringa oleifera* proved effective against oxidative damage. These results are encouraging enough to further pursue the molecular mechanisms involved in the antioxidant protection provided by the plant.
Ethical Clearance: Sought from UDUS Ethical committee

Conflict of interest: The authors have no conflict of interest to disclose

Funding: This study was extracted from the MSc. thesis of the first author at the Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, UsmanuDanfodiyo University, Sokoto. Authors personal money.

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The Effect of Alpinia Galanga Warm Compress in Reducing Rheumatoid Arthritis Pain Intensity in the Elderly: A Quasi-Experimental Study

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Abstract

Backgrounds: Rheumatoid arthritis is a chronic inflammatory disease that causes inflammation and swelling of the synovial membrane. In patients with advanced stages, it will make the patient unable to carry out daily activities and his quality of life decreases. The study at to know the effect of Alpinia Galanga Warm Compress in Reducing Rheumatoid Arthritis Pain Intensity in the Elderly.

Methods: The method of the research was used quasi experiment by using one group pre-test and post-test design without control group. This study was conducted on 45 patients with Rheumatoid Arthritis in Guna Bakti Foundation in Medan, Indonesia. The research process began with measuring the intensity of pain in all of patient using an observation sheet and numerical pain intensity scale before being given a warm galangal compress (pretest), then a warm galangal compress was carried out for 20 minutes, after which it was measured again (post-test) the intensity of the pain scale.

Results: Based on the results of the study, 80% of respondents experienced a decrease in the Rheumatoid arthritis pain scale as a result of giving warm galangal compresses. From the significance value of the Wilcoxon test results obtained p-value <0.001 which value is smaller than the error level (α) 0.05 or with a significance of 95%, then the hypothesis Ho is rejected while Ha is accepted. The conclusion of this study is the effect of Alpinia galanga warm compress in reducing rheumatoid arthritis pain (p<0.001).

Conclusion: Galangal warm compresses are effective in reducing Rheumatoid Arthritis pain. Therefore, such interventions are recommended for these patients.

Keywords: Alpinia Galanga, Warm Compress, Rheumatoid Arthritis

Introduction

The prevalence of rheumatoid arthritis (RA) was 0.4%, 0.37%, 62%, 1.25% and 0.42% in Southeast Asia, Eastern Mediterranean, Europe, America and the Western Pacific1. RA is a chronic multi-system inflammatory autoimmune disease of indefinite...
etiology\(^2\) and the disease is more common among women and elderly\(^3\). The current treatment with immunosuppression aims to reduce symptoms and to prevent further joint destruction\(^4\).

RA is a chronic inflammatory disease that causes inflammation and swelling of the synovial membrane. Inflammation is characterized by redness, burning, pain, and swelling. RA generally attacks the joints of the fingers, wrists, shoulders, knees, and feet. In patients with advanced stages, it will make the patient unable to carry out daily activities and his quality of life decreases\(^5\). Patients with RA often ask their physician for specific dietary advice and many report that different food items improve or worsen the disease symptoms. Experimental results demonstrate a positive effect of the proposed anti-inflammatory diet on disease activity in patients with RA\(^6\).

According to Andriani (2016), the results showed that giving warm compresses such as lemongrass leaves can be used as an alternative to reduce the intensity of pain felt by elderly people suffering from rheumatoid arthritis\(^7\). Meanwhile, the results of the research conducted by Olviani and Sari (2020) found that there was an effect of lemongrass compresses on reducing the intensity of RA pain in the elderly\(^8\). The results of another study from Margowati showed the effect of cinnamon compresses to change the level of pain in rheumatoid arthritis\(^9\). In addition, a study conducted by Cho, et al, in 2018 found that Korean red ginseng can also reduce the rate of progression of RA\(^10\) and there is concern that its immune-enhancing effects may negatively affect patients with rheumatoid arthritis (RA).

Alpinia Galanga can be used in the world of health because it contains transconiferil disacetate which is useful for preventing cancer and inflammation\(^11\). Alpinia Galanga is a member of the Zingiberaceae family which is used in several countries as herbal medicine. This plant in Thailand is used as an additive in food and other countries in Asia Alpinia galanga has long been used to cure rheumatic pain, inflammation, nasal mucous membranes, bronchial, colds, coughs, throat infections and fever. The content of Alpinia Galanga and Acetoxychavicol acetate in galangal has spicy properties and excellent antimicrobial properties\(^12\).

Traditionally, Alpinia Galanga has anti-bacterial and anti-fungal properties. Alpinia Galanga also increases appetite, warms and cleanses the body. While the chemical content of galangal, namely galangun, astirioil, kaempfena and eugenol is effective in overcoming digestive disorders, treating tumors, and rheumatoid arthritis. In addition, galangal juice at a concentration of 90% has maximum potential in inhibiting the growth of Staphylococcus aureus ATCC 25923 with an inhibition zone diameter of 16 mm\(^13\). Several research results regarding the effect of giving warm compresses can affect the intensity of RA pain, from the results of Santos’s research it was found that there was an effect of ginger warm compresses therapy on pain intensity in the elderly suffering from RA\(^14\). So that researchers are interested in conducting research on warm galangal compresses for reducing the intensity of RA pain in the elderly because previously no one has done this research.

**Materials and Methods**

This study uses a quasi-experimental design\(^15\). This research was conducted at Guna Budi Bakti Foundation, Medan. The population in this study was 45 elderly people suffering from RA. The sampling technique used was total sampling, and the research sample was 45 people.

The research process began with measuring the intensity of pain in all elderly people using an observation sheet and a NRS (numeric rating scale) which is divided based on the classification of mild pain (numbers 1-3), moderate (numbers 4-6) and severe (numbers 7-10) before being given a warm galangal compress (pretest), then a warm galangal compress was carried out for 20 minutes, after which it was measured again (post-test). Before compressing, first prepare the tools and materials, boil the galangal, namely prepare 350 grams of Alpinia Galanga, peel the Alpinia Galanga and then wash it thoroughly, then grate the galangal, turn on the stove fire, prepare a pot and fill with enough clean water, about two liters for 350 grams of Alpinia galanga, heat the water until it boils, then mix the grated Alpinia galanga.

The compressing process starts from informed consent, then washes hands, First clean the painful area to be compressed, then pour warm water in a
thermos into the basin, mix a little clean water into a basin that has been filled with boiled Alpinia galanga water, with a temperature of 40-50 degrees celsius, this is done so that the galangal water is not too hot, then put a towel into the warm water, wait a few seconds before the towel is squeezed, then squeeze the towel and stick it on the painful joint area, lift a small towel when it feels cold, compressing is done for 20 minutes at room temperature 20-25 degrees celsius, then change the warm compress every 5 minutes.

**Results**

The results of this study obtained univariate and bivariate analysis of data. The pain experienced before being given warm ginger compresses in Table 1 shows that the majority of respondents had a moderate pain scale of 26 people (57.8%) and the minority had a mild pain scale of 2 people (4.4%). After the intervention by giving warm ginger compresses, the results showed that the majority of the mild pain scale were 29 people (64.4%) and the majority of severe pain scales were 5 people (11.1%) as shown in Table 2.

**Table 1: Pain Scale before Applying Warm Alpinia Galanga Compress (Pre Test)**

<table>
<thead>
<tr>
<th>No</th>
<th>Pain Scale</th>
<th>Frequency (f)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mild pain</td>
<td>2</td>
<td>4.4</td>
</tr>
<tr>
<td>2</td>
<td>Moderate pain</td>
<td>26</td>
<td>57.8</td>
</tr>
<tr>
<td>3</td>
<td>Severe pain</td>
<td>17</td>
<td>37.8</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>45</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table 2: Pain Scale Prior to Alpinia Galanga Warm Compress (Post Test)**

<table>
<thead>
<tr>
<th>No</th>
<th>Pain Scale</th>
<th>Frequency (f)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mild pain</td>
<td>29</td>
<td>64.4</td>
</tr>
<tr>
<td>2</td>
<td>Moderate pain</td>
<td>11</td>
<td>24.5</td>
</tr>
<tr>
<td>3</td>
<td>Severe pain</td>
<td>5</td>
<td>11.1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>45</td>
<td>100</td>
</tr>
</tbody>
</table>

Based on the analysis of the pre-test data, the Mean value was 2.33 and the Standard Deviation was 0.603 and in the intervention group (post-test) the Mean value was 1.56 and the Standard Deviation was 0.755. This can be seen in Table 3 below.

**Table 3: Data Analysis of Pretest and Posttest Experimental Group**

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Test</td>
<td>45</td>
<td>2.33</td>
<td>0.603</td>
</tr>
<tr>
<td>Post Test</td>
<td>45</td>
<td>1.56</td>
<td>0.755</td>
</tr>
</tbody>
</table>

Based on the results of the study, it was found that the significance value of p-value = <0.001, then H0 was rejected. This means that there is the effectiveness of Alpinia Galanga Warm Compress in Reducing Pain Intensity in RA which can be seen in Table 4 below.

**Table 4: Effectiveness of Alpinia Galanga Warm Compress in Reducing Pain Intensity in RA**

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>mean</th>
<th>Std. Deviation</th>
<th>Z</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Test</td>
<td>45</td>
<td>2.33</td>
<td>0.603</td>
<td>-5.688</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Post Test</td>
<td>45</td>
<td>1.56</td>
<td>0.755</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

The results of this study found that there is effectiveness Alpinia Galanga Warm Compress in Reducing Pain Intensity in RA. RA is a chronic inflammatory autoimmune disease influenced by both genetic, epigenetic and environmental factors. Over the last few years, particular attention has been given to novel genes polymorphisms associated with disease susceptibility and with different stages of the disease. That is characterized by symmetric inflammation of the synovial joints, which may lead to damage to the cartilage and bone, and a progressive loss of function. It typically manifests as poly articular pain with a symmetric distribution mainly affecting the hands and the feet. In the hands, it has a preference for the wrists, as well as the metacarpophalangeal and proximal interphalangeal joints. These joints are not only painful but also often swollen and warm.

Alpinia Galanga is an important genus that
has complex taxonomic diversity and contains bioactive molecules that can act as natural enzyme inhibitors for several deadly diseases and can block metabolic activity pathways. The results highlight the little-known facts of the Alpinia genus in terms of bioactive molecules and their significant therapeutic applications to help fight major human diseases. Alpinia Galanga also contains flavonoids which can help prevent or treat allergies, bacterial infections, arthritis, and certain inflammatory conditions.

According to research by Grzanna, et al., ginger extract from Zingiber officinale (family Zingiberaceae) and Alpina galanga (family Zingiberaceae) has a better therapeutic profile and has fewer side effects than non-steroidal anti-inflammatory drugs. Zingiber officinale (family Zingiberaceae) and Alpina galanga (family Zingiberaceae) inhibit the induction of several genes involved in the inflammatory response. These include genes encoding for cytokines, chemokines, and the inducible enzyme cyclooxygenase-2. These findings provide the first evidence that ginger and galangal modulate activated biochemical pathways in chronic inflammation. Based on the results of research by Latha, et al., crude extract of acetone rhizome Alpinia galanga showed antiplasmid activity against Salmonella typhi, Escherichia coli, and vancomycin-resistant Enterococcus faecalis with efficiency of 92%, 82% and 8%, respectively, at 400 g/ml SIC. The content of 1-Acetoxychavicol acetate mediated R-plasmid curing significantly reduces the concentration of inhibitory antibiotics required to inhibit bacterial growth, thereby making antibiotic treatment more effective.

The content of Alpinia Galanga and Acetoxychavicol acetate in galangal has spicy properties and excellent antimicrobial properties. Traditionally, Alpinia Galanga has anti-bacterial and anti-fungal properties. Alpinia Galanga also increases appetite, warms and cleanses the body. While the chemical content of Alpinia galanga, namely galangun, essential oils, kaempfena and eugenol are effective in overcoming digestive disorders, treating tumors, and rheumatoid arthritis.

Giving a warm compress to the body area will give a signal to the hypothalamus through the spinal cord. When heat-sensitive receptors in the hypothalamus are stimulated, the effector system signals the onset of sweating and peripheral vasodilation. Changes in the size of blood vessels are regulated by the vasomotor center in the medulla oblongata of the brain stem, under the influence of the anterior hypothalamic so that vasodilation occurs.

Based on research conducted by Hillyard et al. (2018), it is explained that complications that can occur in patients with chronic rheumatoid arthritis can result in insufficiency fractures, although this frequency is very rare, it will be very risky for the sufferer. For this reason, an accurate examination is needed to detect the occurrence of insufficiency fractures in patients with rheumatoid arthritis. MRI examination is a very appropriate choice in detecting the occurrence of multiple fluorid insufficiency and lesions that occur on the feet and ankles in patients with RA. A radiological examination can only see signs and symptoms and complications that occur. Frequent radiological examinations can even worsen the condition of patients with RA.

Conclusion

The conclusion of this study is that there is an effectiveness of Alpinia galanga warm compresses on reducing the intensity of the RA pain scale in the elderly by 80%. This is because the warm water of galangal is effective for reducing pain in RA and the content of Alpinia galanga, alpine, camphor, galangin, methycinnamate, has anti-bacterial and fungal properties.

Ethical Clearance: Ethical clearance taken from Ethic Committee in Universitas Prima Indonesia, Indonesia with registration number 125/KEPK/UNPRI/IV/2021

Conflicts of Interest: None declared.

Source of Funding: Self

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Relationship Between Agenesis of Third Molars Skeletal Malocclusion

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Abstract

Background and Aim: Among the agenesis of permanent teeth the most affected tooth is the third molar Mandibular third molar is an unusual tooth characterized by considerable variability in formation, timing, variation in crown and root morphology and not infrequently, by agenesis. Our aim of this study is to find the frequency of third molar agenesis as well as correlation of skeletal malocclusion and third molar agenesis.

Material and Methods: A total of 594 patients (242 males, 352 females) aged between 11 and 25 years were reviewed retrospectively. A tooth was considered developmentally missing when there was no evidence of surgical tooth extraction, when there was no any sign of tooth crown mineralization or crypt development with radiolucency on panoramic radiographs. Patients were divided into five groups according to agenesis of third molars: Patient’s skeletal malocclusion was determined by SNA, SNB and ANB angles as well as Wit’s appraisal and divided into class I, II, III malocclusion.

Results: Third Molar Agenesis was more common in maxilla than in mandible. Third Molar Agenesis was more common in females than males. Significant association was observed between Molar tooth agenesis and Gender. Highest number was observed in females at only 1 third molar was missing followed by 2 third molar missing. Prevalence of third-molar agenesis was more in skeletal class III malocclusion followed by class II and I and it was significant statistically. (p≤0.05).

Conclusion: Third molar agenesis was most commonly observed in maxillary arch than in mandibular arch and more common in skeletal Class III followed by Class I and Class II. It was also observed that Third Molar Agenesis was more common in females than males. Significant association was observed between Molar tooth agenesis and Gender.

Keywords: Third molar agenesis, Maxilla, Mandible, Skeletal Malocclusion.
Introduction

The most common anomaly in the development of human dentition is tooth agenesis, which is defined as the congenital absence of one or more deciduous or permanent teeth.\(^1\) Agenesis of this tooth is frequent, although its frequency ranges widely, varying from zero among an unspecified sample of craniums in Tasmania to 49% in an unspecified sample of Hungarian craniums.\(^2\)

Among the agenesis of permanent teeth the most affected tooth is the third molar (Larmour et al., 2005).\(^3\) Mandibular third molar is an unusual tooth characterized by considerable variability in formation, timing, variation in crown and root morphology and not infrequently, by agenesis.\(^4\)

Genetic factors play a role in tooth agenesis as suggested by familial occurrence, different prevalence data between populations, strong association with hereditary syndromes and defects found in several genes by molecular studies.\(^5\) Specific gene polymorphisms in Msx 1, Pax 9, TGFA are reported for their association to tooth agenesis.\(^6\) Besides genetic factors, environmental factors, systemic diseases, and dietary habits can play an etiological role in the occurrence of dental anomalies including agenesis.\(^7\)

There was an inter-relationship between sagittal skeletal malocclusion and third molar agenesis among orthodontic patient sample.\(^8\) Third molars are the best biological indicator for assessment of juvenile age and they also provide forensic specimens.\(^9,10\)

Our aim of this study is to find the frequency of third molar agenesis as well as correlation of skeletal malocclusion and third molar agenesis.

Material and Method

A total of 594 patients (242 males, 352 females) aged between 11 and 25 years were reviewed retrospectively. A tooth was considered developmentally missing when there was no evidence of surgical tooth extraction, when there was no any sign of tooth crown mineralization or crypt development with radiolucency on panoramic radiographs. Radiographs which show pathologies such as cysts or tumors were excluded from the study. Panoramic images reflecting any artifact, and evident orthodontic treatment or surgery in the past were also excluded from the study.

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

Out of 594 patients 423(71.21%) patient showed no any third molar tooth agenesis whereas 171(28.79%) patients shows third molar agenesis. Among 171 patients 76 patients showed 1 third molar agenesis, 40 patients showed 2 third molar teeth agenesis, 21 patients showed 3 third molar teeth agenesis and 34 patients showed all 4 third molar teeth agenesis. Patients were divided into five groups according to agenesis of third molars:

- Group A (35 males and 41 females), patients with agenesis of 1 missing third molar;
- Group B (14 males and 26 females), patients with agenesis of 2 missing third molars;
- Group C (11 males and 10 females), patients with agenesis of 3 missing third molars;
- Group D (16 males and 18 females), patients with agenesis of 4 missing third molars and
- Group E (166 males and 257 females), patients without agenesis of missing third molar

Out of 594 patients 423(71.21%) patient showed no any third molar tooth agenesis whereas 171(28.79%) patients shows third molar agenesis. Among 171 patients 76 patients showed 1 third molar agenesis, 40 patients showed 2 third molar teeth agenesis, 21 patients showed 3 third molar teeth agenesis and 34 patients showed all 4 third molar teeth agenesis. Patient’s skeletal malocclusion was determined by SNA, SNB and ANB angles as well as Wit’s appraisal and divided into class I, II, III malocclusion.

Statistical analysis

The recorded data was compiled and entered in a spreadsheet computer program (Microsoft Excel 2007) and then exported to data editor page of SPSS version 15 (SPSS Inc., Chicago, Illinois, USA). For all tests, confidence level and level of significance were set at 95% and 5% respectively.
Results

Table 1 Describes Distribution of Third Molar Agenesis in Maxilla and Mandible. Third Molar Agenesis was more common in maxilla than in mandible

| Table 1: Distribution of Third Molar Agenesis in Maxilla and Mandible |
|------------------|------------------|------------------|
| Maxilla | Frequency | Percentage |
| 102 | 59.64 |
| Mandible | 69 | 40.35 |
| Total | 171 | 100 |

Table 2: Distribution Third Molar Agenesis according to Gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>74</td>
<td>43.27</td>
</tr>
<tr>
<td>Female</td>
<td>97</td>
<td>56.72</td>
</tr>
<tr>
<td>Total</td>
<td>171</td>
<td>100</td>
</tr>
</tbody>
</table>

According to Table 2 Third Molar Agenesis was more common in females than males.

Table 3: Association of Molar tooth agenesis by Gender

<table>
<thead>
<tr>
<th>Variable</th>
<th>Male N (%)</th>
<th>Female N (%)</th>
<th>Frequency N (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1Third molar tooth agenesis</td>
<td>32 (42.10)</td>
<td>44 (57.8)</td>
<td>76 (44.4)</td>
<td>0.02*</td>
</tr>
<tr>
<td>2Third molar teeth agenesis</td>
<td>19 (47.5)</td>
<td>21 (52.5)</td>
<td>40 (23.39)</td>
<td></td>
</tr>
<tr>
<td>3Third molar teeth agenesis</td>
<td>10 (47.6)</td>
<td>11 (52.3)</td>
<td>21 (12.28)</td>
<td></td>
</tr>
<tr>
<td>4Third molar teeth agenesis</td>
<td>13 (38.23)</td>
<td>21 (61.76)</td>
<td>34 (19.88)</td>
<td></td>
</tr>
</tbody>
</table>

Test applied: Chi square test, *p ≤ 0.05 statistically significant

Chi-square value: 5.59, df = 5

Table 3 Describes Association of Molar tooth agenesis by Gender. Significant association was observed between Molar tooth agenesis and Gender. Highest number was observed in females at only 1 third molar was missing followed by 2 third molar missing.

Table 4: Distribution of Molar tooth agenesis by Malocclusion

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total no of patients</th>
<th>Male no of patients with teeth agenesis N (%)</th>
<th>Female no of patients with teeth agenesis N (%)</th>
<th>no of patients with teeth agenesis N (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I</td>
<td>214</td>
<td>20 (41.6)</td>
<td>28 (58.33)</td>
<td>48 (28)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Class II</td>
<td>195</td>
<td>26 (44.06)</td>
<td>33 (55.93)</td>
<td>59 (34.5)</td>
<td></td>
</tr>
<tr>
<td>Class III</td>
<td>185</td>
<td>28 (43.75)</td>
<td>36 (56.25)</td>
<td>64 (37.4)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>594</td>
<td>74 (43.27)</td>
<td>97 (56.72)</td>
<td>171 (100)</td>
<td></td>
</tr>
</tbody>
</table>

Test applied: Chi square test, *p ≤ 0.05 statistically significant

Chi-square value: 12.04, df = 6

According to table 4 it was observed that Prevalence of third-molar agenesis was more in skeletal class III malocclusion followed by class II and I and it was significant statistically.(p≤0.05)

Discussion

Third molar is a tooth that develops after birth and also the last tooth to erupt. It is characterized by the variability in the time of its formation, its widely varying crown and root morphology, and its varying presence or absence in the oral cavity. A total of 594 patients (242 males, 352 females) aged between 11 and 25 years were reviewed retrospectively.

In this study minimum age was set at 11 years because third molar crypt formation starts at 3 to 4 years of age, calcification begins from 7 to 10 years, crown calcification completes at 12 to 16 years of age.
and eruption occurs between 17 to 25 years of age.\textsuperscript{12,13} Upper age limit was set at 25 years because until this age complete eruption of 3rd molar occurs in the oral cavity. If any patient had undergone surgical removal of a third molar, those patients were excluded from the study. The clinical implications of the associated dental anomalies are relevant, since early detection of a single dental anomaly may call the attention of professionals to the possible development of other associated anomalies in the same patient or in the family, allowing timely orthodontic intervention.

Females presented a higher prevalence of third molar agenesis than males. As the dimensions of dental arch of females were generally smaller than males and growth of maxilla and mandible in females were slower after 12–13 years but in case of male growth continues until age of 16 years.\textsuperscript{14} Racial variations, dietary habit, masticatory function and genetic inheritance can affect jaw size and facial growth. In an animal study, Yamada and Kimmel\textsuperscript{15} reported that diet and masticatory function had a direct relationship with craniofacial growth, specifically effecting the mandible, which could in turn affect the presence/agenesis of third molar.

Significant association was observed between Molar tooth agenesis and Gender. Highest number was observed in females at only 1 third molar was missing followed by 2 third molar missing which is similar to the studies done by Banks\textsuperscript{16} and Afzal et al.\textsuperscript{17} In Contrast, other studies found the order of frequency of Molar agenesis to be one, two, three and four respectively.\textsuperscript{18,19} In addition, there was a significant difference in the occurrence of Molar agenesis between upper and lower jaw (p=0.001).

According to table 4 it was observed that Prevalence of third-molar agenesis was more in skeletal class III malocclusion followed by class II and I and it was significant statistically (p≤0.05). This findings were in accordance with Celikoglu\textsuperscript{16} who reported that the prevalence of third molar agenesis was more in skeletal class III followed by class I and II, this may be due to polygenetic inheritance on formation of third molar germs that control maxillary and/or mandibular dimensions which was different in different Population.

**Conclusion**

Third molar agenesis was most commonly observed in maxillary arch than in mandibular arch and more common in skeletal Class III followed by Class I and Class II. It was also observed that Third Molar Agenesis was more common in females than males. Significant association was observed between Molar tooth agenesis and Gender.

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

**References**

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Bone and its Considerations in Implantology: A Review

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Abstract

Bone is an organ that is able to change in relation to a number of factors and has its implications in Implantology especially when dealing with the edentulous arches. This review will elaborate on various aspects of bone and its clinical implication

Keywords: Bone, implantology, resorption, remodelling.

Introduction

Bone is an organ that is able to change in relation to a number of factors, including hormones, vitamins, and mechanical influences. Wolff, in 1892, elaborated on these concepts and published, “Every change in the form and function of bone or of its function alone is followed by certain definite changes in the internal architecture, and equally definite alteration in its external conformation, in accordance with mathematical laws. The maxilla and mandible have different biomechanical functions. The mandible, as an independent structure, is designed as a force-absorption unit. The maxilla is a force distribution unit. Any strain to the maxilla is transferred by the zygomatic arch and palate away from the brain and orbit. As a consequence, the maxilla has a thin cortical plate and fine trabecular bone supporting the teeth.

Consequences of tooth loss:

Bone density in the jaws decreases after tooth loss. This loss is primarily related to the length of time the region has been edentulous and not loaded appropriately, the initial density of the bone, flexure and torsion in the mandible, and parafunction before and after tooth loss.

Bone Remodelling and Density:

Remodeling is a process of resorption and formation at the same site that replaces previously existing bone and primarily affects the internal turnover of bone, including that region where teeth are lost or the bone next to an endosteal implant. Frost proposed a model of four histologic patterns for compact bone as it relates to mechanical adaptation to strain.¹
Acute Disuse Window

The bone in the acute disuse window loses mineral density, and disuse atrophy occurs. The microstrain of bone for trivial loading is reported to be 0 to 50 microstrain. A cortical bone density decrease of 40% and a trabecular bone density decrease of 12% also have been reported with disuse of bone.

Adapted Window

The adapted window (50 to 1500 microstrain) represents an equilibrium of modeling and remodeling, and bone conditions are maintained at this level. Bone in this strain environment remains in a steady state, and in homeostatic window of health. This is the range of strain ideally desired around an endosteal implant.

Mild Overload Zone

The mild overload zone (1500 to 3000 microstrain) causes a greater rate of fatigue microfracture. As a result, the bone strength and density may eventually decrease. The histologic description of bone in this range is usually woven or repair bone.

Pathological Overload Zone

Pathologic overload zones are reached when microstrains are greater than 3000 units. Cortical bone fractures occur at 10,000 to 20,000 microstrain (1% to 2% deformation). Therefore pathologic overload may begin at microstrain levels of only 20% to 40% of the ultimate strength or physical fracture of cortical bone. The bone may resorb and form fibrous tissue.

Classifications related to bone density:

Linkow\textsuperscript{2} classified bone density into three categories:

Class I bone structure: This ideal bone type consists of evenly spaced trabeculae with small cancellated spaces.

Class II bone structure: The bone has slightly larger cancellated spaces with less uniformity of the osseous pattern.

Class III bone structure: Large marrow-filled spaces exist between bone trabeculae.

Lekholm and Zarb\textsuperscript{3} listed four bone qualities found in the anterior regions of the jawbone.

Quality 1 is composed of homogenous compact bone

Quality 2 has a thick layer of cortical bone surrounding dense trabecular bone

Quality 3 has a thin layer of cortical bone surrounded by dense trabecular bone of favorable strength.

Quality 4 has a thin layer of cortical bone surrounding a core of low-density trabecular bone.

Misch\textsuperscript{3,4} proposed four bone density groups independent of the regions of the jaws, based on macroscopic cortical and trabecular bone characteristics. In combination, these four increasing macroscopic densities constitute four bone categories described by Misch (D1, D2, D3, and D4) located in the edentulous areas of the maxilla and mandible. The regional locations of the different densities of cortical bone are more consistent than the highly variable trabecular bone. A very soft bone, with incomplete mineralization and large intertrabecular spaces, may be addressed as D5 bone.

Bone Density based Treatment Planning

Bone density is directly related to the strength of bone before microfracture. A tenfold difference in bone strength may be observed from D1 to D4 bone. D2 bone exhibited a 47% to 68% greater ultimate compressive strength, compared with D3 bone. In other words, on a scale of 1 to 10, D1 bone is a 9 to 10 relative to strength. D2 bone is a 7 to 8 on this scale. D3 bone is 50% weaker than D2 bone and is a 3 or 4 on the strength scale. D4 bone is a 1 to 2 and up to 10 times weaker than D1 bone.

Influence on bone-to-implant contact

The bone implant contact (BIC) percentage is significantly greater in cortical bone than in trabecular bone. The very dense D1 bone of a C–h resorbed anterior mandible or of the lingual cortical plate of a Division A anterior or posterior mandible provides the highest percentage of bone in contact with an endosteal implant and may approximate more than 85% BIC.

Treatment Planning

Bone density is an implant treatment plan modifier in several ways—prosthetic factors, implant
size, implant design, implant surface condition, implant number, and the need or method of progressive loading. To decrease the incidence of microfracture of bone, the strain to the bone should be reduced.

1. cantilever length may be shortened or eliminated,
2. RP-4 restorations, rather than fixed prostheses
3. RP-5 prostheses permit the soft tissue to share the occlusal force and reduce the stress on the implants.
4. Night guards and acrylic occlusal surfaces distribute and dissipate parafunctional forces on an implant system.
5. As the bone density decreases, the occlusal loads should be oriented more axially.
6. Increase the number of implants
7. Increase the width of the implant to provide more surface area to dissipate crestal loads
8. Macro-design of implant – an implant body designed for softer bone should have more and deeper threads than an implant design for hard bone.
9. Surface coatings enhance bioadhesion, rougher surfaces are indicated for soft bone.
10. The softer the bone, higher the need for progressive loading

**Available Bone**

Available bone describes the amount of bone in the edentulous area considered for implantation. It is measured in width, height, length, angulation, and crown height space. As a general guideline, 1.5 to 2 mm of surgical error is maintained between the implant and any adjacent landmark. This is especially critical when the opposing landmark is the mandibular inferior alveolar nerve. However, the implant may be placed without complication through the cortical plate of the maxillary sinus or inferior border of the mandible. The implant may also be positioned closer to the cribiform plate of a natural tooth. If the implant should become mobile or affected by perimplant disease, the adjacent landmark may be adversely involved. Likewise, if the sinus becomes infected or the adjacent tooth suffers from periodontal disease, the implant may be affected.

Importance of evaluating dimensions of available bone-

Manufacturers describe the root form implant in dimensions of width and length. The implant length corresponds to the height of available bone. The width of a root form implant is most often related to the diameter and mesiodistal length of available bone. Most root form implants have a round cross-sectional design to aid in surgical placement; therefore the diameter of the implant corresponds to the implant width. Many manufacturers propose implants with a crest module wider than the implant body dimension. Yet the often stated dimension of the manufacturer is the smaller body width. The clinician should be knowledgeable of all implant dimensions, especially because the crestal dimension of bone (where the wider crest module dimension is placed) is usually the narrowest region of the available bone and where the implant is closest to an adjacent tooth.

**Available Bone Width**

The width of available bone is measured between the facial and lingual plates at the crest of the potential implant site. The crest of the edentulous ridge is most often supported by a wider base. In most areas, because of this triangular-shaped cross section, an osteoplasty provides greater width of bone, although of reduced height. Crest reduction affects the location of the opposing landmark, with possible consequences for surgery, implant height selection, appearance, and the design of the final prosthesis. This is particularly important when an FP-1 prosthesis is planned, with the goal of obtaining a normal contour and proper soft tissue drape around a single tooth replacement. The crestal aspect of the residual ridge is often cortical in nature and exhibits greater density than the underlying trabecular bone regions, especially in the mandible. This mechanical advantage permits immediate fixation of the implant, provided this cortical layer has not been removed by osteoplasty.

**Available Bone Height**

The available bone height is first estimated by radiographic evaluation in the edentulous ideal and optional regions, where implant abutments are required for the intended prosthesis. The height
of available bone is measured from the crest of the edentulous ridge to the opposing landmark.

Anatomical Limitations-

The anterior regions are limited by the maxillary nares or the inferior border of the mandible. The anterior regions of the jaws have the greatest height, because the maxillary sinus and inferior alveolar nerve limit this dimension in the posterior regions. The maxillary canine eminence region often offers the greatest height of available bone in the maxillary anterior.

The mandibular first premolar region is usually anterior to the mental foramen and provides the most vertical column of bone in the posterior mandible. However, on occasion, this premolar site may present a reduced height compared with the anterior region, because of the anterior loop of the mandibular canal (when present) as it passes below the foramen and proceeds superiorly, then distally, before its exit through the mental foramen. The available bone height in an edentulous site is the most important dimension for implant consideration, because it affects both implant length and crown height. Crown height affects force factors and esthetics.

**Available Bone Length**

The available bone length determines in part the diameter of the implant but most importantly the number of implants that maybe placed in a given edentulous space. The mesiodistal length of available bone in an edentulous area is often limited by adjacent teeth or implants. As a general rule, the implant should be at least 1.5 mm from an adjacent tooth and 3 mm from an adjacent implant. This dimension not only allows surgical error, but also compensates for the width of an implant or tooth crestal defect, which is usually less than 1.4 mm. As a result, if bone loss occurs at the crest module of an implant or from periodontal disease with a tooth, the vertical bone defect will not spread to a horizontal defect and cause bone loss on the adjacent structure.

**Available Bone Angulation**

Bone angulation is the fourth determinant for available bone. The initial alveolar bone angulation represents the natural tooth root trajectory in relation to the occlusal plane. Ideally, it is perpendicular to the plane of occlusion, which is aligned with the forces of occlusion and is parallel to the long axis of the prosthodontic restoration. The maxillary anterior teeth are the only segment in either arch that does not receive a long axis load to the tooth roots, but instead are usually loaded at a 12-degree angle. As such, their root diameter is greater than the mandibular anterior teeth. In all other regions, the teeth are loaded perpendicular to the curves of Wilson or Spee.

Anatomical Considerations-

Rarely does the bone angulation remain ideal after the loss of teeth, especially in the anterior edentulous arch. In this region, labial undercuts and resorption after tooth loss, often mandate greater angulation of the implants or correction of the site before insertion.

In the posterior mandible, the submandibular fossa mandates implant placement with increasing angulation as it progresses distally. Therefore, in the second premolar region, the angulation may be 10 degrees to a horizontal plane; in the first molar areas, 15 degrees; and in the second molar region, 20 to 25 degrees.

**Crown Height Space**

The crown height space (CHS) is defined as the vertical distance from the crest of the ridge to the occlusal plane. It affects the appearance of the final prosthesis and the amount of moment force on the implant and surrounding crestal bone during occlusal loading. The CHS may be considered a vertical cantilever.

The greater the CHS, the greater the moment force or lever arm with any lateral force or cantilever.

Therefore, as the CHS increases, a greater number of implants or wider implants should be inserted to counteract the increase in stress.

**Available Bone and Treatment Planning**

In 1985, Misch and Judy established four basic divisions of available bone for implant dentistry in the edentulous maxilla and mandible, which follow the natural resorption phenomena of each region, and determined a different implant approach to each category.
**Division A (Abundant Bone)**

Features-
1. Width > 6 mm
2. Height > 12 mm
3. Mesiodistal length > 7 mm
4. Angulation of occlusal load (between occlusal plane and implant body) < 25 degrees
5. Crown height space ≤ 15 mm

Treatment Planning-

The prosthetic options for Division A span the full gamut. An FP-1 restoration requires a Division A ridge. However, an FP-2 prosthesis most often also requires a Division A bone. An FP-2 restoration is the most common posterior restoration supported by multiple adjacent implants in partially edentulous patients, because of either bone loss or osteoplasty before implant placement. An FP-3 prosthesis is most often the option selected in the anterior Division A bone when the maxillary smiling lip position is high or a mandibular low lip line during speech exposes regions beyond the natural anatomical crown position.

**Division B (Barely Sufficient Bone)**

As the bone resorbs, the width of available bone first decreases at the expense of the facial cortical plate, because the cortical bone is thicker on the lingual aspect of the alveolar bone, especially in the anterior regions of the jaws. There is a 25% decrease in bone width in the first year and a 40% decrease in bone width within the first 1 to 3 years after tooth extraction.

Features-
1. Height > 12 mm
2. Mesiodistal length > 6 mm
3. Angulation < 20 degrees
4. Crown height space < 15 mm

Treatment Planning -

Three treatment options are available for the Division B edentulous ridge:
1. Modify the existing Division B ridge to another division by osteoplasty to permit the placement of root form implants 4 mm or greater in width (Figure4.9). When more than 12 mm of bone height results, the bone converts to Division A. When less than 12 mm of bone height results, the bone converts to Division C-h.
2. Insert a narrow Division B root form implant.
3. Modify the existing Division B bone into Division A by augmentation.

**Division C (Compromised Bone)**

Features –
1. Width (C-w bone): 0 to 2.5 mm
2. Height (C-h bone) < 12 mm
3. Angulation of occlusal load (C-a bone) > 30 degrees
4. Crown height space (CHS) > 15 mm

There are seven treatment options for the Division C bone:
- Osteoplasty (C-w)
- Root form implants (C-h)
- Subperiosteal implant (C-h, C-a partial, or completely edentulous mandible)
- Augmentation procedures before implant insertion
- Disk design implants (posterior mandible, anterior maxilla)
- Ramus frame implant (C-h completely edentulous mandible)
- Transosteal implant (C-h anterior mandible)

**Division D (Deficient Bone)**

Long-term bone resorption may result in the complete loss of the residual ridge, accompanied by basal bone atrophy.

Features-
1. Severe atrophy
2. Basal bone loss
3. Flat maxilla
4. Pencil-thin mandible
5. >20 mm crown height

It is not infrequent that these patients complain of paresthesia of the lower lip, especially during...
mastication. The CHS is greater than 20 mm, which is a significant force multiplier and can rarely be reduced enough to render long-term success.

Treatment Planning-

The partially or completely edentulous patient with a posterior Division D maxilla and healthy anterior teeth or implants may undergo sinus graft procedures with a combination of local autogenous bone, demineralized freeze-dried bone, and calcium phosphate bone substitutes.

It should be the goal of every dentist to educate and treat the patient before a Division D bone condition develops.

Conclusion

The prudent practitioner monitors bone loss in edentulous sites and offers education and treatment before deleterious effects. The bone density, anatomical site and amount of residual bone should become the primary determinants of the multi-faceted treatment plan for an implant supported prosthesis.

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References

Bone Biomechanics and its Considerations in Implantology: A review

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Abstract

Biomechanics is the study of ‘the mechanical laws relating to the movement or structure of living organisms’. The implant supported prostheses is a unique biosimulating device that should function in tandem with the existing masticatory system. This paper will highlight the responses of bone and the implant system to biomechanics.

Keywords: biomechanics, implant, failure, bone, prosthesis

Introduction

Bone is a dynamic living tissue that is capable of continuously adapting to the changes in the oro-facial environment. The biomechanical properties of the bone-implant interface are the key determinants for the implant stability as well as for the evolution of the implant status. The bone-implant interface properties are determined by the quantity of the implant surface in intimate contact with mineralized bone tissue as well as by the mechanical quality of bone tissue around the interface.

What happens after implant placement?

When an intimate surgical fit between bone and the implant surface occurs after surgery, the interfacial bone undergoes remodelling and is substituted with mature lamellar bone. However, when bone and implants are not in intimate contact, rapid woven bone filling occurs and long term implant stability is ensured by bone modelling and remodelling processes.

During bone healing, micromotions at a relatively low level may be responsible for biomechanical stimulation of bone remodelling. However, fibrous tissue may develop instead of an osseointegrated interface when there is excessive interfacial micromotion early after surgery. The critical parameter is the absence of excessive micromotion at the bone-implant interface.

The cortical bone-implant interface is the most important region regarding the implant success due to highest bone stresses occurring in cortical bone around the implant neck. Mechanical loading...
is essential to skeletal health. Control of most bone modelling and some remodelling processes are related to strain history, which usually is defined in micro strain (µε).

It is proposed that (1) subthreshold loading of less than 200 µε results in disuse atrophy, manifested as a decrease in modelling and an increase in remodelling; (2) physiologic loading of about 200 to 2500 µε is associated with normal, steadystate activities; (3) loads exceeding the minimal effective strain (about 2500 µε) result in a hypertrophic increase in modelling and a concomitant decrease in remodelling; and (4) after peak strains exceed about 4000 µε, the structural integrity of bone is threatened, resulting in pathologic overload.¹

Normal function helps build and maintain bone mass. Sub-optimally loaded bones atrophy as a result of increased remodelling frequency and inhibition of osteoblast formation. Under these conditions, trabecular connections are lost and cortices are thinned from the endosteal surface. Eventually the skeleton is weakened until it cannot sustain normal function.

When flexure (strain) exceeds the normal physiologic range, bones compensate by adding new mineralized tissue at the periosteal surface.

From a dental perspective, occlusal prematurities or parafunction may lead to compromise of periodontal bone support. Localized fatigue failure may be a factor in periodontal clefting, alveolar recession, tooth oblation (cervical ditching), or TMJ arthrosis.

**Implant Biomechanics**

Dental implants are subjected to occlusal loads when placed in function. Such loads may vary dramatically in magnitude, frequency, and duration, depending on the patient’s parafunctional habits. Passive mechanical loads also may be applied to dental implants during the healing stage because of mandibular flexure, contact with the first-stage cover screw, and second-stage permucosal extension.

Perioral forces of the tongue and circumoral musculature may generate low but frequent horizontal loads on implant abutments. These loads may be of greater magnitude with parafunctional oral habits or tongue thrust. Besides, application of non-passive prostheses to implant bodies may result in mechanical loads applied to the abutment, even in the absence of occlusal loads. All these factors make it imperative to understand the biomechanically working of the implant.

A force applied to a dental implant rarely is directed absolutely longitudinally along a single axis. In fact, three dominant clinical loading axes exist in implant dentistry: (1) mesiodistal, (2) faciolingual, and (3) occlusoapical.² A single occlusal contact most commonly results in a three-dimensional occlusal force. Forces may be described as compressive, tensile, or shear. Compressive forces attempt to push masses toward each other. Tensile forces pull objects apart. Shear forces on implants cause sliding. Compressive forces tend to maintain the integrity of a bone-implant interface, whereas tensile and shear forces tend to distract or disrupt such an interface. Shear forces are most destructive to implants and bone compared with other load modalities.

The implant body design transmits the occlusal load to the bone. Threaded or finned dental implants impart a combination of all three force types at the interface under the action of a single occlusal load. This “conversion” of a single force into three different types of forces is controlled completely by the implant geometry. The prevalence of potentially dangerous tensile and shear forces in threaded or finned implants may be controlled optimally through careful engineering design.

**Stress-Strain Relationship**

A relationship is needed between the applied force (stress) that is imposed on the implant and surrounding tissues and the subsequent deformation (strain) experienced throughout the system. The closer the modulus of elasticity of the implant resembles that of the contiguous biological tissues, the less the likelihood of relative motion at the tissue-implant interface.

**Force Delivery and Failure Mechanisms**

The manner in which forces are applied to implant restorations within the oral environment dictates the likelihood of system failure. The duration
of a force may affect the ultimate outcome of an implant system. Relatively low-magnitude forces, applied repetitively over a long time, may result in fatigue failure of an implant or prosthesis.

Moment Loads

The moment of a force about a point tends to produce rotation or bending about that point. Torques or bending moments imposed on implants because of, for example, excessively long cantilever bridge or bar sections may result in interface breakdown, bone resorption, prosthetic screw loosening, or bar or bridge fracture.

**Clinical Moment Arms**

A total of six moments (rotations) may develop about the three clinical coordinate axes previously described. Three clinical moment arms exist in implant dentistry: (1) occlusal height, (2) cantilever length, and (3) occlusal width. Minimization of each of these moment arms is necessary to prevent unretained restorations, fracture of components, crestal bone loss, or complete implant system failure.

**Occlusal Height**

Occlusal height serves as the moment arm for force components directed along the faciolingual axis—working or balancing occlusal contacts, tongue thrusts, or in passive loading by cheek and oral musculature.

**Cantilever Length**

Large moments may develop from vertical axis force components in prosthetic environments designed with cantilever extensions or offset loads from rigidly fixed implants.

**Occlusal Width**

Wide occlusal tables increase the moment arm for any offset occlusal loads.

**Fatigue Failures**

Fatigue failure is characterized by dynamic, cyclic loading conditions. Four fatigue factors significantly influence the likelihood of fatigue failure in implant dentistry: (1) biomaterial, (2) macrogeometry, (3) force magnitude, and (4) number of cycles.

If an implant is subjected to an extremely high stress, then only a few cycles of loading can be tolerated before fracture occurs. Alternatively, an infinite number of loading cycles can be maintained at low stress levels. The stress level below which an implant biomaterial can be loaded indefinitely is referred to as its endurance limit. Titanium alloy exhibits a higher endurance limit.

**Biomechanics at the Implant-Abutment Interface**

The implant abutment interface determines joint strength, stability and lateral and rotational stability. Of particular concern is the problem of screw loosening, which is primarily a biomechanical failure.

**Abutment screw mechanics**

The mechanics of the tapered interference fit, use two types of connection methods: (a) a screw and (b) a tapered interference fit (also called Morse taper)

Screw loosening occurs when the joint-separating forces acting on the screw joint are greater than the clamping forces holding the screw unit together.

**Joint clamping forces (preload) v/s joint separating**

A screw is tightened applying torque. The applied torque develops a force within the screw called the preload. As a screw is tightened, the screw elongates, producing tension. Elastic recovery of the screw pulls the two parts together, creating a clamping force. Hence, established preload is directly proportional to applied torque. Too little torque leads to separation of the joint, screw loosening and failure of connection and too large implant interface and the missing of a passive adaptation torque results in stripping of screw threads and screw fatigue.

To be effective, the level of preload must be less than the elastic limit or proof load, i.e. the maximum load at which implant with no permanent deformation occurs. Depending on the stiffness of the screw material and whether or not the screw is being reused, preloads of 75% to 90% of the elastic limit may be required to prevent loosening under moderate lateral loads. Excessive forces cause slippage between threads of the screw and threads of the bore, resulting in a loss of preload.
Separating forces may include, occlusal contacts, lateral excursive contacts, interproximal contacts between natural teeth and implant restorations, protrusive contacts, parafunctional forces and non-passive frameworks that attach to the implants.

**Settling effect/ embedding relaxation**

No matter how carefully machined an implant surface is, it is slightly rough when viewed microscopically. Settling occurs as the rough spots flatten under load, since they are the only contacting surfaces when the initial tightening torque is applied. When the total settling effect is greater than the elastic elongation of the screw, the screw works loose there are no longer contact forces to hold it in place. To reduce the settling effect, implant screws should be retightened 10 minutes after the initial torque application. This technique should be used as a routine clinical procedure.

**Patient related force factors**

An implant foundation should be designed to support the load and resist the stresses while the restoration is in service. The treatment plan is modified dependent on the force factors of the individual patient.

**Parafunction –**

Parafunctional forces on teeth or implants are characterized by repeated or sustained occlusion and have long been recognized as harmful to the stomatognathic system. This occurs with greater frequency in the maxilla, because of a decrease in bone density and an increase in the moment of force.

**Bruxism**

Bruxism primarily concerns the horizontal, nonfunctional grinding of teeth. The forces involved are in significant excess of normal physiologic masticatory loads. Bruxism may affect the teeth, muscles, joints, bone, implants, and prostheses.

**Clenching**

Clenching is a habit that generates a constant force exerted from one occlusal surface to the other without any lateral movement. The increase in force magnitude and duration is a significant problem, whether by bruxism or clenching. In addition, the clenching patient may suffer from a phenomenon called creep, which also results in fracture of components. Creep occurs in a material when an increasing deformation is expressed as a function of time, when subjected to a constant load.

A common cause of implant failure during healing is parafunction in a patient wearing a soft tissue-supported prosthesis over a submerged implant. The tissue overlying the implant is compressed during the parafunction. The premature loading may cause micromovement of the implant body in the bone and may compromise osteointegration.

**Prosthesis Planning**

- The time intervals between prosthodontic restoration appointments may be increased to provide additional time to produce load-bearing bone around the implants through progressive bone-loading techniques.
- Additional implants are indicated, preferably of greater diameter.
- The excursions are canine guided if natural, healthy canines are present. Mutually protected occlusion, with additional anterior implants or teeth distributing forces, is developed if the implants are in the canine position or if this tooth is restored as a pontic.
- Narrow posterior occlusal tables to prevent inadvertent lateral forces and to decrease the occlusal forces are beneficial.
- Enamoplasty of the cusp tips of the opposing natural teeth is indicated to help improve the direction of vertical forces, within the guidelines of the intended occlusion.

**Tongue Thrust**

Parafuctional tongue thrust is the unnatural force of the tongue against the teeth during swallowing. Although the force of tongue thrust is of lesser intensity than in other parafunctional forces, it is horizontal in nature and can increase stress at the permucosal site of the implant. The tongue thrust may also contribute to incision line opening, which may compromise both the hard and soft tissues.

A potential prosthetic complication for a patient with a lateral tongue thrust is the complaint of inadequate room for the tongue once the mandibular
Implants are restored. A prosthetic mistake is to reduce the width of the lingual contour of the mandibular teeth. A reduction in the width of the posterior teeth often increases the occurrence of tongue biting and may not dissipate with time.

Crown Height Space (CHS)-

The CHS for implant dentistry is measured from the crest of the bone to the plane of occlusion in the posterior region and the incisal edge of the arch in the anterior region. The ideal CHS needed for a fixed implant prosthesis should range between 8 and 12 mm. This measurement accounts for the biological width, abutment height for cement retention or prosthesis screw fixation, occlusal material strength, esthetics, and hygiene considerations around the abutment crowns. Removable prostheses often require a CHS greater than 12 mm for denture teeth and acrylic resin base strength, attachments, bars, and oral hygiene considerations. The CHS is a vertical cantilever when any lateral or cantilevered load is applied and, therefore, is also a force magnifier.

When a cantilever is placed on an implant, there are six different potential rotation points (i.e., moments) on the implant body. When the crown height is increased from 10 to 20 mm, two of six of these moments are increased 200%.

Hence, with an increase in CHS, the cantilever gets magnified when offset loads are applied leading to fatigue failure of the implant and more commonly crestal bone loss. Hence, all efforts should be made to decrease the stress on the surrounding bone when CHS is in excess of 15 mm. Following are some important features of the prosthesis design that will decrease stresses:

1. Shorten cantilever length
2. Minimize offset loads to the buccal or lingual
3. Increase the number of implants
4. Increase the diameters of implants
5. Design implants to maximize the surface area of implants
6. Fabricate removable restorations that are less retentive and incorporate soft tissue support
7. Remove the removable restoration during sleeping hours to reduce the noxious effects of nocturnal parafunction
8. Splint implants together, whether they support a fixed or removable prosthesis.

Masticatory Dynamics

Masticatory muscle dynamics are responsible for the amount of force exerted on the implant system. Several criteria are included under this heading: patient size, gender, age, and skeletal position. The size of the patient can influence the amount of bite force. Large, athletic men can generate greater forces; patients of weak physical condition often develop less force than athletic patients. In general, the forces recorded in women are 20 lb less than those in men. The skeletal arch position may influence the amount of maximum bite force. The brachiocephalic, with a stout head shape, may generate three times the bite force compared with a regular head shape. This is especially noteworthy when accompanied by moderate to severe bruxism or clenching. The maximum bite force decreases as muscle atrophy progresses throughout years of edentulism.

As a general rule, the implant treatment plan should reduce other force magnifiers when masticatory musculature dynamics increase.

Arch Position

The Mandible is a Class III lever, the condyles being the fulcrum and the masseter and the temporalis muscles supplying the force to the teeth and implants. The maximum biting force is greater in the molar region and decreases as measurements progress anteriorly. When the posterior teeth are not in contact, two thirds of the temporalis and masseter muscles do not contract their fibers. As a consequence, the bite force is reduced. Hence, the treatment plan should incorporate measures to account for the increased masticatory stress depending upon the arch location of the tooth being replaced.

Opposing Arch

Natural teeth transmit greater impact forces through occlusal contacts than soft tissue–borne complete dentures. The maximum force generated in an implant prosthesis is related to the amount of tooth or implant supporting the opposing arch. A complete implant fixed prosthesis does not benefit from proprioception as do natural teeth, and patients
bite with a force four times greater than with natural teeth. Thus, the highest forces are created with implant prostheses. In addition, premature contacts in occlusal patterns or during parafunction on the implant prostheses do not alter the pathway of closure, as occlusal awareness is decreased with implant prostheses when compared with natural teeth. Therefore continued stress increases can be expected to occur with the implant restoration.

Partial denture patients may record forces intermediate between that of natural teeth and complete dentures. In the partially edentulous patient with implant-supported fixed prostheses, force ranges are more similar to those of natural dentition, but lack of proprioception may magnify the load amount during parafunctional activity.2

**Conclusion**

The most common complications in implant-related reconstruction are related to biomechanical conditions. Implant healing failures may result from micromovement of the implant from too much stress. To ensure effective healing and long-term maintenance of the implants in the oral cavity, the practitioner should base his prosthesis design on a sound evaluation of the patient’s force factors coupled with an understanding of the biomechanics of the bone-implant interface.

**Conflict of interest:** Nil

**Source of Funding:** Nil

**References:**

An Autopsy Based Study on Fatal Road Traffic Accidents in the Mortuary of Patna Medical College, Patna (Bihar)

Ramanand Choudhary1, Anil Shandil2, Sanjeev Kumar3, Shabbir Ahmad Choudhary4

1Associate Professor, Department of FMT, Patna Medical College, Patna (Bihar), 2Associate Professor, Dept. of FMT, Patna Medical College, Patna, 3PG 2nd year student, Dept. of FMT, Patna, 4PG 3rd year student, Dept. of FMT, Patna Medical College, Patna

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Abstract

Road Traffic Accidents (RTA) continue to be one of the leading causes of death in developing countries and constitute a medico-legal autopsy case conducted in India.

Objective: To study the demographic profile and pattern of injuries among victims of RTA.

Materials and Methodology: Autopsy based study was carried out in the department of Forensic Medicine & Toxicology, in the Mortuary of Patna Medical College, Patna from September 2018 to September 2021. The data source included post-mortem reports, police inquest reports. History noted in the sheet as given by relatives and friends of the deceased, crime scene, photographs, hospital, case records and laboratory investigation reports. The data collected was analyzed and descriptive statistics such as percentages, ratios and proportion were used.

Observation and Results: A total of 8120 autopsies were conducted during the study period and among them 2030 (25%) cases were of RTA of these 6334 (78%) were males and 1786 (21.99%) female. Majority of the victims 23.49% were in the age group of 21-30 years. Head injury 76.99% was the most common cause of death and subdural haemorrhage (80.68%) was the commonest among the intracranial haemorrhages.

Conclusion: Road traffic accidents are preventable cause of mortality and there is urgent need to enhance road safety by multi sector approach along with improving trauma care management in hospitals.

Keywords: road traffic accident, autopsy, injuries

Introduction

About 1.25 million people die every year as a result of RTA across the globe and it is expected to become the seventh leading cause of death by the end of 2030, if proper intervention is not made India accounts for the highest number of road traffic accidents in the world, with over 130,000 people dying every year.[1]
The main contributing factors as revealed by World Health Organization restraints and inefficient law enforcement. Trucks and two wheelers constitute for the majority of the cases. The most common occurrence is during peak afternoon and evening.\cite{2} The present study was undertaken with an objective to study the demographic profile and pattern of injuries among victims of fatal RTA.

**Material and Methodology**

The analytical study was carried out in the department of Forensic Medicine & Toxicology, in the Mortuary of Patna Medical College, Patna (Bihar). The data collected for study from Sept. 2018 to Sept. 2021. The data source included post-mortem reports, police inquest reports, history noted in sheet as given by relative and friends of the deceased, crime scene photographs, hospital case records and laboratory investigation reports. A structured performa was designed to enter the data which was pretested and validated by subject experts. The performa consisted three part, part X, Y and Z, part X included data of post-mortem examination, age of the deceased, sex of the deceased, marital status. Part Y consisted of injuries sustained and part Z cause of death. The data collected was analyzed and statistical methods used are descriptive statistics which includes percentages, ratio and proportion.

**Observation**

<table>
<thead>
<tr>
<th>Age in years</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upto 10</td>
<td>36</td>
<td>0</td>
<td>36</td>
</tr>
<tr>
<td>11-20</td>
<td>350</td>
<td>60</td>
<td>410</td>
</tr>
<tr>
<td>21-30</td>
<td>477</td>
<td>75</td>
<td>552</td>
</tr>
<tr>
<td>31-40</td>
<td>300</td>
<td>102</td>
<td>402</td>
</tr>
<tr>
<td>41-50</td>
<td>200</td>
<td>65</td>
<td>265</td>
</tr>
<tr>
<td>51-60</td>
<td>150</td>
<td>50</td>
<td>205</td>
</tr>
<tr>
<td>61-70</td>
<td>90</td>
<td>65</td>
<td>156</td>
</tr>
<tr>
<td>&gt;70</td>
<td>07</td>
<td>03</td>
<td>10</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1610</td>
<td>420</td>
<td>2030</td>
</tr>
</tbody>
</table>

**Table 2 : Cause of death in RTA Victims**

<table>
<thead>
<tr>
<th>Cause of death</th>
<th>No. (%) (n = total 2030)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head injury</td>
<td>1561 (76.89%)</td>
</tr>
<tr>
<td>Multiple injuries</td>
<td>242 (11.92%)</td>
</tr>
<tr>
<td>Chest injury</td>
<td>61 (3.00%)</td>
</tr>
<tr>
<td>Abdominal and genitor urinary injury</td>
<td>15 (0.73%)</td>
</tr>
<tr>
<td>Spinal injury</td>
<td>30 (1.48%)</td>
</tr>
<tr>
<td>Fracture of Long bones and complication</td>
<td>101 (4.97%)</td>
</tr>
<tr>
<td>Neck injury</td>
<td>20 (0.99%)</td>
</tr>
</tbody>
</table>

**Table 3: Intra Cranial haemorrhage in RTA Victims**

<table>
<thead>
<tr>
<th>Cranial haemorrhage</th>
<th>No. (%) (n = total 2030)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subdural haemorrhage</td>
<td>1638 (80.68%)</td>
</tr>
<tr>
<td>Sub arachnoids haemorrhage</td>
<td>71 (3.49%)</td>
</tr>
<tr>
<td>Extra dural haemorrhage</td>
<td>206 (10.14%)</td>
</tr>
<tr>
<td>Intra cerebral and intra ventricular haemorrhage</td>
<td>115 (5.66%)</td>
</tr>
</tbody>
</table>

**Table 4: Skull Fractures in RTA Victims**

<table>
<thead>
<tr>
<th>Skull Fracture</th>
<th>No. (%) (n = total 2030)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fissured</td>
<td>605 (29.80%)</td>
</tr>
<tr>
<td>Comminuted</td>
<td>302 (14.87%)</td>
</tr>
<tr>
<td>Depressed</td>
<td>167 (8.22%)</td>
</tr>
<tr>
<td>Sutural</td>
<td>106 (5.22%)</td>
</tr>
<tr>
<td>Intact (without fracture)</td>
<td>850 (41.87%)</td>
</tr>
</tbody>
</table>

**Observation and Results**

A total of 8120 autopsies were conducted during the study period and among them 2030 (25%) cases were of RTA of these 78% were males. Majority of the victims were in the age group of 21-30 years followed by fourth decade 19.80%. Head injury 76.89% was the most common cause of death. Followed by multiple injuries 11.92% subdural haemorrhage 80.68% was the commonest type among intracranial haemorrhage followed by extra Dural haemorrhage 10.14%, 5.66% intra cerebral and intra ventricular haemorrhage. Fissured fracture 29.80% outnumbered other types of skill fractures.
Discussion

In the present study, RTA constituted 25% of the total cases of medico legal autopsies thereby emerging as the most common variety for which medico legal autopsies are conducted. This is in contrast with the study conducted by James et. al. in Fiji where only 7% of the autopsies conducted were due to RTA during the study period.[3]

This study shows male predominance over females, similar to studied by Suresh et. al., Yogesh et. al. and Mariam et. al. where males outnumbered females.[4,5,6] Major portion of the victims were in age group between 21-30 years. Which is in accordance with the study done by Yogesh G, Shruti et al, Singh and Mishra.[5,7,8,9] This can be explained by the fact that historically India is a male dominated society where men are exposed to outside as compared to women, who spend most of the time inside the house. Third decade is the most vulnerable age group as they encounter more stress, strain and pressure due to personal and professional commitments and more so is the fact that it is in this age group people tend to start driving two and four wheelers, begin to consume alcoholic beverages, tend to take more risks, prefer to go outings as compared to elderly people who tend to take less risks and stay indoors.

In the present study, head injury was the most common type of regional injury encountered and also the most common cause of death, which is similar to the study done by Mariam, et. al. and Mishra et. al.[6,9] However this study differs from the study made by Yogesh G where multiple injuries was the most common cause of death and extremities were the most common body region affected, head injury is a morbid stage resulting from gross or subtle structural change in scalp, skull and/or contents of the skull due to application of mechanical forces.[5] Brain is the most susceptible visceral organ to trauma.[10] In another study by Khajuria et al, limb injuries were the most common accounting for 31%.[11]

Among the various types of intracranial haemorrhages, subdural haemorrhage was the most common type followed by EDH haemorrhage. This is in contrast with the study by James, et. al. where ED haemorrhage was the most common type, followed by subdural haemorrhage in the present study, there was 10-18% incidence of which is in sharp contrast with the study done Khajuria et. al. where extra Dural haemorrhage constituted for 10.18% of cases.[3,11]

Among the fractures of the skull, fissured fracture was the most common, followed by comminuted type, which is in contrast with observation made by James, et. al. where comminuted variety was the most common.[3]

With respect to seasonal variation, April month recorded the highest number of cases and January recorded the least. This may be attributed to summer vacation for schools and colleges where families plan for outings leading to traffic congestion and mishaps.

Conflict of Interest: NIL
Financial Assistance: None
ETHICAL CLEARANCE NOT Required As this study is based on Hospital Mortuary Records

Conclusion

Road traffic accidents are preventable causes of mortality and morbidity. Hence ensuring road safety which includes avoiding over speeding and drunken driving along with use of helmets and children restraints is the need of the hour. There should be strict law enforcement to check road mishaps.

Reference


The Effect of Puzzle Stimulation on Fine Motor Development of Preschool Aged Children

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Abstract

Background: The growth and development of children involve physical changes and other several aspects, namely fine and gross motor, cognitive, language, emotional and social. Growth and development of children is influenced by internal and external factors. Children who received positive stimulations from the surrounding environment will help them grow and develop optimally. Puzzle play can be an option to stimulate the growth and development of preschool aged children.

Aim: This study is aimed to determine the effect of puzzle stimulation on fine motor development of preschool aged children.

Methodology: The design of this study was quasi-experimental which involved one group pretest and posttest design approach, using observation sheet with DDST (Denver Developmental Screening Test) rating scale instrument. The study was conducted at Leuwiliang Village in Bogor District with a total sample of 23 parents or caregivers of children. The data analysis techniques used were univariate and bivariate with Wilcoxon Rank Test.

Result: Out of 23 participants, 14 participants (60,9%) had suspected delay before stimulation and 19 participants (82.6%) had normal fine motor development after puzzle stimulation.

Conclusion: There was a significant effect of puzzle stimulation in fine motor development of preschool aged children.

Keywords: Fine motor, Puzzle, Preschool aged children, Stimulation

Introduction

Early childhood is defined as period from the child is born up until the age of 6 years old. This period is the age which determines the formation of the character and personality of a child. At this stage, children experience rapid growth and development and it is called as the golden age period. Foods that are nutritious and well-balanced as well as stimulation are needed for growth and development.¹

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E-mail: wijayahusada@gmail.com
Development is the increase of the structure and function of the body to a complex state, in the ability of motion, rough, smooth motion, speech and language socialization, and independence. Early childhood development is divided into four primary areas: social and emotional, language/communication, physical development, and cognitive development.

Stimulation should be given early according to development of each child. According to research by Panzilio, et.al (2020), there are many preschool aged children who have not received sufficient stimulation, while only about 48-72% of family households provide stimulation for their children. Stimulation should be given early according to the development of each child. According to research by Panzilio, et.al (2020), there are many preschool aged children who have not received sufficient stimulation, while only about 48-72% of family households provide stimulation for their children. One group pre-post test equivalent without control design. The results of the univariate analysis with brain gymnastics and playing puzzles intervention showed the 15 respondents before doing brain gymnastics and puzzle, there were nine children.

Fine motor skill is the coordination of small muscles in movement with the eyes, usually involving the synchronization of hands and fingers. Motor skill can be developed through games and daily life activities. During games, the activity of the sensory motor is the largest component used by children and active play is very important for the development of muscle function.

According to Hartshorne (2016), 8.1% of toddlers were found with developmental disorder and 1.92% of preschool aged children were diagnosed with mental retardation. Data from Indonesia Ministry of Health (2016) showed that 16% of children under five years old in Indonesia experienced developmental disorders. Approximately 40% of the total population of Indonesia consists of children and adolescents aged 0-16 years and 13.5% of children under five years old is the age group with high risk of developmental disorders.

Hayuningtyas (2020) found that out of 73 children who participated in the study, 40 children (54.8%) had low cognitive skill and 50 children (68.5%) experienced delayed fine motor skill.

Puzzle is the method of collecting the pieces of small pictures into a complete large image. Image is something that is manifested visually in the form of two-dimensional as the outpouring of feelings and thoughts. Puzzle is one of educational tools that can be used to develop fine motor skills. Puzzle can also be used to stimulate cognitive development, language development and fine motor skill.

### Material and Method

Based on the preliminary study that has been conducted at Leuwiliang Village in Bogor District, there were 23 families with preschool aged children. It has recently been reported that out of 10 preschool aged children, 6 children had developmental disorders and 4 children had impaired fine motor skills.

This type of research used Quasi experimental, with one group pre-test and post-test design. The population in this study were parents or caregivers of preschool aged children at Leuwiliang Village in Bogor District with a sample of 23 participants.

The study inclusion criteria were parents or caregivers of preschool aged children and willing to participate in the research. The exclusion criteria were participants who are unwilling to be a participant. The sampling technique used was Total Sampling. After the number of samples was identified, then the first participant was given code 1, the next sample number was coded 2, and so on until all the samples were fulfilled by 23 participants.

Researchers submitted a research permit issued by Academy of Midwifery Wijaya Husada to the head of Leuwiliang Village in Bogor District. After obtaining research permit, researchers met participants to ask for permission and explained the instruments to be used for the research. The primary data were collected through sheet checklist and DDST (Denver Development Screening Test) observation, while the secondary data were obtained through local government data to determine the number of preschool aged children.

The DDST observation contained personal identity. The nominal dispatch scales for fine motor development is categorized as follows:

1. Unstable
2. Suspect
3. Normal
The magnitude of the effect is determined by Wilcoxon Rank Test.

Results

This research was conducted in April 11-18, 2021. Majority of participants (16 children) were aged 4-5 years old and 15 (65.2%) participants were female.

Table 1: Normality Test of Fine Motor Development of Preschool Aged Children

<table>
<thead>
<tr>
<th>Group</th>
<th>Shapiro-Wilk Statistic</th>
<th>df</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Test</td>
<td>0.043</td>
<td>23</td>
<td>0.049</td>
</tr>
<tr>
<td>Post-Test</td>
<td>0.037</td>
<td>23</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Table 1 indicated that the data were normally distributed, because the p-value in the pre-test and post-test groups fine motor development was significant (>0.05).

Table 2: Homogeneity Test Fine Motor Development of Preschool Aged Children

<table>
<thead>
<tr>
<th>Levene Statistic</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Based on Mean</td>
<td>1.156</td>
</tr>
<tr>
<td>Based on Median</td>
<td>1.125</td>
</tr>
<tr>
<td>Based on Median and with adjusted df</td>
<td>1.235</td>
</tr>
<tr>
<td>Based on trimmed mean</td>
<td>1.271</td>
</tr>
</tbody>
</table>

Table 2 showed that the data is homogeneous (0.357>0.05).

Table 3: Frequency Distribution of Fine Motor Development of Preschool Aged Children Before Puzzle Stimulation

<table>
<thead>
<tr>
<th>Development</th>
<th>Total</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncertain</td>
<td>4</td>
<td>17.4</td>
</tr>
<tr>
<td>Suspect</td>
<td>14</td>
<td>60.9</td>
</tr>
<tr>
<td>Normal</td>
<td>5</td>
<td>21.7</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3 showed that most of the participants had suspect development delay with a total of 14 (60.9%) participants.

Table 4: Frequency Distribution of Fine Motor Development of Preschool Aged Children After Puzzle Stimulation

<table>
<thead>
<tr>
<th>Development</th>
<th>Total</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncertain</td>
<td>1</td>
<td>4.3</td>
</tr>
<tr>
<td>Suspect</td>
<td>3</td>
<td>13.1</td>
</tr>
<tr>
<td>Normal</td>
<td>19</td>
<td>82.6</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 4 showed that after puzzle stimulation, most of the participants experienced normal fine motor development with a total of 19 (82.6%) children.

Table 5: Effect of puzzle stimulation in Fine Motor Development of Preschool Aged Children

<table>
<thead>
<tr>
<th>Paired Differences</th>
<th>Mean</th>
<th>T</th>
<th>Df</th>
<th>Correlation</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-test and Post-test</td>
<td>5.7233</td>
<td>12.082</td>
<td>23</td>
<td>0.152</td>
<td>0.000</td>
</tr>
</tbody>
</table>

The table 5 above showed that the p-value = 0.000, which means p-value < 0.05 and Ha is accepted.

Discussion

A. Fine Motor Development of Preschool Aged Children Before Puzzle Stimulation

Based on the frequency distribution of fine motor development of preschool aged children before puzzle stimulation, the majority of participants had experienced suspect development delay with a total of 14 (60.9%) children.

This study is in line with research conducted by Darmayasa (2020) which stated that around 76% of preschool aged children experienced problems with fine motor development.14

Delayed motor development means that motor development stage is below the normal age of the child. It can be resulted from brain damage at birth or other unfavorable conditions before birth and unpleasant environment after birth. Developmental delays are more often caused by lack of time to learn motor skills, due to overprotection of caregivers or lack of motivation to learn.15
Impaired fine motor development without receiving early and adequate treatment, most likely will end up with a disability. Frequent health check-up to neonatal specialist is also very important to detect developmental disorders in children.16

B. Fine Motor Development of Preschool Aged Children After Puzzle Stimulation

Based on the frequency distribution of fine motor development of preschool aged children after puzzle stimulation, the majority of participants had experienced normal development with a total of 19 (82.6%) children.

Puzzle game relies on instinct or intelligence. The game is made by dismantling and re-installing the suitability of shape, pattern or color. With this game children can practice finding, rearranging and making something seemingly unrelated to being a form of unity that is meaningful.11

C. Effect of Puzzle Stimulation in Fine Motor Development of Preschool Aged Children

Table 5 showed that $p$-value = 0.000, which indicated that there was significant effect of puzzle stimulation in fine motor development of preschool aged children at Leuwiliang Village in Bogor District.

This study is in line with a research conducted by Erni Yuniati (2018) which stated that there was significant difference in the average fine motor skill before and after the puzzle game. Puzzle comes in various materials, pictures, shapes and size. Puzzle play also has different difficulty level and can be used by all children according to each child’s ability. Some wooden puzzles have small knobs that involve children to move their fingers by pinching it. Fine motor skills require small and precise movements and also essential to children’s ability to handle smaller objects and to write for future school preparation. Puzzle is a form of educational games that is attractive and should be introduced to children.12

The purpose and function of motor development is the mastery of skills that are reflected in the ability to complete the task of specific motor. The child will develop optimally when the success rate in performing motor tasks is high.

Conclusion

There was a significant effect of puzzle stimulation in fine motor development of preschool aged children at Leuwiliang Village in Bogor District, Indonesia.

Ethical Clearance: Ethical clearance was not required hence was not obtained

Source of Funding: Self-funded

Conflict of Interest: There was no conflict of interest in the research

References


Genetic Analysis of Genealogy from Dental Y-STR DNA with Raman Spectra Method: Literature Review

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Abstract

The role of Forensic odontologist in genealogical analysis is important identification. This is useful in knowing whether someone has the same or different family lineage. Genealogical analysis can use Y-STR DNA from teeth with Amelogenin. This gene is present in hydroxyapatite crystals on the surface of enamel, dentin and tooth roots. Recent methods obtained from several literatures show that this examination can be performed using a Raman spectra technique which provides a laser wave stream during PCR analysis. The results of the conclusions from several literatures obtained good accuracy.

Key words: Forensic Odontologist, Genetic Genealogy, Y-STR, DNA, Y Chromosome, Dental Samples

Introduction

Forensic odontologist is a new and growing field of forensic medicine. Coupled with the application of technological advances to the detection and investigation of criminal activity, it facilitates the administration of the judiciary, which requires the coordinated efforts of interdisciplinary teams 1. Personal identification using teeth is something that has been done for a long time 2. It is hardest organ of human body and composed of pulp tissue contained in the enamel of the crown tooth and the hard layer of calcified dentin covered with root cement. These properties of the pulp provide high mechanical resistance to environmental invaders and surrounding microorganisms. The pulp is a non-calcified oral tissue composed of soft connective tissue, blood vessels, lymph, and nerve elements that occupy the pulp cavity of each tooth 3.

In connection with criminal cases, there is usually no evidence that can be used as a source of DNA testing in large quantities, but in limited numbers. So that the analysis of DNA examination samples really requires careful handling, considering that it will determine the success of subsequent DNA examinations. Bones and teeth are the strongest tissues of all human organs, which can be used as a
source of forensic identification using DNA analysis. Tooth debris (such as tooth samples) is generally affected by these variables and can be the only source of DNA if viable core DNA is not available for soft tissue degradation. The odontologist may be asked to provide a sample for DNA analysis in most cases it can be a source of saliva, mucosal swabs, and teeth. The implementation of an optimized workflow that combines extraction, quantification, and massively parallel sequencing (MPS) protocols was evaluated as access performance on forensic dental samples.

At the boundary between molecular genetics and genealogy is the genetic genealogy survey, also known as molecular genealogy. The high level of public interest in genealogy creating complex research disciplines may be due to the so-called identity crisis of globalization. Loss of identity, one of the main problems in the age of globalization, encourages people to find their place across generations. Information about your ancestors can help you find your lost ID. Molecular genealogy opens up the possibility of such identification with the help of DNA. This applied science can be thought of as a field of genealogy, known as social genetics, or historical genetics.

The genetic characteristics of the human Y chromosome provide markers of parent-child relationships in the form of a single haplotype that is passed directly from father to son. Haplotypes are a collection of short tandem repeat (STR) alleles typed on a single Y chromosome. For a long time, Y-chromosome analysis has been relatively neglected in forensic casework because it does not provide the security of identification that autosomal DNA can provide. The unilaterality of this marker can be useful in certain situations (sexual violence, missing persons, victim identification, complex kinship analysis, population reasoning). In the current study, the Raman spectral method is a molecule that can be used to identify gender identity by modeling PC scores, especially when modeling different tooth types (molars and premolars) separately. It is a test method. Meanwhile, Kumari etc. (2017) Raman microspectroscopy is an alternative to age determination by using tooth samples of dentin and cement sections. Based on a review of existing literature, the author is interested in reviewing several journals related to genetic genealogy analysis of tooth samples using YSTR-DNA and Raman spectral methods.

**Raman Spectroscopy**

Fourier Transform Infrared (FTIR) and Raman spectroscopy are vibration spectroscopy and serve as an ideal platform for identifying skeletal debris. Vibration spectroscopy can quickly and inexpensively extract large amounts of spectral information by capturing the vibrational motion of functional groups with simple sample pretreatment or without sample pretreatment.

Raman spectroscopy itself is based on Raman scattering or the Raman effect. That is, inelastic dissipation (about 1/106 to 1/108 photons) from visible monochromatic (400/700 nm) or laser (700/850 nm) or UV light (less than 270 nm). Due to the discrete changes in bright light above and below the wavelength of incident photons due to the vibrational frequencies (1/119) of the biomolecules that make up the tissue.

Raman spectroscopy has useful properties for forensic applications that identify body fluids (BFs) that non-invasively characterize a sample using light irradiation and highly selective spectral patterns based on the molecules that make up the sample. Detailed peak assignments show that the BF spectrum has a characteristic spectral pattern that can be interpreted with knowledge of the physiological components. The use of Raman spectroscopy for biomedical applications, including dentistry, has increased significantly.

Raman spectroscopy and FTIR spectroscopy are sensitive to skeletal changes and provide viable techniques for estimating the end of postmortem changes. This makes this approach a diagnostic triage tool and could be a viable option for DNA analysis.

**Y-STR DNA**

Most human nuclear chromosomes are inherited from both parents; only the Y chromosome does not. The unique role of this chromosome as a genetically dominant sex-determining factor makes it a constitutive and male-specific haploid. The human Y chromosome has two pseudoautosomal
regions (PARs) with a short Yp arm and a long Yq arm, separated by a centromere (Figure 1). PARs, the short arm and the proximal portion of the long arm consist of light chromatin enriched with the PAR1 and PAR2 genes, both of which recombine with areas on the X chromosome; the remaining region does not recombine and contains about 70 genes in the male-only region (MSY).

Figure 1: A structural diagram of the human Y chromosome identifying the relative location of the PowerPlex Y23 short-tandem repeat (STR) locus used routinely in forensic DNA casework. Estimated areas identified in diagrams are sometimes subject to variable size omissions.

Genetics Genealogy

Genealogical studies were conducted in the case of President Thomas Jefferson (1743-1826), a slave plantation in Virginia. This is an early application of the catalyst for the use of Y chromosome analysis in family history. The segregation of the Y-chromosome haplotype between the proven male ancestor of Jefferson’s paternal uncle and his son’s ancestor is suspicious.

Figure 2: DNA Y analysis used the Raman spectro method

More generally, the relationships between the haplotype relationships of the Y chromosome names have practical implications. Predicting surnames based on the Y-chromosome haplotype is useful in unexpected criminal cases and has proven feasible in principle, but in practice it does have a related Y-chromosome haplotype. You need a very large database of family names you have. Participant surnames appear to be predictable from published whole-genome sequence data when combined with published non-genetic data, thus raising privacy concerns regarding the anonymity of enrollment in medical genetics research.

MSY analysis is the gold standard for forensic researchers, and the most common approach is to use YSTR to determine if the required samples match. Learning family history is a very popular hobby, and DNA analysis has been enthusiastically accepted by the so-called pedigree genetics community. The genetic characteristics of the human Y chromosome provide a parent-child relationship marker in the form of a single haplotype that is passed directly
from the father to the son. Haplotypes are a collection of short tandem repeat (STR) alleles typed on a single Y chromosome. Y chromosome analysis has been relatively neglected for some time in forensic casework because it does not provide the close certainty of identification that autosomal DNA can offer. The uniparental nature of this marker can be useful in certain circumstances: sexual assault; missing person; identification of disaster victims; complex kinship analysis, and population inference.

Dental Amelogenin

Amelogenin accounts for about 90% of the total enamel matrix protein and plays an important role in enamel calcification and morphological changes. This gene is found on the X chromosome of Xp22.1p22.3 and the Y chromosome of Yp11.2, with 90% of the transcript being expressed on the X chromosome and the rest on the Y chromosome. Homologous recombination has become the most popular genetic marker for sex determination in modern forensics.

Amelogenin Locus

This region encodes for extracellular matrix proteins involved in tooth enamel formation. Mutations in the AMEL gene can cause an enamel defect known as amelogenesis imperfecta. Amelogenesis imperfecta is a disorder that causes abnormal tooth enamel formation in both primary and permanent teeth. The formed tooth enamel is soft and thin; so it is easily damaged.

Table 1: Sex Type Markers and Gene Homologs on Human X and Y Chromos

<table>
<thead>
<tr>
<th>Gebe Symbol</th>
<th>X Chromosomal Location</th>
<th>Distance from Xpter (Mb)</th>
<th>Gene Symbol</th>
<th>Y Chromosomal Location</th>
<th>Distance from Ypter (Mb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMELX</td>
<td>Xp22.2</td>
<td>11.3</td>
<td>AMELY</td>
<td>Yp11.2</td>
<td>6.7</td>
</tr>
<tr>
<td>DXYS156</td>
<td>Xq21.3</td>
<td>88.9</td>
<td>DXYS156</td>
<td>Yp11.2</td>
<td>3.2</td>
</tr>
<tr>
<td>SOX3</td>
<td>Xq27.1</td>
<td>139.6</td>
<td>SRY</td>
<td>Yp11.31</td>
<td>2.7</td>
</tr>
<tr>
<td>STS</td>
<td>Xp22.31</td>
<td>7.1-7.3</td>
<td>STSP1</td>
<td>Yq11.221</td>
<td>17.7</td>
</tr>
<tr>
<td>TSPYL2</td>
<td>Xp11.2</td>
<td>53.1</td>
<td>TSPY1</td>
<td>Yp11.2</td>
<td>9.2-9.3</td>
</tr>
</tbody>
</table>

The AMEL locus has two homologous genes: AMELX (Xp22.2) located on the human X chromosome and AMELY (Yp11.2) located on the human Y chromosome. Although the genes are homologous pairs, they differ in size and sequence.

The most commonly used gender typing method at the AMEL locus was the detection of a 6 bp deletion in AMELX intron 3. This deletion does not exist in AMELY. Sex can be determined using primers that specifically amplify the AMEL locus region. The primer set was developed to amplify both alleles in one PCR. The two most commonly used sets of amelogenin primers yielded 106 and 112 bp or 212 and 218 bp amplicons for the AMELX and AMELY loci, respectively. Amplicons generated from AMELX and AMELY were separated by electrophoresis. Observation of AMELX fragments only showed female, while observations of AMELX and AMELY fragments indicated male.

PCR

PCR was used to amplify, the advent of polymerase chain reaction (PCR) has drastically changed the science of biology since it was first discovered. To amplify a small number of sufficient short target DNA sequences by utilizing sequence-specific oligonucleotide primers and thermostable Taq DNA polymerase.

The DNA amplification techniques used were PCR, RTPCR, and nested PCR. Polymerase chain reaction (PCR) allows individual molecules of target DNA to be amplified to analytical amounts. A limited number of samples in the case of forensic medicine can be solved by changing the amplification. The number of PCR cycles was increased by 10 to properly amplify the degraded DNA. This will result in 100% recovery of genomic DNA. RTPCR and nested PCR show high sensitivity, automation potential, and high throughput. RTPCR technology,
the X or Y chromosomes are identified by counting the number of chromosomes to determine sex. The X chromosome was counted using the comparative method 28 and the Y chromosome was counted using SRY 22.

Nested PCR studies have found that the sexual sensitivity of the amelogenin gene is 9.5-fold, based on the amelogenin ratio before and after nested PCR. Therefore, the degraded DNA can be analyzed by this method. The drawbacks of RTPCR, its susceptibility to false-positive results compared to PCR, and random dropouts when the number of copies is very small, can initiate real-time PCR and fail sex determination23.

Amplification PCR Analysis of Amelogenin and Agerose Gel

Investigation of the amelogenin allele was performed using a different primer pair (for PCR amplification followed by agarose gel electrophoresis) than that provided by the AmpFISTR® IdentifierTM kit. The primer pair used amplifies different introns of one amelogenin gene. (X: p22.122.3 and Y: p11.2) Based on BLAST (Basic Local Alighmen Search Tool), a dot matrix showing similar regions of the AMELY gene and MALEX gene is displayed. A sequence of primers using capillary electrophoresis and primers using PCR amplification and agarose gel electrophoresis 29.

For PCR amplification with amelogenin primers, a disk with an FTA card section of approximately 1.2 mm (> 5 ng DNA) (after cleaning with whatman FTA cleaning reagent according to the manufacturer’s protocol) or 1 ng DNA was used. Already used. The PCR reaction was performed in a volume of 25 ml. Each PCR reaction mix includes 1 ml serum bovine albumin (BSA) (2mg/ml), 12.5 ml TaqDNA Polymerase Master MIX 2x (Qiagen), and 1 ml each amelogenin primer (forward and recognition 10 M) (Sigma). Make a final volume of 25 ml with 3 ml of template DNA (1 ng) or 2 FTA disks (> 5 ng DNA) and nuclease-free water 29.

The PCR reaction conditions were the same as those used to amplify the amelogenin gene using the AmpFISTR Identifier kit, and that amplicons were produced using modified primer pairs. PCR was performed on a thermosizer at an initial temperature of 95°C for 11 minutes, followed by denaturation at 95°C for 1 minute, annealing at 59°C for 1 minute, and elongation at 60°C for 1 minute for 27 cycles. Then hold at 60°C for 80 minutes at 4°C. PCR amplification bank (DNA replaced with an equal volume of extraction reagent blank control) was used as the negative control. The PCR product was analyzed at 120 V for 1 hour on a 1.5% agarose gel electrophoresis, then stained with ethidium bromide (Sigma) for 30 minutes and visualized under UV light29.

Discussion

The human Y chromosome plays an important role in understanding human evolution and genetics. NRY is one of the most obvious regions of the human genome and is a powerful tool for studying paternal inheritance. NRY single nucleotide polymorphisms (SNPs) and short tandem repeats (STRs) have been used as important markers to track direct ancestors from paternal ancestors and reflect male historical behavioral traits 24. In these cases, samples such as bone, teeth, skin, and muscle tissue usually provide sufficient DNA for analysis. In general, a 1 cm² section of such a biological material is suitable for testing 25.

Studies of Priekule et. al. (2021), Studying the Degree of Mineralization of Dental Enamel through
Raman Spectroscopy in Various Spectral Ranges, the method of vibration spectroscopy using IR Spectroscopy and Raman spectroscopy, the main method for studying various structures and properties of crystallized mineral compounds, such as tooth enamel and bone.

Gamulin et. al. (2021), conducted a study on gender analysis of dental samples using Raman spectroscopy on samples of premolars and molars. The results showed that the tooth root and neck anatomy could be used in sex analysis using a DNA molecular approach.

Meanwhile, Miyomari et. al. (2021), used Raman spectra in age analysis by imaging samples from the skin. Shows a less significant result, but is the latest result in a study using protein biomarkers in the skin.

Two of three studies conducted by Prikule (2021) and Gamulin (2021) mainly used examination of the amelogens of the tooth surface. In forensic DNA typing the detection of markers associated with amelogenin (AMEL). The locus has two homologous genes on the X and Y chromosomes: AMELY codes for a protein involved in demineralization of tooth enamel and has a paralogue on the X chromosome (AMELX). AMELX’s first intron is six base pairs (bp) shorter and primers targeting both genes are included in many forensic DNA kits to reveal donor gender.

Sometimes the deletion will cover a wider area resulting in an incomplete Y chromosome STR locus in the profile routinely used in forensic analysis of the Y chromosome. This has led to other markers, such as DYS391, being included in standard autosomal DNA analysis kits because this marker resides in long arm of the Y chromosome and is not involved in the deletion event. Therefore, the examination of Y chromosomes and geneology (side) can be done using dental samples and also the spectrometric Raman method.

PCR examination was carried out with the addition of a laser as a tool for detecting spectra on the results of PCR analysis and to determine the significant similarity of results between gender Y in dental samples with DNA examination using the Raman spectra method in genealogy analysis.

**Conclusion**

Raman Spectra is a method that is quite old and can be used for the analysis of various biological components (blood, teeth, bones and skin) for DNA analysis. The combination of determining the family lineage of tooth samples using the Raman spectra method is still lacking in data in published research results. However, in the literature, the Raman spectra can be used in the detection of Y chromosomes from dental samples which can be used as a sideline identification.

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**References**


Assessing the Psychometric Properties of Hindi version of Experiences in Close Relationships – Revised (ECR-R) scale

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Abstract

Experiences in close relationships – revised (ECR-R) is a widely used questionnaire to measure adult attachment in romantic and marital relationships. Despite its popularity, a Hindi version of ECR-R is missing. Hindi is a dominant language in India; absence of Hindi ECR-R hinders both research and practice in the Indian context. The present study was aimed to fill this gap by providing culturally validated ECR-R. The study (N = 337, community sample, 53.41% females) evaluated the psychometric properties of Hindi ECR-R. The confirmatory factor analysis supports the presence of two-factor model as originally theorized in ECR-R. The support for discriminant validity is provided by negative correlation with inclusion of other in self (IOS) scale. The study contributes methodologically by providing an adequate instrument to measure attachment styles in the Indian context, which could be a valuable resource for practitioners and researchers alike.

Keywords: Attachment theory, Assessment, Culture, Interpersonal relationships, Measures, Scale.

Attachment theory is one of the most popular theories of interpersonal relationships. Although scholars have developed several scales and questionnaires to measure attachment patterns, ‘Experiences in close relationships (revised)’ (ECR-R)1 is often the first choice of researchers. ECR-R contains 36 items that measure insecure attachment styles—anxiety and avoidance. The reliability and validity of ECR-R are evidenced as better than that of other attachment measures. Fraley et. al. found the Cronbach’s alpha value above .90 for anxiety and avoidance.1 Several western and cross-cultural studies have replicated the findings of Fraley et. al.1 The scale has been translated and modified into multiple languages, including French,2 Greek,3 Italian,4 Korean,5 Serbian,6 Romanian,7 Slovak,8 Dutch,9 Spanish,10 and Chinese,11. Most of these studies have confirmed the original two-dimensional structure of attachment consisting of anxiety and avoidance. However, some cross-cultural studies have yielded contrasting results. For instance, Al Tamimi’s12 evaluation of the ECR-R scale in Arabic did not confirm the original findings. Similarly, Jaiswal’s13 attempt to
translate the ECR-R scale in Hindi did not produce any interpretable results. Jaiswal reasoned that the failure to replicate the original model could have been because of social desirability, item wordings, or the plausibility that the two-dimensional model of attachment is applicable mostly in the western context and not entirely for all cultures.

The present work was aimed at re-assessing the psychometric properties of Hindi ECR-R. Support for the validity of Hindi ECR-R would strengthen the cross-cultural support for the two-dimensional model of attachment. Although studies on attachment have grown multifold, a large number of these studies have remained focused on the WEIRD sample. The lack of culturally validated instruments and measurement tools is one of the reasons for the gap in our knowledge about relationship functioning from diverse cultures. Hindi is a dominant language in India, particularly in the northern regions. A culturally validated attachment scale would be highly beneficial to relationship research and practice in the Indian context.

Methods

We started by translating ECR-R items from English to Hindi. Items were modified to measure the attachment of married individuals with their spouses. An example of an anxiety item is “I often worry that my husband/wife will not want to stay with me.” And an example of an avoidance item is “I prefer not to show my husband/wife how I feel deep down.” Three forward and one backward translation were done for each of the questionnaires with the help of volunteers who had university-level education and were fluent in Hindi and English. The items with clear meanings were selected. These translated and modified items were pilot tested on a small sample (N = 30) to ensure that the items were meaningful and comprehensible for the participants. This translated scale was then used to collect data.

We recruited 337 participants (157 males and 180 females) from two states of northern India, i.e., Bihar and Uttar Pradesh, to participate in the study using snowball sampling. Participants’ age ranged from 18 to 63 years (M = 36.20yrs., SD = 7.543). While 152 participants (45.1%) came from rural areas, 185 (54.9%) lived in urban areas. All the participants were literate, and they were all living in arranged marriages. The mean years of being in the relationship were 12.72 yrs. (SD = 7.67). Participants were briefed about the study, and their consent was sought. They had the option to drop out of the study at any point during the data collection process.

The instruction for the ECR-R scale read: “Following statements concern how you feel about your relationship with your husband/wife. Respond to each statement by circling the number that best indicates how much you agree or disagree with those statements. Here ‘1’ refers to ‘strongly disagree’ and ‘5’ refers to ‘strongly agree.’” Twelve items of avoidance dimension and two items of anxiety dimensions are reverse coded. The scores of these items are reversed before calculating the aggregate score that reflects the attachment insecurity of the individual.

We also used ‘Inclusion of other in self scale’ (IOS) to test the discriminant validity of Hindi ECR-R. IOS is a one-item pictorial scale that contains seven options, each consisting of two overlapping circles in different degrees. The degree of overlap indicates the degree to which individuals have incorporated their partners in their self-concept. We reasoned that since IOS measures the level of intimacy in the relationship, it should negatively correlate with insecure attachment styles. Constant et al. have found a statistically significant but negative correlation between anxious and avoidant attachments and intimacy indicators. The instruction provided to participants read, “Each of the seven options provided below has two overlapping circles. One circle represents your personal self, and the other represents the self of your husband/wife. Please circle the option that best represents the degree of inclusion of your partner in your personal self.”

Results

Data analysis was carried out in SPSS (v. 20) and Amos (v. 20). We carried out both exploratory and confirmatory factor analysis (EFA and CFA). Principal component analysis and varimax rotation were used for EFA. We forced the extraction of two factors. The KMO and Bartlett's test of sphericity indicated that factor analysis was meaningful.
(KMO=.80; for Bartlett’s test =10354.55, df=703, sig < 0.01). Most items loaded significantly on their respective components—anxiety and avoidance. However, four anxiety items (items 1, 5, 6, and 7) significantly loaded on avoidance dimension (> 0.3), and three avoidance items (27, 29, and 36) overlapped on anxiety dimensions. Since the model was majorly aligned with the two-dimensional attachment model, we decided to confirm the findings using confirmatory factor analysis.

![Figure 1: Factor loading of items](image)

CFA was carried out in Amos (v. 20). The model fit indices indicated that the original two-factor model with 36 items did not have adequate model fit indices (TLI = 0.65, CFI = 0.67, RMSEA = 0.07). The factor loadings indicated that some items had low loadings on their respective components. We removed these items. A total of eight avoidance items and five anxiety items that had low loading (< 0.40) were removed. We also drew covariance between error terms of avoidance items 12 and 13. This improved the model fit index substantially (TLI = 0.89, CFI = 0.90, RMSEA = 0.05). The final model had ten avoidance items and 13 anxiety items.

The Cronbach’s alpha was .84 for both avoidance dimension and anxiety dimensions. The confirmatory factor analysis itself provides support for the internal consistency and structure of the construct. We calculated the discriminant validity using Henseler’s hetero-trait mono-trait (HTMT) method. The HTMT score is recommended to be lower than .85. The HTMT score for the modified Hindi ECR-R was .34. Thus the discriminant validity was supported. The discriminant validity was also supported by analyzing the correlation between the attachment styles and the IOS scale. We found a statistically significant but negative correlation between avoidant attachment and IOS (-0.44, p < .01) as well as between anxious attachment and IOS (-0.32, p < .01). It is reasoned that individuals with insecure attachment (anxiety and avoidance) tend to behave in ways that adversely affect intimacy in their relationship.

Discussion

The study’s findings support the two-dimensional model for the Hindi ECR-R. The findings also
suggest adequate reliability, internal consistency, and discriminant validity for the measure. Jaiswal\textsuperscript{13} had failed to find an interpretable structure for Hindi ECR-R that led her to question the applicability of ECR-R in a cross-cultural context. She reasoned that items’ wording and social desirability could have negatively influenced her study. Issues with item wordings of ECR-R have also been observed in Kim et. al.\textsuperscript{5}, where the authors found positive and negative worded items emerge as separate factors. Item wordings and social desirability could have influenced the current study as well. It is possible that the wording of items affects the meaning or content it aims to investigate. Despite this, in the present study, the two-dimensional structure was interpretable. The findings indicate that the Hindi ECR-R can be meaningfully used to explore adult attachment in the Indian context.

Hindi ECR-R can be a valuable resource for both practitioners and academicians. The family and couple counselors would benefit from the availability of the Hindi version of these scales. The availability of culturally validated instruments opens new avenues for testing and extending attachment theory propositions in the Indian context.

There are some limitations to the current study. Although the sample size was moderate, a larger sample would have provided a more accurate picture. Using other measures and scales to explore convergent and discriminant validity rather than relying only on HTMT and CFA could have provided a more accurate picture of the construct validity. The future direction would be to modify the ECR-R items that had lower loadings on the anxiety and avoidance dimensions and test the influence of item wording.

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**References**


Prosthetic Considerations for an Aesthetic Rehabilitation in the Lower Anterior Region: A Confluence of Art and Science

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Abstract

Developments in adhesive dentistry have provided the dental profession with new restorative materials and technology to restore esthetics and function to the missing anterior dentition. Interdisciplinary treatment planning, knowledge of available restorative materials, sequencing treatment modalities, and adequate communication between the disciplines involved are a key to a successful treatment outcome when pursuing restorative rehabilitation.

This clinical report describes the challenges faced in the prosthetic rehabilitation of a partially edentulous patient to achieve a successful restoration in terms of biomechanics and function.

Key words: adhesive dentistry, rehabilitation, metal ceramic, aesthetic.

Introduction

Replacement of missing teeth in the anterior region becomes utmost important esthetically. Various treatment options are available for replacement of missing lower anterior like implant, removable partial denture (RPD) and fixed dental prosthesis. RPDs generally tend to cause bone resorption and flattening of the interdental papillae on prolonged use. However, they can be used as interim prosthesis for initial esthetics. Conventional bridges require more amount of tooth preparation all around the abutment tooth causing trauma to the pulp and hypersensitivity. Newer methods have indicated a more conservative and less invasive resin bonded fixed prosthesis, helping to preserve the remaining alveolar ridges and the soft tissue and also the vitality of the abutment teeth.

Resin bonded bridges (RBBs) are a type of fixed dental prostheses that need a minimum amount of tooth preparation. The bonding is achieved by directly cementing over the tooth structure with the help of resin cement when the preparation is restricted to the enamel surface only. These restorations were first described in 1973 when the
natural extracted tooth of the patient was cemented directly to the etched enamel surface with composite resin for a limited time period to provide esthetics. Then Rochette Bridge was designed by Rochette for periodontal splinting of the mandibular anterior teeth. However, due to the bulky framework and limited adhesion to the enamel surface its use was restricted. Later Moon and Hudgins developed ‘Virginia bridge’ at Virginia Commonwealth University in 1984. Lost salt technique was utilized to provide the macroscopic mechanical retention to the framework and the tooth surface. In spite of adequate retention of Virginia bridge, this framework was bulky. The ‘Maryland Bridge’ was developed at the University of Maryland. The bridge retention has been enhanced by the development of resin cements which bond chemically to both the tooth surface and the etched metal alloy. It provides micro mechanical retention. Thompson and Livaditis in 1983 developed the technique of electrolytic etching of Ni-Cr and Co-Cr alloy. However, the Maryland bridges are alloys specific. It is used only for non-precious alloys because precious alloys cannot be etched to provide micromechanical retention.

Case Report

A 28 year old female reported to the Department of Prosthodontics complaining of missing teeth and wanted them to be replaced. The patient was unwilling for any removable prosthesis and wanted a fixed prosthesis.

On examination it was revealed that the lower anteriors 41 42 and 31 were missing. The abutment teeth were deemed unfit for full coverage FDP. The bone in the anterior region was insufficient for implants and the patient was unwilling for extensive surgical procedures. Thus after thorough discussion with the patient Maryland bridge was decided as the treatment for replacement of the missing teeth.

The abutment teeth were prepared following regular protocol on the lingual surfaces only and an elastomeric impression using putty and light body was recorded for fabrication of the prosthesis and sent to laboratory for fabrication of the metal framework with wings. Metal try-in was done and speech and mastication was evaluated. Shade matching using Vita 3D was performed. After the successful metal trial, it was processed for ceramic build up. The final restoration was cemented using Rely-X cement. Occlusion verification was done in centric and eccentric. Post - cementation instructions were given and the patient was recalled for follow-up.
Discussion

Conventional techniques of fixed dental prosthesis require considerable amount of tooth preparation. Expected transition in gingival position and the patient’s age were the main deciding factors along with the patient’s unwillingness for undergoing extensive surgical procedure for correction of deficient bone and implant placement, to rehabilitate using resin bonded prosthesis.

The resin bonded prosthesis, traditionally, are known to have compromised bonding and consequently shorter life span. However, newer self-etch adhesive systems have improved the longevity of the prosthesis. The main threats to the associated with resin-bonded prosthesis are debonding (21%), tooth discoloration (18%) and caries (7%).6,7

The 1 year period uneventful follow-up showed encouraging signs regarding its longevity and after its tenure, a more conventional fixed prosthesis maybe planned.8-10

Conclusion

RBBs have been very effective in rehabilitating lost teeth, particularly in short span ridges, restoring esthetics and oral function without much invasive techniques and consequently achieving can be high patient satisfaction. With newer and improved self-etch adhesive systems, thorough patient assessment and use of proper techniques can achieve longer lasting, more esthetic restorations in cases of short spans.

Conflict of Interest: Nil

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References

Periodontitis and Leukoplakia: Is There an Interconnection?

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Abstract

Background: Leukoplakia is an asymptomatic, potentially malignant lesion in the oral mucosa which often develops into oral cancer. Various follow-up studies done show that between <1 and 18% of oral pre-malignant lesions will develop into oral cancer. Periodontitis is related to different pathological states in the oral cavity including pre-malignant and malignant lesions. Frequently, periodontal sites are involved in proliferative types of leukoplakia. Therefore, Periodontitis increases the risk of oral leukoplakia and, therefore, the risk of mucosal lesions predisposing to oral cancers.

Methods: A 39 year old male patient reported to the Department Of Periodontology with chief complaint of bleeding of gums and whitish patch on his right and left buccal mucosa since 6 months. A Complete clinical examination and detailed case history was recorded for the same. Proper treatment plan was planned and implemented. This cased showed a strong correlation between periodontitis and oral leukoplakia.

Conclusion: Poor oral hygiene and the ensuing of plaque accumulation results in a chronic inflammatory process, creating an environment that promotes the development premalignant lesions and oral carcinoma. Our study supports the hypothesis that periodontitis is an individual risk factor for Oral premalignant lesions. Periodontal disease is a chronic inflammatory condition that may be prevented with regular dental visits and with the maintenance of the Oral hygiene. Dentists play an important role in preventing oral cancer by evaluating the socioeconomic status of each patient and appraising their lifestyle and habits. Therefore this case report mainly focuses on interrelationship between maintenance of good periodontal health and development of oral premalignant lesions.

Keywords: Premalignant lesion, Risk factors, Leukoplakia, Periodontitis.

Introduction

One of the most common oral potentially malignant disorders (PMDs) affecting the oral cavity is oral leukoplakia (OL). In the first international conference on OL (1984) in Malmo, Sweden, OL was defined as “a white patch or plaque that cannot be characterized clinically or pathologically as any other disease and is not associated with any physical or
chemical causative agent except use of tobacco.” In the year 1997, the WHO defined leukoplakia as “a predominantly white lesion of the oral mucosa that cannot be characterized as any other definable lesion. van der Waal in 2007\(^1\) suggested a new definition that includes histological confirmation, but this has not been yet assessed by the WHO. “A predominantly white lesion or plaque of questionable behaviour having excluded, clinically and histopathologically, any other definable white disease or disorder.

Periodontitis is related to different pathological states in the oral cavity including pre-malignant and malignant lesions. This is also true for smoking, the most important risk factor for oral leukoplakia and for periodontitis\(^2\) Frequently, periodontal sites are involved in proliferative type. Various reports suggested that an association may exist between periodontitis and premalignant lesions\(^3\) Data from the NHANES study suggest associations between periodontal disease and the risk of precancerous lesions\(^4\)

The association between periodontal disease and oral neoplasm is biologically plausible and may be explained by:

1. Broken mucosal barrier in the presence of periodontal disease and consequent enhanced penetration of carcinogens such as tobacco and alcohol.
2. Increased cellularity in blood vessels and connective tissue in chronic inflammation. Association between chronic inflammation and cancer is coupled with the development of chronic diffuse epithelial hyperplasia which is regarded as a common precursor to intraepithelial neoplasia.
3. Immunosuppression as a common mechanism leading both to periodontal disease and oral cancer. For example, major concentrations of defensins (which have antibacterial, antiviral, and antitumor activities and are likely to play an important role in killing periodontal pathogens) found in neutrophils and epithelia suggest potential implications for critical immune surveillance within periodontal attachment.
4. Viruses such as human papilloma virus (HPV) and herpes simplex virus 1 (HSV 1) or Candida albicans that are found both in oral cancer and periodontal disease.
5. Bacterial overgrowth in patients with poor oral hygiene may lead to an increased rate of metabolites with possible carcinogenic potential. For example, higher microbial production of carcinogenic acetaldehyde from ethanol has been shown in patients with poor oral hygiene.

Periodontitis is an inflammatory response to oral microbiome and is associated with bone loss and tooth loss.\(^5\) Exposure to inflammatory conditions promotes the infiltration of immune cells to the oral mucosa. Inflammation and immune related mediators have been accepted as the hallmarks of malignant transformation

**Case Report**

A 39 year old male patient reported to the Department Of Periodontology with chief complaint of bleeding of gums and whitish patch on his right and left buccal mucosa since 6 months. A Complete clinical examination and detailed case history was recorded for the same. On eliciting personal history, the patient has a habit of smoking since the last 12 years, 3 times a day. Patient had no significant medical history and was not under any medication.

On clinical examination, no abnormalities were detected extraorally. Inspection of the lesion intraorally revealed a uniform whitish patch on the right and left buccal mucosa at the line of occlusion, measuring approximately 3 cm × 2 cm at its greatest diameter [Figure 1&2].

![Figure 1 & 2: White Patch Seen on Right and Left Buccal Mucosa](image)

Cracked mud appearance was seen with the overlying mucosa whitish in colour. Colour was non scrapable, non-stretchable and non-palpable.
VAS score for burning was 0. The examination of oral mucosa for lesions – oral leukoplakia status was done and covered all areas of the oral cavity including the tongue and lips.

Periodontal status was assessed. The examination was conducted under sterile clinical conditions keeping all safety protocols in mind. Assessment included pocket probing depth, clinical attachment loss (CAL), Plaque index, bleeding on probing (BOP).

All fully erupted teeth were assessed excluding third molars. Attachment loss and probing depth were assessed with a periodontal probe.

Colour of the gingiva was pale pink everywhere and bleeding was present even on slightest provocation. The lesions were non-scrapable and non-tender. It was raised 0.5 mm over the surface. No bleeding from the site was noticed.

Based on the history and clinical examination, a provisional diagnosis of bilateral homogeneous leukoplakia was considered.

Excisional biopsy of the lesions was performed and the specimen was submitted for histopathological examination which revealed Oral Leukoplakia.

A final diagnosis of Oral Leukoplakia was confirmed based on the history, clinical examination, and histopathological report. Elevation of salivary interleukin-6 (IL-6) concentrations in leukoplakia cases with coexisting periodontitis and the additional effect of smoking supports the role of periodontal inflammation.

Figure 3 & 4: Showing Labio-Lingual Recession Seen In Mandibular Anteriors

Figure 5: Orthopantomogram of the Patient

Periodontal Status: Orthopantomogram was advised for the patient which revealed the following—

1. Recession was present w.r.t 31,32,33,34, 41,42,43,44,45 both on labial and lingual aspect.
2. Periodontal pocket were present with vertical or angular bone loss seen with maxillary and mandibular posterior teeth with average pocket probing depth of 5-7mm.
3. Miller’s grade 1 mobility was seen with 31,32,41,42.
4. Average clinical attachment level was 3-4mm.
5. Plaque Index (Silness and Loe) was calculated which came out to be poor.

Treatment Plan

Patient was advised habit cessation and counselling of the patient was done making him aware of his current oral health and its possible consequences if he didn’t stop his habit of tobacco chewing. Velscan examination was done. Vital staining of the lesion was also performed. Cap Aquasol A twice a day for 15 days was prescribed. Tab A to Z OD was given was advised. After treatment of the lesion periodontal treatment was given followed by scaling and root planning. Patient was recalled after 15 days to check for all the clinical parameters which include pocket probing depth, clinical attachment loss, bleeding on probing, mobility. Regenerative procedures will be done after taking patient informed consent followed by treatment of recession and mobility. Patient will be recalled after every 7 days and will be kept on the maintenance phase.
Results and Discussion

In the present case report, we found evidence that gingivitis and periodontitis (as characterized by bleeding on probing, pocket depth, bone loss, clinical attachment loss) is associated with the occurrence of leukoplakia in a dose-dependent manner. This relationship was independent of smoking despite the fact that smoking contributes significantly to the odds of having leukoplakia.

Possible pathogenetic mechanisms for the associations shown are completely unknown and therefore speculative. A possible explanation could be the accumulation of carcinogenic metabolites produced by periodontopathogenic bacteria. From the results it may be concluded that there is a continuously increasing risk of leukoplakia with increasing severity of periodontitis or gingivitis. Increased concentrations of inflammatory markers suggest that tissues irritated by defence processes such as periodontitis are vulnerable to premalignant transformations. Thus, studies reported associations between chronic infections or inflammation and cancer. Such associations were also reported with periodontitis. Elevation of salivary interleukin-6 (IL-6) concentrations in leukoplakia cases with coexisting periodontitis and the additional effect of smoking supports the role of periodontal inflammation and relationship to tobacco use.

Ever since Seymour stated in 2010 that poor oral hygiene may affect the risk of OC, a link between PD and the development of OC has been suspected[6].

Periodontitis is an inflammatory process affecting the supporting structure of the teeth, including the gingiva, periodontal ligaments and the bone [7].

Elevated plasma levels of several proinflammatory cytokines, acute phase proteins and proteinases can be observed in periodontal disease [8]. The development of a malignant lesion can be associated with inflammation itself, peculiarly as it causes oxidative damage on the cell’s DNA [9].

The recent studies of Gopinath et. al. and Geng et. al. on the development of Oral cancer suggests that there is a direct link between periodontal pathogens and the carcinogenesis.[10] In Clinical Attachment level and Pocket probing depth were used to evaluate the existence/lack of periodontitis and its severity (periodontal stage), ensuring a more reliable result. There is a clear positive link between the severity of periodontitis and the incidence of Oral lesions.

Moreover, according to Colotta et. al., periodontal lesions include some inflammatory mediators (for example, IL1-ß and TNF-α) associated with carcinogenesis [11].

Alcohol consumption, ageing, smoking and poor oral hygiene are important risk factors for periodontitis [12,13,14]. It should be noted that the same factors can also be associated with carcinogenesis in the oral cavity.

According to the observation of Hashim et. al., good oral hygiene may reduce the risk of Oral premalignant lesions and oral carcinomas[15]

Conclusion

Poor oral hygiene and the ensuing accumulation of plaque accumulation results in a chronic inflammatory process, creating an environment that promotes the development of Oral premalignant lesions. Our study supports the hypothesis that periodontitis is an individual risk factor for Oral premalignant lesions and oral cancer. The risk of the Oral Squamous Cell Carcinoma increases at more severe stages of periodontitis. Periodontal disease is a chronic inflammatory condition that may be prevented with regular dental visits and with the maintenance of the Oral hygiene. Dentists play an important role in preventing oral cancer by evaluating the socioeconomic status of each patient and appraising their lifestyle and habits. Motivating patients at risk of Oral carcinoma to maintain sufficient oral hygiene is crucial and easily achievable. Preserving the periodontal health and monitoring the individuals with lifestyle risk factors who are periodontally compromised may minimize the risk of Oral Cancer.

In conclusion, our findings give new hints into the complex inter-relationship between systemic and local diseases. Periodontal inflammation may be considered as an additional risk acting synergistically with smoking and/or metabolic.
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References


Pivotal Role of Vitamin D on Periodontium

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Abstract

Vitamin D is a fat-soluble vitamin with pleiotropic effects on the body. Major portion of the vitamin is synthesized in the epidermis under the influence of sunlight. Vitamin D is essential for the maintenance of periodontal health. It plays an important role in maintaining oral health through bone and mineral metabolism and innate immunity. While vitamin D deficiency has been associated with periodontitis, little information exists regarding its effect on wound healing and periodontal surgery outcomes. Vitamin D and its receptor, the vitamin D receptor (VDR), maintains the integrity of the periodontium. Owing to the immunomodulatory, anti-inflammatory, and antibacterial properties of 1,25(OH)2 D / VDR signalling, a sufficient serum level of vitamin D is necessary to maintain proper periodontal health. In cases of established chronic periodontitis, vitamin D supplementation is associated with reduction in the severity of periodontitis. Vitamin D has a ‘perio-protective’ effect and can decrease susceptibility to periodontal diseases. This review addresses the basics of Vitamin D metabolism and underlines the role of Vitamin D on periodontal health, disease, and therapy. The rationale for using vitamin D supplementation to help maintain periodontal health and as an adjunct to standard periodontal treatment has also been discussed.

Key words: Alveolar bone metabolism, Immunomodulatory effect, Vitamin D, Vitamin D receptor polymorphism, Wound Healing

Introduction

Vitamin D is a fat-soluble vitamin. In the last two decades there is major improvement in the understanding of the bioactivities relating to vitamin D. It has a major role in various functions such as promoting growth, remodelling of bone and teeth, controlling the regulation of calcium as well as phosphate metabolism etc. It exerts favourable effects on the oral health, this is mainly done by regulating the anti-microbial peptide production. Vitamin D deficiency can cause anomalies in tooth development, defects in enamel and dentin and also cause increase in dental caries incidence.¹

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Individuals with inadequate levels of vitamin D are at greater risk for osteoporosis as well as infectious and inflammatory diseases. Insufficient vitamin D leads to reduced density of bone mineral. Intake of fatty foods which have inadequate vitamin D levels not only leads to obesity, but also increases the incidence of periodontal diseases in both children as well as adolescents. On the other hand, consumption of foods that are rich in riboflavin, fibre, calcium and vitamin D tends to lower the chances of gingivitis. It has been established that consumption of appropriate amounts of vitamin D which may be of either exogenous or endogenous in origin, leads to the preservation of periodontal health.\(^{(1)}\)

**Synthesis of Vitamin D**

Vitamin D synthesis takes place in the skin. It is initiated through ultraviolet radiation from sunlight. Vitamin D can be obtained exogenously as well, this through foods such as oily salt fish (mackerel, salmon, and tuna), cod liver oil and egg yolk. There are several countries which are in short supply of these natural food sources, hence in order to compensate this, fortification of dairy products with vitamin D is done. There are various over-the-counter dietary supplements available too. Proper vitamin D synthesis is crucial for the proper physiological functioning of various systems of the body.\(^{(2)}\)

The Ultraviolet B radiation comes in contact with the skin and causes the conversion of 7-dehydrocholesterol in to Vitamin D3 (cholecalciferol). The rest is absorbed from the gut either as Vitamin D3 or as Vitamin D2 (ergocalciferol).\(^{(1)}\) Vitamin D2 and D3 are biologically inactive forms. Firstly, hydroxylation takes place in the liver leading to the formation of 25(OH)D. Then this formed 25(OH)D, gets hydroxylated again in the kidney, giving rise to 1, 25(OH)\(_2\)D, which is biologically active. The process of synthesis of Vitamin D has been shown in (Figure 1)

**Figure 1: The process of synthesis of Vitamin D**

400-600 IU is known to be the recommended daily consumption of vitamin D for adults. 800 - 1,000 IU is advised for the prevention of osteoporosis. Almost a billion people across the world are estimated to have Vitamin D deficiency. Vitamin D and periodontal health has a strong relation. This can be explained by its various functions, which includes its role in metabolism of alveolar bone, the modulation of host response and vitamin D receptor polymorphism.\(^{(2)}\)

**Vitamin - D Effect on the Periodontium**

Previous studies indicate that insufficient Vitamin D can causes a greater risk of periodontal diseases. It has also been noted that supplementation of Vitamin D can cause improvement in periodontal health, increase in the density of bone in maxilla and mandible and may also cause reduction in alveolar bone resorption. A study was conducted by Dietrich et. al. (2004) based on the “US National Health and
Nutrition Examination Survey”, in which an inverse association between the concentrations of vitamin D and the amounts of clinical attachment loss was reported. This study included individuals who were greater than fifty years of age. The participants with reduced amounts of vitamin D had more loss in periodontal attachment when in relation to those with high amounts of vitamin D.\(^{(3)}\)

It was also noted that Vitamin D showed an increased protective characteristic in men. The anti-inflammatory property of Vitamin D was investigated by Jönsson et. al. (2013).\(^{(4)}\) The Third US National Health and Nutrition Examination Survey data was used in order to study the synergistic effect of hormone replacement therapy (HRT) and serum Vitamin D on the loss of clinical attachment and tooth in the case of postmenopausal women. It was observed that women who were in HRT and who also had high plasma 25-hydroxy Vitamin D levels, seemed to have reduced loss of clinical attachment. On the other hand, women who had reduced Vitamin D levels showed greater loss of clinical attachment. The additive beneficial effects of HRT and adequate levels of Vitamin D also showed a reverse relation to the number of tooth lost in postmenopausal women.

Millen AE et. al. (2014) conducted a prospective study which was five year long. This study included postmenopausal women. There was no association found between the levels of Vitamin D and periodontal disease was observed. It was reported that adequate levels of Vitamin D did not provide any protective effect against the progression of periodontal disease.

Jimenez M et. al. (2014) conducted a cross-sectional study on 11,202 patients of the “NHANES III (National Health and Nutrition Examination Survey)“\(^{(6)}\) The study included older patients (over fifty years of age). A reverse association was observed between vitamin D level in serum and clinical attachment loss (CAL). This was seen in men as well as in women. Thus, observations from earlier studies suggest that to prevent periodontal disease progression in case of middle age group adults, it is important to provide Vitamin D supplementation. This was suggested in accordance to the key function of vitamin D in bone metabolism. This also took into account that elderly patients are at a greater risk for diseases relating to the bone.\(^{(6)}\) The various effects of Vitamin D on Periodontal health has been shown in (FIGURE 2)

![Figure 2: The various effects of Vitamin D on Periodontal health](image-url)
Schulze-Späte et al. (2015) conducted a longitudinal study and reported that although men suffering from severe periodontitis revealed deficiency in Vitamin D, the measurement of Vitamin D could not be considered as a good predictor for the progression of the disease, when taking chronic periodontitis in to consideration.\(^{(7)}\)

Adegboye A.R et al. (2016) conducted longitudinal study in the U.S. under the name of “Health Professionals Follow-Up Study”.\(^{(8)}\) Patients of the age group 40 – 75 years were included in this study. A reverse relation was present between the 25(OH)D score and the number teeth lost and periodontal disease cases. This association was dose-dependent in nature.\(^{(8)}\)

Laky M et. al. (2017) conducted a longitudinal study on 1904 participants in order to study the association of poor Vitamin D status in relation to periodontitis effected individuals and healthy controls.\(^{(9)}\) Follow up was done for a period of 5 to 6 years. There was a 13% reduction in the mortality of tooth with every simultaneous 10μg/L increase in 25-hydroxy Vitamin D in the serum. Anti-inflammatory effects of Vitamin D is exerted on the synthesis process of prostaglandin as well as cyclooxygenase pathways. It causes Inhibition of matrix metalloproteinases (MMPs), which in turn reduces the tissue destruction which is seen in periodontitis.\(^{(9)}\)

**Vitamin D and Wound Healing**

Bacterial insult causes a host immune response which leads to alveolar bone loss which is characteristic on Periodontitis. Vitamin D plays a crucial role in maintaining bone as well as regulation of immunity. It has been suspected that deficiency in vitamin D could have a negative impact on the periodontium. It has been indicated that to achieve perfect wound healing, there is a need to have appropriate Vitamin D levels, especially in the postsurgical time period.

Bashutski et. al. (2011) conducted a study to assess the outcomes of periodontal surgery and teriparatide administration in vitamin-D-sufficient and -insufficient individuals.\(^{(10)}\) They reported that there was a greater gain in clinical attachment level in addition to a reduction in pocket-depth in case of placebo patients with sufficient vitamin D in comparison to vitamin D deficient patients. This study concluded that vitamin D status influenced postsurgical healing. It was seen that at the time of periodontal surgery, having inadequate levels of vitamin D could lead to a negative effect on the treatment outcomes for up to one year. These findings are in line with other studies in which vitamin D deficiency was related with delayed periodontal healing. Thus it is stated that vitamin D may have a role in bone healing in the oral cavity.\(^{(10)}\)

Garcia et. al. (2011) conducted a study to determine whether the patients in periodontal maintenance programs taking vitamin D and calcium supplementation had a trend for better periodontal health compared to patients not taking supplementation.\(^{(11)}\) It was observed that periodontitis related parameters were higher in case of non-takers compared to takers. So, it was concluded that there is potential that vitamin D may exert a positive impact on periodontal health.\(^{(11)}\)

In a case report by Bashutski et. al. (2012), a patient suffering from periodontitis and an intrabony defect was treated by open flap debridement surgery.\(^{(12)}\) The patient was prescribed with 20 mg of teriparatide systemically and oral vitamin D supplements for a duration of six weeks. This case was followed up after four years, where in the patient showed improved clinical as well as radiographic outcomes. It was deduced that the administration of Teriparatide along with oral vitamin D and open-flap debridement surgery could be used as an appropriate treatment for severe intrabony defects.\(^{(12)}\)

Schulze-Späte et. al. (2016) conducted a study where in the treatment group referred to the patients who underwent maxillary sinus floor augmentation and received 5000 IU of Vitamin D3, which was compared to a placebo group.\(^{(13)}\) There was an increased number osteoclasts, which surrounded the graft material in case of the vitamin D group. This observation reflected the presence elevated metabolic activity in sites which were augmented, in case of the treatment group which received Vitamin D3. Vitamin D deficiency has proved to show a detrimental effect on wound healing. In the early phase of tissue healing, it has been noted that the keratinocyte proliferation...
as well as differentiation may be influenced by vitamin D. It also exerts effect on the mobilization of macrophages. (13)

Dental implants coated with Vitamin D3 have shown improved osseointegration with alveolar bone (Javed F. et. al. 2016). It is also observed that vitamin D3 when injected intraperitoneally could further enhanced orthodontic tooth movement, thus helping in the acceleration orthodontic treatment in patients who are undergoing bisphosphonate therapy. (14) Further studies are needed in order to substantiate the link between surgical / non-surgical periodontal therapy and the intake of vitamin D.

**Vitamin D and Alveolar Bone Metabolism**

Periodontal infections act as reservoirs for different bacterial antigens, cytokines, Gram-negative bacteria, and other pro-inflammatory mediators, these are responsible for the spread of diseases throughout the body. The action of pro-inflammatory cytokines and prostaglandins have led to investigate the association between periodontitis and osteoporosis.(15) Inadequate Vitamin D is known to be a major risk factor leading to the development of osteoporosis. Vitamin D helps in regulating the metabolism of calcium. It is an important part of the immune system as well. The utilization of calcium from the body doesn’t continue increasing, once it crosses the threshold value, hence its regulation by vitamin D is crucial.

The chief role of 1, 25(OH)2D is to maintain the homeostasis of calcium and bone. Reduction in the resorption of alveolar bone is noticed, in the presence of optimal levels of 1,25(OH)2D, this is considered to be the osteoprotective nature of vitamin D. Osteoblasts produce Receptor activator of nuclear factor kappa-B ligand (RANKL) and Osteoprotegerin (OPG). Expression of Receptor activator of nuclear factor kappa-B (RANK) occurs on the osteoclast progenitor cells. The conversion of an osteoclast precursor cell into a functional mature osteoclast, is caused due to the RANKL to RANK binding. (1) OPG has the ability to aggravate the RANK-RANKL interaction. Vitamin D receptor expression is contained in the RANKL gene promoter structure. The interaction of Vitamin D and its receptor, causes an elevated expression of RANKL in the bone marrow-derived stromal cells as well as in the osteoblasts. Vitamin D also tends to reduce the OPG. Thus, causing activation of osteoclastic activity and resorption of bone. (1)

Kitazawa et. al. (2003) reported that though vitamin D initially decreases the OPG expression, on continuous exposure the OPG levels get elevated. (15) It was concluded that Vitamin D had a transient nature of osteoclastic activity. In addition to this, Vitamin D also has effect on the osteoblasts, by stimulating the osteopontin and alkaline phosphatase activity in. On prolonged exposure it tends to promote osteoblastic proliferation and differentiation. (15)

**Immunomodulatory Effect of Vitamin D in Periodontium**

The discovery of VDR (Vitamin D receptor) on the immune system related cells, lead to ascribe the definite immunomodulatory effect of Vitamin D. The transformation of biologically inactive vitamin D to the bioactive form is regulated by 1α-hydroxylase enzyme, which in turn is stimulated by the macrophages as well as dendritic cells, also known as “antigen-presenting cells”. Thus through its antibacterial properties and antigen presenting abilities, Vitamin D has the potential to modulate the innate immune response of the body. (16) In the innate immune response, the toll-like receptors senses the pathogen-associated molecular patterns, then there is an increase in the synthesis of 1,25-(OH) 2D3 in macrophages. This eventually causes the production of antibacterial substances. The antibacterial substances that may be produced are cathelicidin and beta-defensins. (16)

An in vitro study conducted by Gauzzi MC. et. al. (2005) reported that 1,25-(OH) 2D3 lead to the impediment of the proliferation as well as the maturation of dendritic cells. (17) A reduction in major histocompatibility complex molecules was also observed. Vitamin D dampens the ability of antigen presentation and T-cell activation of macrophages and dendritic cells.

In vitro studies conducted by Korf et. al. (2012) showed that silencing of the Vitamin D receptors lead to increased hyperresponsiveness of macrophages on stimulation of LPS. (18) Vitamin D supplementation may cause a modulated response of antibacterial
properties, against the putative periodontal pathogens. Macrophages is categorized into M1 and M2, where in M1 is referred to the pro-inflammatory phenotype and M2, as the anti-inflammatory phenotype. The pro-inflammatory M1 phenotype causes the production of nitric oxide, tumour necrosis factor-alpha (TNF-α), as well as interleukin (IL-1 beta). On the other hand, phenotype M2 causes the production of anti-inflammatory cytokine-IL-10.\(^{(18)}\)

Vitamin D has the ability to cause a switch from M1 to M2 (Zhang et. al. 2015).\(^{(19)}\) Vitamin D influences the T - helper cells by effecting its proliferation, differentiation, along with their function. Vitamin D tends to regulate the immune response by causing selectively stimulating and inhibiting specific T-helper cells. It causes the production of IL-4, IL-5, and IL-10, which leads to the stimulation of the development of Th2 cell.\(^{(19)}\) It also causes the production of interferon-gamma cells, and resulting in the inhibition of Th1cells. Vitamin D provides enhanced antimicrobial as well as anti-inflammatory effects along with its role in T-cell differentiation, makes an aggregate of its pleiotropic immunomodulatory influence.\(^{(19)}\)

**Vitamin - D Receptor Polymorphism and Periodontium Association**

Vitamin D receptor gene polymorphisms is believed to influence the remodelling of alveolar bone. It also regulates the host response in the presence of periodontopathic bacteria. Polymerase chain reaction as well as restriction enzyme digestion is used to study the Vitamin D receptor polymorphisms taking place in the genes.\(^{(20)}\) There are various VDR restriction fragment length polymorphisms also known as RFLPs, that are known to be in association with periodontal disease. Taq I-Bsm I, Apa I-, and Fok I are few of the VDR polymorphisms that have been studied in detail. These are investigated on their ability to increase the susceptibility to periodontal diseases. There are mixed conclusions obtained in this regard and further studies are needed in order to have a stronger comprehension of the functional relevance of VDR RFLPs and its relation with the pathogenesis of periodontal disease.\(^{(21)}\)

**Conclusion**

Vitamin D brings into play a multitude of functions beyond its capability to maintain calcium homeostasis, and thereby by becoming an important factor in skeletal health. There is strong evidence suggesting a relation between osteoporosis and the oral bone mineral density. Osteoporosis or reduced levels of bone mass is also considered to be a risk factor for the development of periodontitis. It has been well established the deficiency in vitamin D could increase the chances of infectious diseases with inflammatory pathologic components, this includes diseases such as arthritis, cardiovascular diseases and periodontitis.\(^{(21)}\)

The characteristic of vitamin D to causes the release of antimicrobial peptides, including beta-defensins and cathelicidin, results in initiation of the innate immune response, which further causes increased strength of the physical barriers and hence the breach of epithelium by various pathogens extremely difficult. Vitamin D also has the ability to modify the adaptive immune response. This is done by the stimulation of specific Th subsets which results in resolution of inflammation. These properties may be stated as examples of the “perio-protective nature” of Vitamin D. Additional studies are essential to interpret the various functions of vitamin D, and the appropriate dosage needed to induce its beneficial effects. One of the he most intriguing function of vitamin D, is its role in wound healing and in what way the specific appropriate levels may modify the results of the periodontal surgery. Further clinical studies are needed to confirm these findings and eventually explain the workings involved in the processes.

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Comparative Efficacy Analysis of Intubation Profile in Airtraq Video Laryngoscope Versus Macintosh Laryngoscope in Simulated Difficult Airway Using Cervical Collar

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Abstract

Aim: Comparative efficacy analysis of laryngoscopes -Airtraq vs macintosh - using Intubation Time and Intubation difficulty score in simulated difficult airway using cervical collar.


Results: With airtraq laryngoscope intubation discoursed outcomes intubation time is irrelevant with difficult airway where it is highly associated in case of macintosh laryngoscope. Intubation time & difficulty score is commenting use of Airtraq laryngoscope.

Conclusions: The enhanced visual interiors expedite in such a way that intubation time in Airtraq is not dependent on IDS like Macintosh but on neuromuscular reflexes of performer, commenting the superiority of Airtraq over Macintosh.

Keywords: airway, intubation, cervical collar, Airtraq, Macnitos, Laryngoscope

Introduction

Successful & faster intubation in first attempt without causing morbidities & mortalities in emergency or planned surgery has been age-old challenge.¹,²,³ Macintosh laryngoscope played pivotal role for decades till the evolution of fiber optic video
graphic laryngoscope. The Airtraq (Prodol Meditec, Vizcaya, Spain) is an intubation device developed to expedite tracheal intubation in patients with normal or difficult airways by enhancing visuals of interior anatomy. Literature commentating advantages of Airtraq over Macintosh laryngoscope in simulated difficult intubation scenarios in the manikin when used, are either inexperienced or novice trying intubate. This study was aimed to assess the effectiveness of Airtraq for tracheal intubation using cervical collar over Macintosh Laryngoscope comparing different intubation profile variables like intubation time, intubation difficulty score & checking their association etc. cervical collar are mostly used in trauma cases where pathological factors affecting outcome already, but in this study applied in normal subjects for simulating difficult airway management where tracheal study environment is not affected in advance by inflammatory mediators or secretion due to trauma.

Materials and Method

A prospective interventional randomized control study was carried at tertiary care centre in Western Indian population after taking permission from Scientific Research Committee and Institutional Ethics Committee for Human Research. Sample size was calculated with the help of GraphPad software, considering mean difference of SDs = 14.4, Type I alpha error as 0.05 and Type II beta error as 0.1, confidence level interval 95% and power of the study 90%, that came to be Sixty (60). We included 60 participants of both genders in the age group of 18-60 years, belonging to American Society of Anesthesiologists (ASA) physical status I and II, Mallampati grade I and II that were scheduled for planned surgeries and require endotracheal intubation in the study with duly signed informed consent form in vernacular language. We excluded participants with any systemic diseases like cardiac disease, hepatic disease, renal dysfunction, neurological disorder, lung disease, psychiatric illnesses, endocrine disorders on clinical assessment. We also excluded participants with anticipated difficult intubation, thyromental distance less than 6 cm, Inter-incisor gap less than 3 cm, sternomental distance less than 12 cm, neck circumference more than 42 cm, pregnant and obese patients, and risk of gastric aspiration. Patients were explained in detail about the purpose, procedure of the study and possible side effects. They were also explained about the devices. Operation theatre preparation: Anesthesia machine, AIRTRAQ Size -3/MACINTOSH Size-4 airway devices, routine and emergency anesthesia drugs and equipment, multipara monitor and cardiac defibrillator were checked and kept ready. Multipara monitor was attached and baseline vital parameters were recorded. Premedication: All the patients were premedicated with following medications at the time of induction. 1. Inj. Glycopyrrolate 0.2mg IV 2. Inj. Dexmedetomidine 1 µg/kg IV 3. Inj. Ondansetron 4 mg IV. 4. Inj.Tramadol 1 mg/kg.

Participants were randomly allocated into two groups by envelope method. 1. Group AVL (n=30): patients were intubated using AIRTRAQ video laryngoscope. 2. Group ML (n=30): patients were intubated using MACINTOSH laryngoscope. Induction: Preoxygenation was done with 100% oxygen for 3 min with Bain’s circuit. Inj. Propofol 2 mg /kg IV was given till the loss of eyelid reflex followed by Inj. Vecuronium bromide 0.1mg/kg IV. Lungs were ventilated using Bain’s circuit for 3 minutes with O2 + N2O (50: 50) and Sevoflurane 2-2.5%. Peculiarity is that cervical collar is used mostly in trauma cases where pathological events interfere with outcome.

Laryngoscopy and intubation were done as per the group randomization. For the group AVL: Tracheal intubation was carried out using Airtraq video laryngoscope. Airtraq was loaded with ETT size 7.5mm for female and 8.5mm for male. For the group ML: Tracheal intubation was carried out using Macintosh Laryngoscope. Tracheal intubation was carried out with ETT size 7.5mm for female and 8.5mm for male. Intubation with the Airtraq SP laryngoscope: The head was placed in the neutral position. The standard technique (A) consists of sliding the tip of the Airtraq SP laryngoscope into the mouth along the tongue. The blade of the laryngoscope is kept in contact with the tongue, until the epiglottis comes into view. If passage of the laryngoscope into the oropharynx gave difficulty, crawling movements were used to move the blade over the tongue towards the epiglottis. Once the tip
of the laryngoscope is positioned in the vallecula, the laryngoscope is lifted straight up to expose the glottis. For rotational insertion (B), the Airtraq SP laryngoscope is inserted into the mouth from 90 to 180 degree to the usual direction and then rotated into the usual position. The tracheal intubation was considered failed if attempt time exceeds 120 seconds leading to switching of the type of laryngoscope i.e. Macintosh to Airtraq or vice versa. If the Spo2 dropped below 92% during intubation attempt, facemask ventilation was carried out and laryngoscope to be switched. Maximum 2 attempts were allowed. Maintenance with gas mixture: O2 + N2O 50:50, Sevoflurane given up to 2 to 2.5 %, Inj. Vecuronium bromide was given intermittent dose at 0.02, mg/kg. Ventilation was carried out with circle absorber system. Parameters observed: (1) Intubation Time: (in seconds) It is the time taken from removal of face mask for intubation to connection of endotracheal tube to anesthesia circuit and sine wave on capnography. *7 (2) Intubation Difficulty Score: 9 N1 Number of intubation attempts >1 (n-1), N2 Number of operators >1 (n-1) N3 Number of alternative intubation techniques used (n), N4 Glottic exposure (Cormack and Lehane grade 1) (n-1), N5 Lifting force required during laryngoscopy (0= normal; 1=increased), N6 Necessity for external laryngeal pressure (0= not applied; 1= applied), N7 Position of the vocal cords at intubation (0= abduction/not visualized; 1= adduction. Intubation Difficulty score and Intubation Quality: IDS = O - Easy, 0 < IDS ≤ 5 - Slight difficult, 5 < IDS - Moderate to Major difficult, IDS = ∞ -Impossible intubation. The Cormack and Lehane grade at laryngoscopy 10: Grade 1 Visualization of the entire laryngeal aperture, Grade 2 Visualization of only posterior commissure of laryngeal aperture, Grade 3 Visualization of only epiglottis, Grade 4 Visualization of just the soft palate.

All procedures adopted guidelines as per world medical declaration of Helsinki for research in human participants. Statistical analysis was performed by descriptive and inferential statistics using the student’s unpaired t-test, Graph Pad Prism (version 5.0; Graph Pad Software Inc., California, USA). P-values less than 0.05 were considered statistically significant.

**Results**

All data were presented as mean±SD (standard deviation). Demographic data analysis and justification of study population was inferred by student’s t test (p<0.01) - Statistically highly significant, (p<0.05) - Statistically significant, (p> 0.05) - Statistically Not Significant (NS).

**Table 1: Age & sex demography of subjects**

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Group-AVL (n=30)</th>
<th>Group-ML (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>21-40</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>41-60</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>15</td>
</tr>
</tbody>
</table>

**Table 2: Age, weight & ASA parameters are statistically similar in both groups**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group-AVL (n=30)</th>
<th>Group-ML (n=30)</th>
<th>‘p’value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>34.80±10.91</td>
<td>33.16±11.23</td>
<td>P &gt;0.05</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>56.12±7.78</td>
<td>55.82±10.09</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>ASA grading I: II</td>
<td>16:14</td>
<td>15:15</td>
<td>P &gt;0.05</td>
</tr>
</tbody>
</table>

(Note: AVL- Airtraq video laryngoscope, ML - Macintosh laryngoscope, M-male, F- female)
Table 3: Airway parameters of study participants in both groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group AVL(n=30)</th>
<th>Group ML(n=30)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouth opening</td>
<td>3.15±0.51</td>
<td>3.20±0.50</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MPG Mallampati grade</td>
<td>1.83±0.79</td>
<td>1.66±0.71</td>
<td></td>
</tr>
<tr>
<td>Inter-incisor gap</td>
<td>4.31±0.99</td>
<td>3.94±0.71</td>
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</tr>
<tr>
<td>Thyromental distance</td>
<td>6.21±0.46</td>
<td>6.02±0.76</td>
<td></td>
</tr>
<tr>
<td>Neck circumference</td>
<td>38.71±3.42</td>
<td>38.37±3.33</td>
<td></td>
</tr>
<tr>
<td>Teeth (present/absent)</td>
<td>27:3</td>
<td>28:2</td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Comparison of Intubation profile- intubation time-IT, intubation difficulty score- IDS) in two groups

<table>
<thead>
<tr>
<th>Profile</th>
<th>Group AVL(n=30)</th>
<th>Group ML(n=30)</th>
<th>'p' value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intubation time</td>
<td>23.43±8.70</td>
<td>37.26±12.81</td>
<td>0.0001</td>
</tr>
<tr>
<td>intubation difficulty score, IDS: 0 Easy</td>
<td>18 (60%)</td>
<td>12 (40%)</td>
<td>0.034</td>
</tr>
<tr>
<td>0&lt;IDS≤5 Slight difficult.</td>
<td>12 (40%)</td>
<td>18 (60%)</td>
<td>0.0420</td>
</tr>
<tr>
<td>5&lt;IDS Moderate to major difficult</td>
<td>0</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>IDS =∞ Impossible</td>
<td>0</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>Association by regression analysis intubation time vs IDS- correlation coefficient determination r &amp; two tailed p value</td>
<td>r=0.2801 p=0.1338</td>
<td>r=0.6500 p=0.0001</td>
<td></td>
</tr>
</tbody>
</table>

Table 5: Intubation Difficulty Score profile of study participants in both groups

<table>
<thead>
<tr>
<th>IDS Criteria</th>
<th>Group AVL(n=30)</th>
<th>Group ML(n=30)</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>N₁ (No of attempts)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st</td>
<td>25 (83%)</td>
<td>17 (56%)</td>
<td>0.049</td>
</tr>
<tr>
<td>2nd</td>
<td>05 (16%)</td>
<td>13 (43%)</td>
<td>0.04</td>
</tr>
<tr>
<td>N₂ (No of operator)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One person</td>
<td>26 (86%)</td>
<td>25 (83%)</td>
<td>0.09</td>
</tr>
<tr>
<td>Two persons</td>
<td>04 (13%)</td>
<td>05 (16%)</td>
<td>0.97</td>
</tr>
<tr>
<td>N₃ - No of alternative technique applied</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>N₄ (Cormack &amp; Lehane grade)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade I</td>
<td>20 (66%)</td>
<td>08 (26%)</td>
<td>0.004</td>
</tr>
<tr>
<td>Grade II</td>
<td>07 (23%)</td>
<td>14 (46%)</td>
<td>0.1</td>
</tr>
<tr>
<td>Grade III</td>
<td>03 (10%)</td>
<td>08 (26%)</td>
<td>0.216</td>
</tr>
<tr>
<td>Grade IV</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>N₅ (Lifting force required)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (normal)</td>
<td>27 (90%)</td>
<td>17 (56%)</td>
<td>0.007</td>
</tr>
<tr>
<td>1(increased)</td>
<td>03 (10%)</td>
<td>13 (43%)</td>
<td>0.009</td>
</tr>
<tr>
<td>N₆ (External laryngeal pressure)</td>
<td>NA</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Applied</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>N₇ (position of vocal cord)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>abducted</td>
<td>26</td>
<td>25</td>
<td>0.83</td>
</tr>
<tr>
<td>adducted</td>
<td>04</td>
<td>05</td>
<td>0.66</td>
</tr>
</tbody>
</table>
Discussion

In present study we observed that, mean intubation time is shorter with using Airtraq laryngoscope compared to Macintosh which is in consonance with the study performed by PadmajaDurga et al where mean intubation time was 33.27±13.25 s for ML laryngoscopy while 28.95±18.53s for AVL group. Marwa A Tolon et al observed similar results although mean values were on higher side in both groups. The Intubation Difficulty Scale (IDS) score as observed in our study (table no.5) showed that intubation is much easier with Airtraq laryngoscope considering when total score is 0 but referring to moderate difficult intubation scenarios both laryngoscopes are equally effective. None of the patient had IDS score more than 5 so a comment on superiority of either laryngoscope in severely difficult intubation can’t be inferred from our study. There is neither the case of failed tracheal intubation in either group. Our results were in agreement with study by Padmaja Durga et al. They concluded that for Airtraq group the first attempt success rate for intubation was higher, less no of alternate technique required, achieved Cormack and Lehane grade I in more no of patients and least lifting force was required. In group AVL, 25 patients trachea was intubated at first attempt and in 5 patient’s second attempt was required. While in group ML in 17 patient’s trachea was intubated at first attempt and in 13 patients second attempt was required. Statistically it was significant (p = 0.045). In current study too we observed similar findings. In Group AVL, 26 patient’s trachea was intubated by one operator and in 4 patients’ trachea was intubated by two operators, while in Group ML, in 25 patients’ trachea was intubated by one operator and in 5 patients’ trachea was intubated by two operator, which was statistically not significant (p = 0.7 in one person p = 0.2 in two person respectively). In our study, we have not used any other alternative technique like the use of boogie, stylet, removal of cervical collar and external laryngeal pressure or BURP applied during intubation. Our all-stated outcomes in regard of no. of attempts, operators & technique are in course with results of Padmaja Durga et al, Maharaja et al, M.A. Tolon et al. where there was no need for any optimizing maneuvers. In group AVL, Cormack and Lehane grade I was seen in 20 patients, grade II in 7 patients and grade III in 3 patients, while it was grade I in 8 patients, grade II in 14 patients and grade III in 8 patients in group ML. So, we observed Cormack & Lehane grade 3 in Group ML become grade 2 in Group AVL and grade II become grade I in Group AVL. There was improved Cormack and Lehane grade from Grade II to Grade I using Airtraq laryngoscope compare to Macintosh which was statistically significant (p = 0.04). These findings were corroborative with Padmaja Durga et al. In group AVL, lifting force during laryngoscopy was not required in 27 patients and only in 3 patients it was required, while in group ML in 17 patients lifting force was not required and in 13 it was required. So increased lifting force was required in ML Group which was statistically significant (p = 0.009). Results were in consonance with Padmaja Durga et al. External laryngeal pressures was not applied because of the presence of cervical collar around the neck in both the Groups. We observed abducted position of vocal cord in 26 patients in Group AVL, 25 patients in Group ML and 6 in Group AVL and 5 patients in Group ML, which was statistically not significant (p = 0.3). Koh et. al. reported higher success rate of intubation with Airtraq in patients with cervical immobilization with collar. Arslan et. al. evaluated the effectiveness of the Airtraq and C-Trach in lean patients with simulated cervical spine injury after application of a rigid cervical collar. Padmaja Durga et. al. 2012 reported less intubation difficulty score using Airtraq Video laryngoscope using rigid cervical collar.

Unique observation in our study (image1) that intubation time in AVL group is not dependent on intubation difficulty score which is different from previous study the reason might be the enhanced visuals by video laryngoscope. But in Macintosh laryngoscope we observed that intubation time is highly associated with intubation difficulty score (image 2) which is in accordance with previous discoveries so i would like to put new hypothesis for future research that enhanced visual smooths the entire process in such a way that intubation time is now dependent on neuromuscular reflexes of performer.
of intubation not on intubation difficulty score.

Conclusions

The Airtraq video laryngoscope reduced the intubation time, had high success rate in first intubation attempts, minimum need for additional maneuvers, and with less intubation difficulty score.

Conflict of interest: Nil

Source of Funding: Self or other source- self

Ethical clearance: Ethical clearance was obtained from institutional human ethical committee SSG Institutional Ethics committee for human research (IECHR) medical college & SSG hospital, Baroda & approval no. ECR/85/INST/GJ/2013

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Statistic Value for Determination of Parents-child Relationship using Single Nucleotide Polymorphisms (SNPs) 44 Loci in Thai Individuals

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Abstract

The current report determined the parent-child relationship based upon 44 SNPs markers by iPLEX® Pro Sample ID Panel using statistic values of paternity index (PI). The sample study had two groups: True parent-child (n= 38 families) and unrelated groups (n=38 families). The results showed that the true parent-child group shared 44 loci (38 families: 100%), and the unrelated groups had loci mismatch between 2 to 13 loci. The four mismatch loci was the highest number of families (10 families: 26.32%). The maximum and minimum of PI values were 1.0120×10⁷ and 83.9371 were among the true parent-child groups. The unrelated group’s maximum and minimum PI values were 5.0065×10⁴ and 7.0750, respectively. The lower limit of the distribution of the true parent-child intersected with the upper limit of the distribution of the unrelated between the values of 83.9371 to 5.0065×10⁴. In this range of values, the distribution of the PI values could not be used to determine the relationship and therefore was recognized as a “gray zone”. However, if eliminated mismatch for calculating relatedness of PI, it made the gray zone not allow for clear identification. This research showed that the difference between true parent-child and unrelated groups using 44 SNPs loci typing could help predict human relationships and be applied in other relationships in the future.

Keywords: SNPs, Parents-child, Paternity index

Introduction

DNA fingerprint in forensic casework is a crucial part of the Criminal Justice Process due to its reliability and accuracy. Currently, the DNA fingerprint determined the familial relationship based on a likelihood ratio (LR) of short tandem repeat (STR) markers. LR is the ratio of the probability of DNA profiles under two alternative hypotheses and is useful for identifying two persons’ relationships, especially a parent/child and sibling relationship.¹⁻³ However, previous studies have looked at another DNA fingerprint, including single nucleotide polymorphisms (SNPs).

SNPs have lower mutation rates and can be genotyped after PCR with shorter amplicons than STRs, which are thought to be potentially helpful markers in forensic DNA analysis.⁴ Bersting et. al. studied the performance of 52 SNPs developed...
for human identification on 124 parent-child trios, showing that the PI values of true parent-child groups were much higher than unrelated groups. Before using SNPs to calculate human relationships, SNPs studies were found for human identification tests. The result shows that the 52-plex SNPs set can be readily adapted to a range of other genotyping methods offering the possibility of high-throughput solutions. Kim et. al. studied 30 SNPs in a total of 8,842 unrelated Korean populations. They found the probability of individual humans duplicating SNPs was $4.83 \times 10^{-13}$, and the probability of duplicating SNPs was reduced by 10 times for every added 5 SNPs. The current study reports pairwise kinship testing with a combination of STRs and SNPs loci found that the 40 STRs with 91 SNPs could also distinguish most full-sibling relationships from half-sibling but cannot distinguish relatives who belong to the same autosomal other kinship such as the first cousin.

However, the SNPs study is currently being applied to calculate the human relationship, including parent-child, full-sibling, half-sibling, and other relationships in many populations except the Thai population. Therefore, the present study analyzed the PI value from 44 SNPs loci of true parent-child in comparison with unrelated groups. The analysis of PI distribution was conducted using manual calculation with the formulae from Peter M. Lee. The data were divided into two groups. The first group was a true parent-child group 38 families, including father, mother, and child. The second group was an unrelated group 38 families that shuffled from true parent-child group consisting of father, mother, and children who are not related to each other.

The results were determined by the Paternity index (PI), which was calculated using manual calculation (with Microsoft excel software) with formulas published by Peter M. Lee in 1997 and used allele frequency of Asian from dbSNP on National Center for Biotechnology Information (NCBI) (Table 1).

### Materials and Methods

Study protocols were approved by the Human Research Ethics Committee of Thammasat University (Science), Thailand in accordance with the compliance to the Declaration of Helsinki, the Belmont report, CIOMS guidelines and the international practice (ICH-GCP) (COA No. 066/2019). The buccal swab was collected from 38 families in Thai individuals. We rub inside the cheek bulge for 15s per each by a cotton swab. The cotton swabs were extracted with QIAamp DNA Mini Kit (Qiagen, USA) and identified 44 SNPs markers (Table 1) using iPLEX® Pro Sample ID Panel (Agena Bioscience, USA). SNPs genotyping was performed on a MassARRAY® System (Agena Bioscience, USA).

The data were divided into two groups. The first group was a true parent-child group 38 families, including father, mother, and child. The second group was an unrelated group 38 families that shuffled from true parent-child group consisting of father, mother, and children who are not related to each other.

The results were determined by the Paternity index (PI), which was calculated using manual calculation (with Microsoft excel software) with formulas published by Peter M. Lee in 1997 and used allele frequency of Asian from dbSNP on National Center for Biotechnology Information (NCBI) (Table 1).

<table>
<thead>
<tr>
<th>SNPs maker</th>
<th>Chro.(10)</th>
<th>Allele frequency</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>T</td>
</tr>
<tr>
<td>rs10495407</td>
<td>1</td>
<td>0.2390</td>
<td></td>
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<td>rs1413212</td>
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<tr>
<td>rs2714854</td>
<td>7</td>
<td>0.5080</td>
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</tr>
</tbody>
</table>

Table 1 Asian’s allele frequency of 44 SNPs makers list
<table>
<thead>
<tr>
<th>SNPs maker</th>
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<th>Allele frequency</th>
<th>Study</th>
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<td>0.4100</td>
</tr>
<tr>
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<td>11</td>
<td>0.6730</td>
<td>0.3270</td>
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<tr>
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<td>12</td>
<td>0.5097</td>
<td>0.4903</td>
</tr>
<tr>
<td>rs1994997</td>
<td>12</td>
<td>0.6540</td>
<td></td>
</tr>
<tr>
<td>rs1335873</td>
<td>13</td>
<td>0.6886</td>
<td>0.3114</td>
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<tr>
<td>rs354439</td>
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<td>rs2040411</td>
<td>22</td>
<td></td>
<td>0.3447</td>
</tr>
</tbody>
</table>

Results and Discussion

1. Parentage allele sharing

Thirty-eight true parent-child groups and thirty-eight unrelated groups were tested with the 44 SNPs multiplex typing method and determined parentage based on loci shared between the child and mother and father. Thus, the outcome of parentage testing is inclusion or exclusion. In addition, this study showed the result of allele shared. The result showed that the true parent-child group shared 44 loci (100%), and the unrelated groups had loci mismatch between 2 to 13 loci (Table 2). The four mismatch loci were the highest families (10 families: 26.32%).
Table 2: Summary of the results of 38 true parent-child groups and 38 unrelated groups with loci shared

<table>
<thead>
<tr>
<th>Relationship</th>
<th>All inclusion</th>
<th>Single mismatch</th>
<th>Two mismatches</th>
<th>Three mismatches</th>
<th>&gt;Three mismatches</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>True parent-child group</td>
<td>38</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>38</td>
</tr>
<tr>
<td>Unrelated group</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>33</td>
<td>38</td>
</tr>
</tbody>
</table>

2. Paternity Index (PI)

PI values produced from two groups were summarized in Table 3. Among the true parent-child groups, the median PI was \(6.9982 \times 10^4\) (log 4.8450) with maximum and minimum values of \(1.0120 \times 10^7\) and 83.9371 (log 7.0052 and 1.9240), respectively.

The unrelated group’s PI was calculated with alleles shared without alleles mismatch. The median PI was 454.8812 (2.6579) with maximum and minimum values of \(5.0065 \times 10^4\) and 7.0750 (log 4.6995 and 0.8497).

Table 3: Statistic value of true parent-child and unrelated groups

<table>
<thead>
<tr>
<th>Relationship</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>True parent-child group</td>
<td>38</td>
<td>6.1286x10^5</td>
<td>1.7162x10^6</td>
<td>83.9371</td>
<td>1.0120x10^7</td>
<td>6.9982x10^4</td>
</tr>
<tr>
<td>Unrelated group</td>
<td>38</td>
<td>3.4292 x10^3</td>
<td>8.9449x10^3</td>
<td>7.0750</td>
<td>5.0065x10^4</td>
<td>454.8812</td>
</tr>
</tbody>
</table>

The distributions of PI values in two groups were compared. In the true parent-child groups, it was found that the lower limit of the distribution of the true parent-child intersected with the upper limit of the distribution of the unrelated between the values of \(83.9371\) to \(5.0065 \times 10^4\)(log 1.9240 to 4.6995). In this range of values, the distribution of the LR values cannot be used to determine the relationship and therefore recognized as a “gray zone” (Figure 1).

The strength of PI thresholds in terms of supporting the prosecution’s position was suggested by Butler JM in 4 positions (numbers of PI thresholds in parentheses); limited support (1 to 10), moderate support(10 to 100), strong support(100 to 1000), and very strong support(1000 and greater)\(^{[1]}\). The true parent-child groups found 3 supports were moderate support, strong support and very strong support.
with 1 (2.63%), 1 (2.63%), and 36 (94.74%) families, respectively. The unrelated groups found 4 supports (families : percentage in parentheses) were limited support (1 : 2.63%), moderate support (10 : 26.32%), strong support (17 : 44.73%) and very strong support (10 : 26.32%).

The threshold for PI values of the true parent-child was $5.0065 \times 10^4$ or higher (log: 4.6995) and unrelated groups was 83.9371 or lower (log 1.9240). Cho et. al. compared the distribution of PI tested among 1209 true parent-child groups and 16 unrelated groups. The log_{10}PI thresholds were analyzed from 50 in 136 SNPs loci. PI thresholds of true parent-child groups was among log 1.25 to 6.39, whereas unrelated groups log 0.63 to 3.57. This study found the overlaps between PI threshold from true parent-child and unrelated groups, similar to our current study. Moreover, this study showed the overlap of PI distribution between true parent-child and unrelated groups called “gray zone” similar to the previous study (6, 54-55). However, if eliminated mismatch for calculating relatedness of PI, it made the gray zone not allow for clear identification.

SNPs can be substituted for correlation calculations using STR because the correlation judgment model is similar to the STR analysis. The decision consists of all shared loci shared by parents and children. The relationship was calculated using the same principle of STRs analysis can differentiate between true parent-child and unrelated groups. To increase the reliability of the relationship prediction, additional STR, SNP and other genetic markers can be incorporated (56-58).

**Conclusion**

The scope of this research showed that the difference between true parent-child groups and unrelated groups using 44 SNPs multiplex typing could help predict human relationships. The results show the highlight of potential problems from eliminated mismatch for calculating the PI value. However, the accuracy of the interpretation should be concerned. Finally, the SNPs study is applied to calculate the human relationship, including full-sibling, half-sibling, and other relationships in the future.

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**Ethical clearance:** Study protocols were approved by the Human Research Ethics Committee of Thammasat University (Science), Thailand in accordance with the compliance to the Declaration of Helsinki, the Belmont report, CIOMS guidelines and the international practice (ICH-GCP) (COA No. 066/2019).

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**References**

Cyber Crime and Role of Law Enforcement to Tackle Online Crime in Jammu and Kashmir

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Abstract

Since the inception of the computer the cybercrime cases have been reported by and large. However, with easily accessible internet services, lawlessness has gained momentum across the world. The infractions are enacted in diverse forms like banking frauds, hacking, online piracy, stealing of IPRs, match-fixing, etc. This review provides comprehensive insights into the cyber crimes across Jammu and Kashmir with illustrations of the copious number of reported crimes. An inclusive and extensive description of criminality in the modern world makes this review indispensable and significant. The numbers presented in the review are precisely unerring and easy to understand. From fake news reporting to social media craziness this review vastly touches the necessary aspects of the cybercrime doldrums.

Keywords: Cybercrime, E-Crime, India, Jammu and Kashmir, Police

Introduction

Cybercrime is the latest and potentially the most complex epidemic in the history of technology. The concept of cybercrime involves any criminal activity that uses a computer as a subject or object, target or medium to reinforce further crimes. Cybercrime can be described in simple words as any criminal act where computers could be used as a weapon or target. Computers may be used to commit a crime in several ways like illegal access to the device, stealing of electronics-related information, e-mail bombing, data mining, salami attacks, logic bombing, Trojan attacks, web-time theft, computer-based theft, physical damage to the computer system, etc.¹. At the 10th United Nations Congress on Crime Treatment and Control of Offenders, devoted specifically to computer-related crime, cybercrime was divided into two categories, I- Cybercrime in a limited sense: any illegal activity involving online operations that affects the security of the computer network and the data created by it. II- Cybercrime in a broader sense: Any illegal action carried out on or in conjunction with a computer system or network, including offences like unlawful possession and the collection or distribution of unauthorized information through a computer system or network². Literature studies have established the following four forms of cybercrime

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1. Use of Computers as a target to commit the crime: stealing of intellectual property, hacking of marketing information like client list, pricing info or promotional plan, and accessing computer files for blackmailing purposes (e.g., personal data, medical information or sexual preference).

2. Use of Computers as a tool of crime: fraudulent use of credit/debit cards; illegal money transfers, illegal stock sales, etc.

3. Use of Computers associated with other crimes: money laundering and fraudulent bank transactions, organized crime reports or documents, match-fixing in sports, etc.

4. Crime correlated with computer prevalence. - Piracy / counterfeit apps, copyright theft, counterfeit goods, black-market hardware and systems, burglary of electronic gadgets, etc.

Common Forms of Cyber Crime

Throughout all times, cybercrimes cost businesses and people billions and billions of dollars annually. Perhaps more appalling is the fact that this statistic still had no limit in sight for the past five years. The advancement of technology and greater usability of intelligent technology ensures that hackers can use multiple access points to get into the user’s online profiles, although law enforcement continues to counter this massive concern, there are still many offenders exploiting Internet anonymity. Below are the most common forms of cybercrime that are continuously growing rapidly.

1. Hacking- Hacking is an act performed by an attacker by accessing the computer device without proper consent. Hackers are essentially computer programmers with sophisticated programming expertise and usually misusing this information for illegal acts. They are typically technology experts with expert-level expertise in one program or another. The motives behind hacking may be greed, popularity, money, etc. Most of the hackers do it simply to show their expertise. In most common cases hacker breaks into systems to steal personal banking information, financial records, etc. They often try to modify processes so they can perform tasks at their will. Hackers with such aggressive activity are often called black hat hackers.

On the other side are ‘White Hat’ hackers, who condemn computer system misuse. another term is ‘Grey hat’ hackers who may sometimes violate laws and have malicious intent as that of black hat hackers.

2. SQL (Structured Query Language) Injections: An SQL injection is a technique that enables hackers to exploit a security vulnerability in the software. This could be used to target any unprotected or insecure SQL database. This method includes inserting SQL code into a web form entry field (usually usernames and passwords), to allow the intruder more exposure to the database of the website or a particular account. It may also be used to collect details including credit card numbers or passwords from insecure pages.

3. Cross-site Scripting (XSS)- Cross-site Scripting is an injection attack on client-side code. The attacker aims to execute malicious scripts in the victim’s web browser by inserting malicious code into the website. The actual attack happens as the victim visits the website or application that executes the malicious code. The web page or application serves as a tool for distributing malicious scripts to the user’s browser. Vulnerable platforms typically used for such attacks are shopping offer web links, chat rooms, and web links for forms and feedback.

4. Logic Bombs- A logic bomb is a type of code purposely introduced into a software program that would set off a destructive operation when requirements are met. E.g., a programmer can hide a piece of code that keeps deleting files from the database of a company. A logic bomb is a malicious code that attackers inject into a program or operating system. This code remains latent until a situation arises. Such conditions may be a fixed time or a particular command the user inputs in. When situations arise, the logic bomb can destroy the operating device (corrupting the hard disk, stealing records, or overtaking the computer). Hackers also use logic bombs of viruses, worms, and Trojan horses to do optimum damage. Indeed, when used as a logic device, certain forms of malware can behave in one manner and radically shift tactics until the conditional requirement is reached.
5. **Virus Dissemination.** Viruses are computer programs that infect or corrupt a device or data and appear to circulate on a network among other devices. They interrupt the system process and damage the storage data, either by changing it or totally removing it. Virus propagation is a destructive malware mechanism connected to certain applications. Virus, spiders, Trojan Horse, Logic Bomb, Rabbit, and Bacterium are instances of malware that breaks the victim’s machine. Some Trojan generation tools enable hackers to construct their own Trojans. These toolkits help hackers develop personalized Trojans. These techniques can be risky and backfire if not properly executed. New Trojans produced by hackers typically have the added benefit of going undetected by virus detection and Trojan scanning tools because they don’t suit any known signatures. Viruses and worms can be used to manipulate software to allow an intruder to gain access. A virus and a worm are similar; both being a form of malicious software (malware). A virus infects another program and uses it to spread itself. The virus code is inserted into the already safe software and shows its effect while operating the application.

6. **Phishing**- Phishing is a cybercrime in which an attacker acting as a legitimate institution contacts to target via an e-mail, telephone, or text message for the purpose of enticing individuals to provide confidential information such as personally identifying information, credit card details, and passwords. The details are then used to access valuable accounts, contributing to data fraud and financial damage. The first phishing case was brought in 2004 against a Californian youth who developed an “America Online” replica website. Using this fake website, he was able to obtain confidential user information and use credit card details to withdraw money from their accounts. Aside from email and website phishing, there are also ‘vishing’ (voice phishing), ‘smishing’ (SMS Phishing), and many other cybercriminal phishing tactics.

7. **The Denial of Service attack (DoS)**- DoS is a type of cyber assault, in which an attacker attempts to disrupt a computer or any device by interrupting the usual operation of the software to its intended users. DoS attacks typically work by flooding a specific system with requests until regular traffic is not handled, which contributes to device denial of service. A DoS assault is characterized by the usage of a single device to attack the target. A DoS assault is specifically aimed at over-saturating the ability of a specified system, leading to subsequent requests being denied.

8. **Email Bombing**- Email bombing is characterized by an attacker sending a large number of emails to a target address leading to the crashing of the victim’s email account or mail servers. The email is irrelevant and too lengthy for network resources to be absorbed. When several mail accounts are targeted, they may have a denial-of-service effect.

9. **Web Jacking**- Web jacking means seeking control over the website of victims. It is simple if someone clones your website and trick you to assume that the cloned site is yours. The malicious link is on your website, waiting for a click. When that link is clicked it is replaced by a malicious web server. It is very different from the normal forms of phishing. Normally, if anyone wants to hijack a Website, the name on the address bar subtly shifts from your original website when the victim clicks on the link. E.g., if the original is www.cyber.com, something like www.ciber.com or something quite similar might be visible.

10. **Identity Theft and Credit card frauds**- Theft of identity and credit card frauds.- Identity theft occurs if a person steals a victim’s identity to claims that he has access to resources such as credit cards, bank accounts, and other benefits in the victim’s name. The impostor can use the victim’s identity to commit other crimes as well. Credit card fraud is a broad term for identity theft crimes where the criminal uses someone’s credit card to finance his or her operations. Credit card fraud is the easiest method of identity theft. The most popular form of credit card theft is the pre-approved card that gets into the possession of someone else.
11. **Salami Attack** - A salami attack occurs where minor attacks add up to a large attack that is not detected. This is also known as salami slicing. Although salami slice is frequently used to carry out criminal acts, it just represents a tactic to achieve an edge in time by building up to it in minor increments, so it can be utilized in a perfectly legal manner. One basic illustration is where an attacker deducts Rs. 0.01 (1 paisa) from an SBI account. Nobody would notice such a small flaw. However, if one paisa is deducted from all Indian bank holders, it generates a large amount of money. Computer computations are rounded to small fractions several times. Most banks attempt to rob money when making these corrections.

12. **Cyberstalking** - Cyberstalking in our society is a common type of cyber-crime where someone is tracked or followed online. A cyberstalker does not follow his victim physically, he practically does so by following his online activity, collecting information about the stake, and harass the victim. It is a breach of one’s online privacy. Often victims are women who are stalked by men and children stalked by paedophiles. Cyberstalkers target their victims by email, chat rooms, directories, forums of conversation, and open websites. The availability of free email/website space and the anonymity of chat rooms and forums have led to an increase in cyberstalking. Everybody has an online profile now so it is very easy to check Google to collect the name, contact number, and address, which leads to the risk of cyberstalking. As the internet is becoming an extremely essential part of our personal and professional lives, stalkers with just a few clicks away can take advantage to obtain information about the victim.

13. **Software Piracy** - With the advancement and development of internet websites and torrents, virtually any video, program or song can be found free of charge. Online piracy is an intrinsic aspect of our existence, to which we all lead intentionally or unintentionally. This affects the income of resource developers. It’s not only about exploiting the intellectual property of anyone else illegally but even handing it over to your mates to reduce their profits. Computer piracy is illegal electronic program use and delivery. Software developers work hard to develop such services and piracy curbs their capacity to produce adequate money to fund the creation of software. It affects the world economy when revenues are diverted from other markets, resulting in less advertisement and analysis spending, software piracy includes installation up the PC with unlicensed software, use of single-licensed software on many computers, use a key generator to disable copy security, distribution of the cracked version of software online and offline.

**Emerging trends of Cyber-crime in Jammu and Kashmir**

For our daily lives, computers and the Internet are becoming relevant. About 1,00,000 persons worldwide had internet access in 1990. There are now approximately 4.57 billion internet users worldwide according to the STATISTA report. Improved and increased use of Information technology gives ordinary people to access, store, and share information across the globe. The cyber-world is a digital world wherein a person is able to perform his personal acts as easily and freely as possible, and the online world serves as the platform for all electronic services and practices. The primary mode of data exchange in the present world is e-mail and websites. This includes not only instructional and informational material but also personal information. India has the 2nd largest internet user after China, with a surge of 564.5 million internet users in the year 2020 (figure 1). Facebook users worldwide overtook the United States and for the first time, India has provided a growing consumer network. The 20th century has brought to life the concept of a global village, with digital technology interconnecting and intertwining the markets, societies, and communities of the planet. India is no different, with over 400 million internet users as of 2018, making it the second-largest internet population in the world and each year internet crimes are touching its skys (figure 2). Although accessibility across the world wide web is promising significant change, it also throws up new threats to our digital communities. Cybercrimes know no bounds and...
are developing at the same pace as new technology. The insertion of mobile networking and expansion of the GPRS system in the erstwhile state of Jammu and Kashmir has developed new mass rhetoric since 2003. The swift advancement of the telecom services blended with the infusion of rapidly growing private networking in Kashmir derived people towards a more advanced type of connectivity. The number of mobile/landline network connections per 100 people in a particular zone or area (teledensity) was found to be 53.22% in 2012 with an estimated number of about 6.1 million network connections (Mobile). The statistics documented by Telecom Regulatory Authority of India (TRAI) in 2015 a teledensity of 79.5% with mobile users of about 9.6 million with netizens touching the figures of 3.5 million. Just by a year or so the subscriber’s number reaching about 117 million and a teledensity of 94.34% in 2017 which kept on growing rapidly. There is a dramatic rise in cybercrime in Jammu and Kashmir, with 73 such crimes reported in the state in 2019. Official figures of the National Crime Records Bureau (NCRB) indicate 73 and 63 cyber-related crimes in Jammu and Kashmir have been observed in 2018 and 2017 respectively. According to figures, 38 cybercrime incidents were registered in 2015, while 25 were registered in 2016 (figure.3). In collaboration with the State Department of Finances and house government, the proposal to alert cyber-crime police stations and the workplace’s growth was under consideration recently. most police stations are provided with new IT gadgets/technology designed to efficiently combat cybercrimes. The latest victim of cyber-attack in J&K was on servers of the state power department where critical information from servers, pushed their engineers into a frenzy situation. However, the attack was then defended and data was backed up by the authorities. Another well-known cyber-attack was carried on the National Institute of Technology (NIT) Srinagar in June 2017 where a group of hackers hacked the NIT website and posted anti-India messages on the website. Following the case, some spam on the online sale of refurnished goods at a much lower price was circulating across the OLX and Facebook accounts, which were stated to be diligent and cautious by J&K cyber cell. Any customer who has an interest in purchasing the item posted by the fraudsters were made to believe that they serve in the military or other defence agency and selling products to a lower price due to their transfer, thereby rendering innocent and unaware of transferring the sum to their e-wallets/bank accounts and eventually deceiving and getting the money from innocent people. With this regard, the Cyber Police of J&K rescued Rs130,000 from fraudsters and also blocked numerous counterfeit bank accounts used in the crime commission.

There are few instances of the way that social media in recent times have been misused and have been a source of false rumours. Many of these stories were circulated by anonymous profiles. This is at a time when the world is trying to combat the latest coronavirus pandemic, and many people rely on social media to improve their safety and stay updated about the pandemic. Administrative agencies, doctors, and journalists often use social media to share new information to stay updated with the safety measures and precautions about the disease.

Several media channels became a victim of false information. For example, many news portals displayed the fake NEWS of the 4G internet order of the Supreme Court. The Cyber Crime Division of J&K has reported nine cases of abuse of social media since the coronavirus outbreak and at least 178 people in the valley were arrested after fake news, videos, and pictures were posted. Cyber Police in Srinagar, Jammu Kashmir has set up its response team and stepped up efforts to tackle cybercrimes in the Valley. Such action came into effect after a string of complaints received by the police through different channels that certain people were using false identities in social media to abuse women. In certain cases, men were using morphing photos and threatening women. There are a few instances where several women were not willing to make official complaints, fearing shame and embracement. however, The J&K Cyber Crime Team has time to encouraged such victims to come forward against cyber-crime and ensured that their identities would remain confidential.

Cybercrime in J&K also involves problems relating to the setting up of fake Facebook accounts, fake online profiles, or unauthorized access to another person’s account through fake identity, with the aim of cheating. The Cyber-crime wing of J&K Police also
seized lakhs of rupees from the hackers disguising themselves as bank representatives and online business operators to target people for financial crimes. The anti-corruption bureau (ACB) arrested the accused in Jammu and Kashmir Co-operative Bank in a huge scam Rs. 223 crore which emerged in March 2020\(^{27}\). Following a six-month inquiry into incidents of violence in Kashmir in 2017, a team of the National Investigation Agency (NIA) found 79 WhatsApp groups, with 6,386 phone numbers, used to scoop up stone-pelting boys. Of these, about 1,000 active numbers were identified from Pakistan or the Gulf States. In different areas of the Valley and neighbouring states, the remaining 5,386 numbers were found involved. Most of these groups had Pakistan-based administrators\(^{28}\). In Kashmir, the number of young people with free access to social media grew from 25% in 2010 to more than 70% in 2015. Since then it can reasonably be concluded that this number has risen. In 2016, the Government for the first time took action by banning internet communications for five months to control violence and terrorism. Online curfews typically suspend 3G and 4G networks and social media services\(^{29}\). 

![Figure 1: Countries with the highest number of internet users worldwide](image.png)

Social media is proving to be a potent tool for militant groups in today’s times. Earlier they had to strive for reaching out to the people through newspaper columns and audio messages on the radio. However, social media has provided them with an easily accessible platform that enhances their outreach. The social sites are filled with posters, pictures, and messages of different individuals and organizations associated with the different outfits. The content is diffused into the world faster than the air via encrypted means like WhatsApp, telegram, etc. The sloganeering near encounter sites, recording of militant’s family phone calls, training videos of militant groups, the self-styled photography displaying arms and ammunition have been considered as acts of charisma by the youth in Kashmir\(^{30}\).
The rebirth of agitation and mass demonstrations has ensued in correspondence with the rapidly growing number of netizens in the last decade. The mass agitation of 2008 was largely influenced the get together of protesters. The footage of the protesters diffused through the internet to the nook and corner of Kashmir captivated protesters towards the agitation. Although, the separatist groups alongside the enraged protesters used this platform in 2010 more extensively so as to mobilize more masses. The more entanglement of mobile users towards social media sites has led to a model shift with regard
to the protests and protesters. The alteration has been swayed by the control and restrictions on the conventional media houses. During the 2010 uprising, the government came heavily on the mainstream media lines and stopped some newspapers, and curtailed the printing process of many publishers. Some of the channels that reported from ground zero and covered the stories related to agitation were also restricted and banned in the context of provocation of violence. This hardened censorship on the news sources compressed the conventional media thus made way for the alternative media sources. Adding to the list of restrictions the government at the same time imposed a ban on SMS services as well in the context of law and order. The ban lasted for about four years and was abrogated in May 2014. After the 2016 unrest in Kashmir, the Government banned various social media sites in the context of law and order. The websites that are banned include YouTube, Telegram, QQ, WeChat, Tumblr, Viber, Reddit, and Flickr. The ban was lifted after the situations were under control, however, since then there were many incidents where the government banned mobile services, social media sites, and/or internet services.

**Role of Law Enforcement to Tackle Cybercrime in J&K**

In the combat against the terror in the newly created union territory, the Jammu and Kashmir (J&K) police are at the forefront, but modern challenges are still present. Kashmir has contended with a lot of false propaganda on websites of social networking, which are being deeply tracked by cyber cells. A lot of this form of social networking propaganda is rooted in Pakistan. The internet was shut down in the valley after the revocation of Article 370 and this led to a variety of propaganda profiles from across the border. Although the action for the establishment of law and order was taken in numerous cases in the valley, the security forces performed well. After the encounters, videos from sites were made viral and people were invited to gather near locations, which have now stopped entirely. Many individuals were arrested and no other events have happened thereafter. Stone Pelting was also a big challenge for security forces. Several WhatsApp groups were used to gather stone-pelters from various locations. The cyber cells have shut down hundreds of WhatsApp groups that are actively involved in these events. In Srinagar, the cyber laboratories are keeping eyes on miscreant social media handlers. IT experts and digital forensic units along with the crime branch of Srinagar are tracking and monitoring cyber crimes. Cybercrime has proven to be a major problem for law enforcement officials in the valley. Cyber teams have done everything possible to combat and hold propaganda content to inspire young people.

**Conclusion**

The immense rise in Internet usage in the world and particularly in India has led to India becoming vulnerable to such crime. Cybercrimes are worldwide and offenders are not related to a single geographical region. Cyberspace is free-flowing, unbounded, and not covered by local regional restrictions. The current way of living has been transformed by new technology. The internet has given us many advantages. Whether connecting with friends, seek information, do financial transfers, access online resources, find a career, find a life partner, or even run whole companies. internet is a basic need. Nearly all facets of our lives come from the Internet. It is often vulnerable for users to a wide variety of attacks. The internet is constantly targeted by new and strong cyber threats. Slight error management of our online life will unlock the gates for cybercriminals. they can steal victim’s money or ruin their reputation. Online fraud and cyberspace abuse are rampant around the world, and in Kashmir, users are not immune to such risks. In the Cashmere Cyber Police Station of Srinagar, several complaints about the exploitation of cyber-space are received, mainly online financial fraud. To cheat/hide the gullible people, cyber fraudsters and scammers continue to change their way of operating. Cyber police Kashmir is actively recommending to the general public to be highly vigilant, not to be exposed to numerous cybercrime frauds such as unexpected prizes or rewards, jobs offerings, the construction of cell phone tower offers, loan offers, insurance incentive offers, etc. and not to exchange bank details /personal information or transfer funds to unknown people. Cyber Police of Kashmir has always encouraged people to stay away from e-crimes and advises people to promptly report such incidents.
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Predictive Equation Model to Estimate Postmortem Interval using Total Body Score

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Abstract

The decomposition process of corpse varies due to many factors. There are several studies conducted to improve the postmortem interval (PMI) by visual inspection of human remains. In this study, a total body scoring method was developed to estimate the PMI based on the decomposition and degree days (DD). One hundred and three cases were used to estimate the PMI. The autopsy reports were scored using the decomposition scoring method. The temperature data was obtained from the Thailand Meteorology Department (TMD). The PMI was estimated using the total body score (TBS), accumulated degree day (ADD) and accumulated degree hour (ADH) from the finding date back until the predicted time since death. To this end, a retroactive approach was taken in which cases from the medical examiner with a known “date last seen” and “date recovered” were compiled. A qualitative analysis was conducted examining the specific decomposition changes which occur in various contexts. Quantitatively, a linear regression analysis was employed to determine if DD or TBS explained more of the variation in decomposition. The ADH, ADD and TBS were determined to be the main components in modelling decay. These results suggest that the TBS can be optimally used to assess PMI. While the ADD and ADH were used to overestimate PMI, indicating inconsistency of the method. In total, a set of time since death estimation formula applicable to indoor, outdoor, and aquatic contexts was produced, and region-specific standards best suited to estimating time since death in Thailand. Due to the subjectivity in all the available methods and decomposition is a highly variable process. Further research is required before an estimated PMI should be considered as evidence in court.

Keywords: Total Body Score, Postmortem Interval, Accumulated Degree Days, Accumulated Degree Hour, Estimation

Introduction

The time of death is sometimes extremely important. It is a question almost invariably asked by police officers, sometimes with a touching faith in the accuracy of the estimate. Determining the time of death is extremely difficult, and accuracy is

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impossible. Considering the variables which influence the rate of body heat loss, the best one can say about the reliability of algor mortis as a postmortem clock is that it permits a rough approximation of the time of death. PMI estimation is one of the majors in forensic pathology and forensic medicine. It is often an exclusion process of available methods which ultimately can lead to an unsatisfactory outcome due to poor reliability. This problem is most acute in the early and late PMI. When decomposition proceeds and some methods (algor, livor, and rigor mortis) are no longer applicable. Several methods, such as forensic entomology, skeletal muscle protein degradation, and the study of body decomposition by application of a morphological scoring, are expected to provide further information. However, all have certain limitations and weaknesses. Availability of a tool of methods allows a case-specific selection of the most appropriate one(s), or eventually provides improvements in the overall accuracy and precision of the PMI estimation by merging and combining methods(1).

Currently, several methods are expected to assist in the estimation of the PMI in the early, late, and advanced stages of decomposition such as forensic entomology(2), the skeletal muscle protein degradation(3), Biochemistry change and the classification of decomposition morphology by the means of a TBS(4). However, the reliability of these methods is also affected by several sources of inaccuracy, mostly related to biotic and abiotic factors. Moreover, they are validated to varying degrees. Temperature, environment, and access for insects are well known as the most important variables influencing degradation or preservation of body tissues(2). In case work, there is often a certain time point of interest, such as if a person died before or after a specific date. In combination with the numerous variations of the circumstances of death, this requires a tool-box of methods to select the most appropriate ones for case-specific application. Some methods have to be excluded under certain circumstances, under which other approaches work just fine. Ultimately, merging and combining several methods can improve the overall accuracy of the estimation, just as used in the compound method for early-period PMI estimation(5).

The overview of the discussed methods to estimate the PMI. There are two variants to the TBS or total decomposition score (TDS), one that has been modified by Mofatt et al(6) based on Megyesi et. al. (4), and one by Gelderman et al(7). There are two variants to the total aquatic decomposition score, one that has been developed by Heaton et. al. (8), indicated by a subscripted ‘H’, and one by Van Daalen et. al. (9), indicated by a subscripted ‘D’. There is one variant to charred body scale by Gruenthal et al(10). Nowadays, the validity of the method in which TBS, ADD and ADH are used to estimate the PMI is examined. The rate of development of decomposition is temperature dependent. Each stage of development has its temperature requirement hence each stage has its own defined number of ADD or accumulated degree hours (ADH) to complete its development. Once the thermal history is obtained, it can be compared with temperatures at the death scene and PMI can be estimated (11). The aim of this study is to estimate of the method that are based on the visual inspection of the body remains and accumulated degree, by making a comparison of currently available methods.

**Materials and Methods**

**Research study design**

In this retrospective study, the cases were selected from the human identification records which potentially could yield information on rate of postmortem change. All these cases were under the jurisdiction of the forensic medicine section, Lampang hospital, Lampang, Thailand and forensic medicine and toxicology unit, department of pathology, faculty of medicine, Prince of Songkla University, Thailand. Autopsies were performed by the forensic physician in all cases.

**Total body scoring**

The decomposition scoring method was developed by Meewuttisom and Poriswanish’s data(12) was used as a basis. The scoring method separates the human body into three regions (Head, body and limbs), because these body parts decompose in a different rate(7). Each body characterization was assigned six stadia with corresponding scores, with a lowest score of one (Fresh or no visible changes). Every stage contains specific decomposition...
phenomena but these were assigned a separate score, because they can occur at the same time or one by one\textsuperscript{13,14}. The TDS represents the sum of the skin color of facial decomposition score (FDS, 8 scores), the body decomposition score (BDS, 8 scores), and the limbs decomposition score (LDS, 8 scores), swelling (5 scores), blistering and peeling characteristics (5 scores), hair loss (2 scores), bone exposed (10 scores) and insect activity (2 scores) score\textsuperscript{12}. The stages of decomposition were derived from an extensive literature study as shown in Table 1.

\[ \text{TBS} = aH + aT + aL + b + c + d + e + f = 48 \]

Table 1: Categories and developed decomposition scoring method

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin color - Head, Trunk &amp; Limp (a)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>Pink</td>
</tr>
<tr>
<td>3</td>
<td>Marbling</td>
</tr>
<tr>
<td>4</td>
<td>Green</td>
</tr>
<tr>
<td>5</td>
<td>Green/Black</td>
</tr>
<tr>
<td>6</td>
<td>Black and moist</td>
</tr>
<tr>
<td>7</td>
<td>Beyond black 1 (grey and moist)</td>
</tr>
<tr>
<td>8</td>
<td>Beyond black 2 (light brown and dry)</td>
</tr>
<tr>
<td>Swelling (b)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>Begin bloat</td>
</tr>
<tr>
<td>3</td>
<td>Full bloat</td>
</tr>
<tr>
<td>4</td>
<td>Partial deflate</td>
</tr>
<tr>
<td>5</td>
<td>Fully deflate</td>
</tr>
<tr>
<td>Blistering and peeling characteristics (c)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>Minimal bleb</td>
</tr>
<tr>
<td>3</td>
<td>More bleb, minimal slippage (&lt;50%)</td>
</tr>
<tr>
<td>Hair loss (d)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>Hair loss</td>
</tr>
<tr>
<td>Bone exposed (e)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>Facial bone 25%</td>
</tr>
<tr>
<td>3</td>
<td>Facial bone 50%</td>
</tr>
<tr>
<td>4</td>
<td>Facial bone 75%</td>
</tr>
<tr>
<td>5</td>
<td>Facial bone 100%</td>
</tr>
<tr>
<td>6</td>
<td>Extremity - subcutaneous exposed</td>
</tr>
<tr>
<td>7</td>
<td>Extremity - muscle and tendon exposed</td>
</tr>
<tr>
<td>8</td>
<td>Extremity - ligament and tenure structure preserved</td>
</tr>
<tr>
<td>9</td>
<td>Extremity - bone exposed</td>
</tr>
<tr>
<td>10</td>
<td>Bare bone</td>
</tr>
<tr>
<td>Insect activity (f)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Results and Discussion

The 103 autopsy case reports were conducted from the Lampang hospital and department of pathology, faculty of medicine, Prince of Songkla University, Thailand. These cases were autopsied through forensic medicine unit and human identification laboratory records for information on the duration of the PMI, the circumstances of recovery, condition of the body, and the results of the investigation into the cause and manner of death. The lowest and highest of TBS values were 8 and 37, respectively. Using the linear regression model, a predictive equation was developed that can be used to calculate PMI for unknown case (Table 2).

Table 2: The linear regression model

<table>
<thead>
<tr>
<th>Variable</th>
<th>Formula</th>
<th>Coefficient of determination (R\textsuperscript{2})</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMITBS</td>
<td>( y = 0.1925x + 7.0923 )</td>
<td>0.9796</td>
</tr>
<tr>
<td>PMIADD</td>
<td>( y = 0.3321x + 4.385 )</td>
<td>0.9997</td>
</tr>
<tr>
<td>PMIADH</td>
<td>( y = 5.854x + 36.69 )</td>
<td>0.9984</td>
</tr>
</tbody>
</table>

The coefficient of determination of TBS, ADD, and ADH were 0.9796, 0.9997, and 0.9984, respectively. This means that 97, 99, and 99% of the variability in decomposition as reflected by PMI is accounted for by TBS, ADD, and ADH, respectively. The results showed that the most of PMI\textsubscript{TBS} values were in the
PMI\textsubscript{REPORT} range by forensic physician. While, both of PMI\textsubscript{ADD} and PMI\textsubscript{ADH} values were higher than the PMI\textsubscript{REPORT} range. Therefore, ADD and ADH methods are unreliable because they cannot provide accurate PMI in individuals and localities. This reason may be affected from temperature in different season and localities.

Table 3: Categories of TBS and comparison of PMI

<table>
<thead>
<tr>
<th>TBS</th>
<th>PMI\textsubscript{TBS} (Hour)</th>
<th>PMI\textsubscript{REPORT} (Hour)</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>4.72</td>
<td>0.5-12</td>
<td>Land: Indoor remains (88)</td>
</tr>
<tr>
<td>9</td>
<td>9.91</td>
<td>6-16</td>
<td>Land: Indoor remains (1), Outdoor remains (1)</td>
</tr>
<tr>
<td>10</td>
<td>15.10</td>
<td>8-24</td>
<td>Land: Indoor remains (3)</td>
</tr>
<tr>
<td>14</td>
<td>35.88</td>
<td>24-48</td>
<td>Land: Indoor remains (1)</td>
</tr>
<tr>
<td>19</td>
<td>61.86</td>
<td>48-72</td>
<td>Drowning or Submerged or Aquatic (1)</td>
</tr>
<tr>
<td>24</td>
<td>87.83</td>
<td>72-96</td>
<td>Drowning or Submerged or Aquatic (1)</td>
</tr>
<tr>
<td>26</td>
<td>98.22</td>
<td>48-72</td>
<td>Land: Indoor remains (1)</td>
</tr>
<tr>
<td>28</td>
<td>108.61</td>
<td>24-96</td>
<td>Land: Indoor remains (2)</td>
</tr>
<tr>
<td>33</td>
<td>134.59</td>
<td>48-168</td>
<td>Land: Indoor remains (3)</td>
</tr>
<tr>
<td>37</td>
<td>155.36</td>
<td>72-120</td>
<td>Land: Indoor remains (1)</td>
</tr>
</tbody>
</table>

Amount of land indoor, land outdoor and aquatic remains were 100, 1, and 2, respectively. The four cases of land indoor remains PMI\textsubscript{TBS} values were higher than PMI\textsubscript{REPORT} possibly due to the fact that in summer it accelerated the rate of decay (Table 3).

Conclusion

These results suggest that the TBS can be optimally used to assess PMI. While, the method consistently overestimates ADD and ADH, and therefore is not a reliable method for determining an accurate PMI in an individual and localities. The limitation of appropriate temperature applications must also be given consideration when applying this research to cases where a body has decomposed in a temperature-controlled environment. The current study was the lack of complete information on the scene, which led to impossibility to measure all the additional factors affecting the postmortem changes of the body. Extensive data on scene peculiarities, such as airflow level, humidity, and also the more detailed information on the bedding of the corpse, sun exposure, could possibly let us to define their affection level on the PMI evaluation, with further comprehensive modifications to currently used methods. Therefore, the decomposition should be models as being dependent on the accumulated temperature rather than just the elapsed time since death. A fundamental portion of this research methodology is based on the concept of accumulated degree days, which utilize average ambient temperatures. However, in indoor environments with controlled temperatures the ambient temperature remains stable throughout the day, perhaps with only minor variations due to external factors such as airflow from a fan or sunlight shining in through a window. Likely, a more stable temperature environment would lead to better results with less variation as it is a more controlled variable, but the extent to which this would affect the equations produced has not been explored. Finally, it has clearly been established through many other previous published studies that decomposition is a temperature dependent process. Thus, it follows that research of this nature needs to be conducted with respect to various geographic regions. Likewise, other countries would need to conduct studies to determine the differences in PMI due to the variations in their climate. Studies utilizing similar methods have been conducted on a short-term basis in other geographic regions with varying success, but for optimal use and application to a forensic death investigation. These studies would need to be conducted with many more replicate trials and should take seasonal variation into account when establishing the methodology for predicting time since death.
Acknowledgement: We are thankful to Graduate School, Silpakorn University for providing the facilities.

Ethical clearance: Ethical Committee Nation University, Thailand clearance was obtained before conducting the study (NTU.EC.1-005/2564).

Conflict of interest: None

References

**Coffea Canephora Extract as Antibacterial Agent against Escherichia coli**

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**Abstract**

**Introduction:** This research is investigate antibacterial activity of Coffea canephora extract against Gram-Positive Escherichia coli.

**Methods:** An experimental laboratory with post-test only group design, investigating antibacterial activity of Coffea canephora extract in concentrations 4%, 16%, 25%, 33%, 39% and 44% were observed and measured mean diameter inhibitory zone then analysed by statistical and sensitivity test. Sensitivity criteria according standard of Clinical and Laboratory Standards Institute (CLSI). Statistical test using Kruskal-Wallis and Mann-Whitney Test.

**Conclusion:** In The Coffea canephora extract showed lowest concentration to inhibit Escherichia coli was 33% (Mean : 9mm) although categorized as resistant. Greatest concentration 44% (Mean: 13.75mm) was categorized as intermediate. Statistical analysis using Kruskal-Wallis showed significant difference in all concentration and Mann-Whitney showed significant mean diameter inhibitory zone in concentration 33%, 39% and 44%. In this study we showed Coffea canephora extract perform antibacterial activity against Escherichia coli although in current study no sensitive concentration found. Increase concentration correlated with increase mean diameter inhibitory zone which indicated antibacterial activity.

**Keyword:** Coffea canephora, Coffea canephora extract, Antibacterial, Antibacterial agent, Escherichia coli, Gram Positive

**Introduction**

Coffee is one of most popular beverage and important food commodity worldwide. Coffee is produced in over 70 countries¹. Most produce coffee species are Coffea canephora and Coffea arabica². Coffea canephora as known as robust coffee has more bio compound which related to antibacterial and antioxidant effect³.

Based on some researches, coffee has some beneficial effect for human cardiovascular as anti-
inflammation\textsuperscript{4} and related with decrease risk of stroke\textsuperscript{5} and heart failure\textsuperscript{6} Coffee also has benefit for hepatic system to decrease fibrotic risk in steatohepatic patients\textsuperscript{7} and lower risk hepatocellular carcinoma (HCC)\textsuperscript{8}. In human gastrointestinal, coffee has protective effect to carcinoma colorectal\textsuperscript{9} and decrease negative bacterial in gastrointestinal tract\textsuperscript{10}.

\textit{Escherichia coli} is Gram-Negative and facultative anaerobic bacteria which lives as normal flora in human gastrointestinal tract\textsuperscript{11}. Escherichia coli could be pathogenic bacteria and capable to cause diarrhea and extraintestinal disease. Some Escherichia coli strain could produce own toxin such as Shiga toxin (\textit{stx} 1 and 2) which produced by Shiga-Toxin \textit{Escherichia coli} (STEC). Outbreak of disease are common and some of disease have fatal prognoses.\textsuperscript{12}

The aim of this study is find antibacterial effect of \textit{Coffea canephora} extract against Gram Positive \textit{Escherichia coli} bacteria that belong to pathogenic bacteria in human gut.

**Material and Methods**

This was an experimental laboratory with post-test only group design. The study was held in Microbiology Laboratory, Universitas Airlangga

**Coffee Sample**

Coffee beans were harvested in Pupuan, Bali, Indonesia. Coffee beans were purchased from Mokhabika Coffee Roaster, Surabaya, Indonesia. The beans were roasted into dark roast profile. The beans were ground with grinder Baratza Encore (Baratza LLC, Seattle, WA, USA) with finest ground (250 µm).

Grounded coffee was extracted in Pharmacology Laboratory of University Airlangga. The grounded coffee extracted with ethanol 96\% (Onemed, Surabaya, Indonesia). First stage of extraction was mix 500 grams of grounded coffee with 1500ml ethanol 96\% for 24 hours in room temperature. Next, the mixture was filtered with filter paper. Filtered coffee extract was evaporated by waterbath and spent mixture was mix with 1000ml. This stage was done repeatedly for four times.

**Strain utilized**

The extract was tested contamination test in Microbiology Laboratory of Universitas Airlangga. Extract was stroked in Muller-Hinton (MH) Agar (Oxoid Ltd., Basingstoke, Hampshire, England) and incubated in aerobic condition with temperature 37\°C. There was any bacteria growth in Agar indicated no contamination in the extract.

The bacterial strains used were from Microbiology Laboratory of Universitas Airlangga stocks. The bacterial used is \textit{Escherichia coli} which cultured from clinical patients in Department of Microbiology Soetomo General Hospital, Surabaya. \textit{Escherichia coli} was growth in MacConkey Agar (Oxoid Ltd., Basingstoke, Hampshire, England). All stocks were grown in incubator (Binder GmbH, Tuttlingen, Germany) at 37\°C in aerobic condition.

**Culture Medium**

This research used Mueller-Hinton Agar (Oxoid Ltd., Basingstoke, Hampshire, England). This medium had been sterilized in autoclave (Tomy Kogyo Co Ltd, Tokyo, Japan) for 24 hours before used for antibacterial assay.

**Preparation antibiotic substance and aquadest as control**

Antibiotic Chloramphenicol 30µg Agar (Oxoid Ltd., Basingstoke, Hampshire, England) was used as positive control and prepared according to Clinical and Laboratory Standards Institute (CLSI) standard. Aquadest as negative control was made in Microbiology Laboratory of Universitas Airlangga. Inoculation of all bacteria stocks were prepared for all assays using standard method of Clinical and Laboratory Standards Institute (CLSI).

**Preparation and standard inoculant**

The inoculants were made using Mueller-Hinton Broth Agar (Oxoid Ltd., Basingstoke, Hampshire, England) in tubes which standardized to McFarland 0.5 scale corresponding to 10\textsuperscript{8} CFU (Colony Forming Unit) bacteria.

**Antibacterial assay**

Coffee extract antibacterial effect were determined diffusion technique according to CLSI susceptibility test. The extract was resuspended in aquadest in random concentration at final concentration range from 5 to 44\% for all antibacterial assay. Each dilution
series of extract were prepared in volume 100 µl, then added to each Mueller-Hinton Agar Agar (Oxoid Ltd., Basingstoke, Hampshire, England) which had been growth bacteria. Chloramphenicol 30µg and aquadest also added to each Mueller-Hinton Agar (Oxoid Ltd., Basingstoke, Hampshire, England) which had been growth bacteria as positive and negative control. The plates were incubated at temperature 37 ºC for 24 hours.

Antibacterial effect was analysed with measured diameter inhibitory zone of extract and compared with diameter inhibitory zone of Chloramphenicol 30µg as positive control. Diameter was measured in mm and categorized with sensitive, intermediate or resistant according sensitivity of Chloramphenicol 30µg against *Escherichia coli* in CLSI.

<table>
<thead>
<tr>
<th>Group measurements</th>
<th>Mean Diameter Inhibitory Zone (mm)</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-)</td>
<td>0</td>
<td>R</td>
</tr>
<tr>
<td>Group 1 (4%)</td>
<td>0</td>
<td>R</td>
</tr>
<tr>
<td>Group 2 (16%)</td>
<td>0</td>
<td>R</td>
</tr>
<tr>
<td>Group 3 (25%)</td>
<td>0</td>
<td>R</td>
</tr>
<tr>
<td>Group 4 (33%)</td>
<td>9</td>
<td>R</td>
</tr>
<tr>
<td>Group 5 (39%)</td>
<td>12.75</td>
<td>R</td>
</tr>
<tr>
<td>Group 6 (44%)</td>
<td>13.75</td>
<td>I</td>
</tr>
<tr>
<td>Control (+)</td>
<td>23.25</td>
<td>S</td>
</tr>
</tbody>
</table>

Based on CLSI sensitivity criteria of Chloramphenicol 30µg as positive control against *Escherichia coli*: R = Resistant (≤ 12mm), I = Intermediate (13-17mm), S=Sensitive (≥ 18mm).

The result in Table 1, show that in group 1(4%), group 2 (16%) and group 3 (25%), there are no antibacterial activity of *Coffea canephora* extract and categorized as resistant. However, in greater concentration, activity antibacterial are shown by diameter inhibitory zone. In group 4 (33%) and group 5 (39%) mean diameter inhibitory zone are 9mm and 12.75 mm which categorized as resistant. The last group with concentration 44%, is categorized as intermediate.

The present study does not found concentration which categorized as sensitive. Nevertheless, Minimum Inhibitory Concentration (MIC) could not determine, this study is shown *Coffea canephora* extract has antibacterial activity in concentration at least 33%. And this study is shown correlation among concentration and mean diameter inhibitory zone. Increase concentration correlates with greater diameter inhibitory zone associated with greater antibacterial activity (Fig 1).

Statistical analysis

Statistical analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, IL). Kruskal-Wallis Test was used to find differences among each groups and continued with Mann-Whitney Test to find the significance of mean diameters inhibitory zone. All measurements were done in quadruplicate.

**Result and Discussion**

The antibacterial effect of *Coffea canephora* extract against *Escherichia coli* is shown in Table 1. Diameter inhibitory zone indicates ability extract to inhibit growth of *Escherichia coli* which classified as sensitive, intermediate or resistant.
Presence of antibacterial compounds of *Coffea canephora* extract are related to antibacterial activity against *Escherichia coli*. Melanoidin in coffee extract has metal-chelating effect and result in membrane disruption of bacteria. Increase melanoidin compound in extract relates to lower free iron and greater chelated iron which decrease supply iron for bacteria. Iron compound is needed by bacteria to maintain itself and not be eliminated by host defence mechanism\textsuperscript{13}.

Caffeine in coffee extract has antibacterial activity with inhibits RNA synthesis cell. Caffeine also decrease DNA synthesis cell, specifically thymidine intakes and its conversion to dTTP\textsuperscript{14}. Kang *et al.* found caffeine enhanced sensitivity in *Escherichia*
coli mutant strains (DNAQ, holC, holD dan priA). The research found caffeine inhibit DNA replication which need gen repaired by gen recA, recB, recC\textsuperscript{15}AlkA, YzaB, Ogt, KsgA. In other study, Whitney and Weir showed caffeine potentiates methyl methane sulfonate (MMS) inhibit Escherichia coli with disrupt SOS Pathway in Escherichia coli\textsuperscript{16}. SOS Pathway is mechanism DNA repair to prevents further damage.\textsuperscript{17}

Whitney and Weir showed inhibition SOS Pathway in three regulator gene, recA (recombining repair), umuC (trans-lesion synthesis) and uvrA (excision nucleotide repair). SOS Pathway starts when recA binds DNA then degrades LexA and initiates SOS Pathway. Gene uvrA, uvrB dan uvrC work to excision nucleotide repair, especially uvrC which changes damage nucleotide with correct nucleotide. The study showed caffeine only inhibits uvrC gene with decrease synthesis trans-lesion in DNA cell\textsuperscript{16}.

Phenolic compounds in coffee extract showed antibacterial effect specifically Chlorogenic acid (CGA) and Caffeic acid (CA). CGA changes membrane cell permeability\textsuperscript{18} and effects to death cell\textsuperscript{19}. CA has metal- chelating effects with formatting Fe(II)-CA Complex and similar with melanoidin effect\textsuperscript{20}. CA also inhibits RNA polymerase enzyme\textsuperscript{21}.

This current study showed different minimum concentration to inhibit bacteria growth with other study which showed minimal concentration to inhibit bacterial growth is 10%. This current study found minimum concentration to inhibit bacteria is 33%. The difference occurred by different instrument and time of extraction. Other study used rotary evaporator while this current study used waterbath to extraction\textsuperscript{22}. The difference also relates to difference roasting time which impacts to antibacterial compound in coffee extract\textsuperscript{23}. Both studies showed Coffea canephora extract performed antibacterial activity and could be investigated further in the future as alternative antibiotics or drugs whether oral drug or topical drug\textsuperscript{24}.

Conclusion and Acknowledgement

In this study we showed Coffea canephora extract perform antibacterial activity against Escherichia coli although in current study no sensitive concentration found. Increase concentration correlated with increase mean diameter inhibitory zone which indicated antibacterial activity. Antibacterial activity was enhanced by compound in coffee extract such as Melanoidin, Caffeine, Chlorogenic acid (CGA) and Caffeic acid (CA). These data suggest that components of coffee extract might be used as component for skin lotion or hand sanitizer properties to prevent from opportunistic infection. However, coffee extract will be necessary to evaluate in vivo to evaluate its effectiveness as alternative compound as antibiotic drug specially for human gut infection.

Ethical Clearance: This experimental study had been approved by Ethical Committee of Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

Conflict of Interest: The author stated there is no conflict of interest.

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Reference


Cross Sectional Study on Knowledge and Awareness on Informed Consent Among Nurses in Tertiary Care Hospital Hyderabad

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Abstract

Nurses have a legal duty to ensure and obtain informed consent from their patients before undertaking any examination or procedure. Informed consent allows patients to make their decisions with their healthcare providers, this collaborative decision making process is mandatory in medical practice. A cross sectional study on knowledge and awareness on informed consent among nurses working in a tertiary care teaching hospital was conducted at Hyderabad, Telangana, 200 nurses working in various departments of the hospital were participated in this study, a questionnaire was prepared in regard to informed consent and assessed their knowledge levels by grading.

The data was taken into Excel sheet and statistic evaluation was done by using MS Excel software. We found 67.7% are having awareness on informed consent whereas 32.3% are unaware. Similar results were observed in studies conducted in India and abroad, periodical workshops and continuous medical education programs can achieve better results.

Key words: knowledge, awareness, informed consent, Nurses.

Introduction

Consent is a voluntary agreement between a doctor and a patient prior to any procedure. Proceeding without consent is tantamount to assault and battery, even though it is beneficial to the patient and done in good faith¹. Every patient has the right to know information before any procedures and treatments. If adult patients are mentally able to make their own decisions, medical care cannot begin without informed consent.

The main components of the informed consent² are, Voluntariness: The decision to either consent or not to consent must be made by the person themselves and must not be influenced by the

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hospital staff, friends or family members. Capacity: The person must be capable of understanding the information given to them and they are capable to make decision. Knowledge (Rule of full disclosure) patient must be given all the information related to his health condition, treatment procedure, risks involved in the proposed treatment and any alternate treatment available. Informed refusal mean patient’s can change their mind at any time and refuse at any point of time.

According to “Sec 13 of Indian contract act” Consent is defined as two or more parties agreeing on the same thing in the same sense. The practice of consent is ancient in India, Sushrutha samitha an ancient surgeon in his treatise mentions that the permission from the king required before any surgery that endangers to the life of the patient. In 1914 the legal precedent of consent came in to limelight after the judgment of Benjamen Cardoza, he opined that Every human being of adult age and sound mind has the right to determine what shall be done with his own body and the surgeon who performs the operation without consent commits assault for which he is liable for damages.

Consent defined legally in a negative term as per section 90 IPC. Consent given under the following circumstances will not be valid. Consent obtained under fear or threat, by misconception of the facts, from an intoxicated person, person who is unsound mind and by a person who is below the age of 12 years. Informed consent is part in clinical research as well, in which a human subject voluntarily confirms his or her willingness to participate in a particular clinical trial, after having been informed of all aspects of the trial that are relevant to the subject’s decision to participate. Informed consent is documented by means of a written, signed, and dated in a proper consent format. Consent may be implied or explicitly communicated by verbally, nonverbally, in electronic or written form.

Section 2(11) of the Consumer protection Act states that services rendered without consent is considered as deficiency. Nurses deal with informed consent in most of the health care centers. Nurses are typically assigned the task of obtaining and witnessing written informed consent, when delivering routine nursing care nurse must take informed written consent. Blanket consent has no role in medical practice; consent must take in every independent circumstance.

The ideal completion of the informed consent process may be achieved if doctor and knowledgeable nurses collaborate and work as a team. Nurses must be trained in documentation process of informed consent to achieve better results and curb the legal issues.

The aims and objectives of the study is to evaluate the knowledge and awareness on informed consent among the nursing staff of the hospital and to explain how the documentation errors can resulting into a medico legal issues. It helps to create awareness on informed consent among nurses will in turn helps to minimize the documentation errors and legal litigations against the institution.

**Material and Methods**

A Cross sectional and prospective study was conducted on Knowledge and awareness on informed consent among the nurses working in a tertiary care teaching hospital Hyderabad, Telangana. 200 Nurses involved in patient care are selected randomly for this study. Study was conducted from 1st January 2022 to 30th June 2022. Informed written consent was obtained from all the participants before commencement of the study. Names of the Participants were not revealed and remain anonymous. A nurse those are not working in a patient care and not interested to participate in this study was excluded.

Information on knowledge and awareness on informed consent was collected in the form of a questionnaire. The components of the Questionnaire were prepared as per the legal guidelines, fulfilling the legal definition of informed consent.

Information on the following components of questionnaire was collected from each participant.

1. Define consent.
2. Voluntariness in relation to consent.
3. Who is capable of giving consent.
4. What circumstance the consent is not valid.
5. What is the rule of full disclosure.
6. What is informed refusal.
7. Are they aware of documentation of informed consent.
8. Who can sign in consent document.
9. Who can be a witness in consent documentation.
10. What is the legality and procedure of consent under video.

Each question was assessed by grading as Poor, fair, good and excellent, based on their knowledge and awareness. For statistical analysis, the data was taken in to MS excel sheet and analyzed by using computer software MS Excel.

**Results**

Cross-sectional study on knowledge and awareness among nurses working in a tertiary care teaching hospital Hyderabad revealed the following results.

**Table 1: Percentage of results of grading of each individual component of informed consent.**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Component of questionnaire</th>
<th>Poor</th>
<th>Fair</th>
<th>Good</th>
<th>Excellent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Define consent.</td>
<td>28</td>
<td>59.5</td>
<td>12.5</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Voluntariness in relation to consent.</td>
<td>37.5</td>
<td>51.5</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Who is capable of giving consent.</td>
<td>32.5</td>
<td>38.5</td>
<td>28.5</td>
<td>0.5</td>
</tr>
<tr>
<td>4</td>
<td>What circumstance the consent is not valid.</td>
<td>30.5</td>
<td>53.5</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>What is the rule of full disclosure.</td>
<td>40.5</td>
<td>38.5</td>
<td>18.5</td>
<td>2.5</td>
</tr>
<tr>
<td>6</td>
<td>What is informed refusal.</td>
<td>39</td>
<td>37</td>
<td>22.5</td>
<td>1.5</td>
</tr>
<tr>
<td>7</td>
<td>Are they aware of documentation of informed consent.</td>
<td>25</td>
<td>52.5</td>
<td>20</td>
<td>2.5</td>
</tr>
<tr>
<td>8</td>
<td>Who can sign in the consent document.</td>
<td>29.5</td>
<td>42.5</td>
<td>22.5</td>
<td>5.5</td>
</tr>
<tr>
<td>9</td>
<td>Who can be a witness in consent documentation.</td>
<td>29</td>
<td>44</td>
<td>21</td>
<td>6</td>
</tr>
<tr>
<td>10</td>
<td>What is the legality and procedure of consent under video.</td>
<td>31.5</td>
<td>45.5</td>
<td>18.5</td>
<td>4.5</td>
</tr>
</tbody>
</table>

**Figure 1: Results of all the components graded in terms of number.**
Discussion

Cross-sectional study on knowledge and awareness among nurses working in a tertiary care teaching hospital was conducted in Hyderabad, Telangana. 200 nurses have participated in the study and we found only 2.3% are having excellent, 19.1% are good, 46.3% are fair and 32.3% are having poor knowledge. Nurses who are having poor knowledge considered as unaware where as fair, good and excellent knowledge considered as aware of information regarding informed consent.

Consent is defined as compliance or permission; it is a contract between a doctor and patient agreeing on same thing in same sense. In our study we found 28% of nurses are unaware.

Consent must always be voluntary with freedom and informed, it should be obtained without any undue force, misconception and influence. Our study shows 37.5% are unaware of this information. According to Section 87 of Indian Penal Code, an adult above the age of 18 years with conscious mind can give a valid consent. In our study we noticed that 32.5% are unaware.

Consent obtained under influence of a drug or alcohol, misrepresentation of facts, under fear, below the age of 12 years and suffering from insanity is considered as invalid (Section 90 IPC). We observed that 30.5% of nurses are unaware of this information. Consent must be informed; doctor should reveal the complete information regarding the disease, available treatment procedure, any alternate treatment available, risks and complications that may arise by the treatment. Doctor should also explain the outcome of the procedure under the Doctrine of Rule of full disclosure. Nurses should be aware of this information and meticulously prepare the document of informed consent. Our study results shows 40.5% of nurses are unaware.

Patient has the right to accept or refuse any kind of treatment procedure or examination. Patient can refuse even after signing the document of consent and before the commencement of procedure; this has to be explained to the patient by a doctor or a nurse under the doctrine of informed refusal. In our study 39% of nurses are unaware of this information. Informed consent documentation has to be done by a nurse whenever required. 25% of nurses are unaware of documentation procedure.

The consent document should be signed by the patient in the presence of disinterested third party, in case of unconscious, unsound mind patient or under the age of 18 years the consent must be signed by the guardian. We found 29% of nurses are unaware of this information. The documentation of consent must have a witness who is not related to either of the party and who can testify whenever a legal issue arises. Study revealed 29% of nurses are unaware.

The documented informed consent is always better than an oral consent because when litigation arises the document can be produced as evidence in the court of Law. If the documentation procedure recorded under video, it is more transparent and credible to produce as evidence in the court of Law. We found 31.5% nurses are unaware of this information.

Study conducted by Elif Akyuz et. al. on surgical nurses knowledge and practices about informed consent at turkey revealed that only 21.7% nurses are aware of informed consent legal significance. In another study on practice and factors associated with informed consent at south eastern Ethiopia was conducted by wogene Negash shows 45.6% of nurses are having poor knowledge.

Study results from abroad also revealed similar results like our study. 32.3% of nurses this is almost one third of the nursing staff working in the hospital are having poor knowledge; this will impact adversely on documentation of informed consent.
Conclusion

Informed consent is mandatory in medical practice, doctor examine a patient without consent can be treated as assault under section 351 IPC. Documentation is an essential part in medical practice; good documents always give good defense in the court of law. Our study results revealed that 32.3% of nursing staff are unaware of informed consent documentation. Studies conducted at abroad also similar like our study results. It is impossible to practice good documentation without knowledge, for better practice among nurses, Informed consent should teach in their under graduate curriculum. Working nurses should be given periodical trainings and continuous medical education programs at work place will help to minimize the errors and legal litigations.

Conflict of Interest: Nil

Source of fund: Self

Ethical clearance: Taken from Institutional ethical committee.

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The Severity Difference in COVID-19 Symptoms Between Vaccinated and Non-vaccinated People in al Basrah Province-Iraq

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Abstract

Introduction: The coronavirus pandemic has cross the continents creating millions of infected people and hundreds of thousands of dead, subsequently, a race against time to produce an effective vaccine was the humanity goal and hope. Many vaccines were developed with different production strategies, and with different protection margins, and despite the reported high efficacy of vaccines, a small percentage of people who received the food and drug administration (FDA) recommended vaccine doses still acquiring symptomatic or asymptomatic SARS-CoV-2 infection.

Objective: The main objective of this study was to compare the severity of COVID 19 symptoms in vaccinated and unvaccinated persons.

Methodology: Data of a total of 930 volunteers were collected and divided in to two main groups of Covid-19 infected people: 1- vaccinated and 2-non-vaccinated patients each group were subdivided in to two other subgroups: a-healthy patients and b-patients with chronic diseases, performing the research in Al-Basra province. A 17-item questionnaire was established and was covered by a massage explain the research purpose and background. volunteers rate the severity of their symptoms in four degrees scale: intense, intermediate, mild and none for each there was a correspondent number helped to make the statistical analysis from zero to three.

Results: A total of 930 people took part in the survey. In 168 hours, from 6th of October to 22nd of October, 2021, we obtained 950 responses to the questionnaire. Overall, mean age of the responders was 32.8 years (SD ± 4.026), The total sample for validation study was 930 people receiving the questionnaire, (417 registered males and 533 registered females). Only 930 agreed to participate in this study (total response 97.8%), (71.8%) of the participants had a mild-to-moderate COVID-19 in the acute stage.

Conclusion: This study also shows that a large percentage of COVID-19 patients in Al Basrah province do not become infected after receiving the vaccine (74%), and that the symptoms of COVID vaccinated people are less severe than those of unvaccinated people.

Keywords: COVID-19, Symptoms, Vaccine, questionnaire.

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Introduction

For a long time, coronaviruses were believed to be a weak pathogen for humans that cause a flu-like illness; however, with the outbreaks of SARS and MERS in 2002 & 2012 subsequently, and nowadays SARS-CoV-2, their ferocity is very well confirmed globally (1,2).

Coronaviruses belong to the family Coronaviridae and the order Nidovirales and they are the largest family of viruses (3,4); however, our concern is severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) specifically, which affects both lower and upper respiratory tracts with a wide range of symptoms, starting with sore throat, common cold, fatigue, fever, dry cough, nasal congestion, and sometimes diarrhea reaching to severe pneumonia, difficulty in breathing and ends with death (5) beside the disrupted flavor and taste sensation earlier before respiratory involvement (6).

The coronavirus pandemic has crossed continents creating millions of infected people and hundreds of thousands of deaths (7,8); subsequently, a race against time to produce an effective vaccine was the humanity goal and hope. Many vaccines were developed with different production strategies (9,10) and with different protection margins (11,12); and despite the reported high efficacy of vaccines, a small percentage of people who received the food and drug administration (FDA) recommended vaccine doses still acquiring symptomatic or asymptomatic SARS-CoV-2 infection (13,14); this called a vaccine breakthrough infection which defined as the detection of SARS-CoV-2 RNA or antigen in a respiratory specimen collected from a fully vaccinated person after 14 days or more from the last dose of COVID-19 vaccine (15); however, the efficacy of COVID-19 vaccines in reducing the severity of symptoms to vaccinated infected people still a questionable especially by considering human genetic variation is geographically systematized (16).

Method

Data of a total of 930 volunteers were collected and divided into two main groups of COVID-19 infected people: 1- vaccinated and 2- non-vaccinated patients each group were subdivided into two other subgroups: a- healthy patients and b- patients with chronic diseases, performing the research in Al-Basra province. A 17-item questionnaire was established and was covered by a massage explain the research purpose and background. Volunteers rate the severity of their symptoms in four degrees scale: intense, intermediate, mild, and none for each there was a correspondent number helped to make the statistical analysis from zero to four. Other information was asked from each volunteer including age, sex, province, chronic diseases, type of vaccine and the method of COVID-19 infection diagnosis. All the responses were anonymous and the inclusion criteria included age above 18 and belonging to Al-Basra city, any response below the age 18 and/or doesn’t settled in Al-Basra were excluded. The registration period started from 6th of October to 22nd of October, 2021. After data collection were finished, data analysis by using t-test were done to calculate significance. A comparative measure between the symptoms of the four subdivisions were made beside calculations to the percentage of the infection after full vaccination, after single dose vaccination and for non-vaccinated respondents, the percentage of patients admitted to intensive care unit, the most common symptom all were performed.

Content of survey

The period of disease was assessed from its first COVID-19 diagnosis, with the assumption that at least two months should pass after healing to avoid terminology confusion. Patients’ symptoms were classified into the following systems: general (fatigue, fever, and runny nose), respiratory (chest pain, dyspnea, and cough), cardiovascular (high blood pressure, angina, and MI), diabetic, dermatological (hair loss and rash), and GIT (abdominal pain, loss of smell and taste, and vomiting, diarrhea). To assess the severity of the symptoms, participants were asked to rate each symptom on a scale of zero (no problem) to 4 (very severe) (17).

Statistical analysis

Percentages, frequencies, and mean SD were used when applicable. Statistical significance was defined as a p value less than 0.05, using Predictive Analytics Software version 19.0. The t-test was used to compare continuous variables for distributed data and categorical variables in parametric settings.
To identify independent variables of symptoms, multivariate logistic regression was performed.\textsuperscript{(18)}

**Results**

During the study period, 950 people who had recovered from COVID-19 were asked if they wanted to take part in the study, and twenty of them refused to participate in the study. A total of 930 people took part in the survey. In 168 hours, from 6\textsuperscript{th} of October to 22\textsuperscript{nd} of October, 2021, we obtained 950 responses to the questionnaire. Overall, mean age of the responders was 32.8 years (SD ± 4.026) as seen in Table 1. The total sample for validation study was 930 people receiving the questionnaire, (417 registered males and 533 registered females) as shown in Table 2. Only 930 agreed to participate in this study (total response 97.8\%), (71.8\%) of the participants had a mild-to-moderate COVID-19 in the acute stage. The sociodemographic characteristics of the study population are presented in figure 1,2.

For registered people, the percent of healthy peoples (not have risk factor) are (82.3\%). The percentage of patients suffering from respiratory disease, chronic heart disease, and diabetes are (6.1\%), (9.8\%), and (4.4\%), respectively. The most common answers regarding the diagnosis of COVID 19 infection by PCR, CT scan, and others are (64.3\%), (16.6\%), and (19.1\%), respectively. The percentage of people who have received the vaccine is 73.5\%, while the percentage of people who have not received the vaccine is 26.5\%. More than half of the registered people have received the Pfizer vaccine (68.6\%) and about (15.7\%) have received the Sino pharm vaccine while the others takes AstraZeneca (11.2\%). (74.4\%) of those in this study were vaccinated with two doses, while (18.7\%) were vaccinated with a single dose. After receiving two doses of vaccine at different times, approximately (74\%) of participants in this study did not become infected with COVID 19. Majority of registered people agreed to participate in this study and completed the questionnaire. Figures 1-4 illustrate the demographic and clinical characteristics of participants.
Moreover, our results clearly showed that, for vaccinated people, the infection period lasts (1-7) days (10.2%), while (5.3%) for period (8-14) days, and the registered people with infection period longer than 14 days are (10.5%) as in the figure 5.

Figure 5: COVID 19 infection period

Health Status:

About (55.8%) of people registered in this study didn’t become infected with COVID 19 after receiving the vaccine, while (5.2%) of those participating in this study became infected after receiving the first dose of vaccine, and (9.5%) became infected after receiving two doses of vaccine. (25.2%) of participants people in this study didn’t receive vaccination yet, but they reported positive test. Only (8.5%) of people in this study who didn’t take vaccination and do not infected.

Situation in the critical care unit:

The percentage of patients admitted to the intensive care unit (ICU) was (3.2%). While (95.7%) of those who participated in this study did not admitted to the intensive care unit.

Discussion

The information collected in the study will be important in raising public awareness about the consequences of the COVID-19 epidemic. In the current study, in which most data were collected via questionnaire, clearly demonstrated a difference in symptoms between vaccinated and unvaccinated people. The questionnaire contains various questions, the most important of which are (a) symptoms of COVID 19 patients before taking vaccine and (b) symptoms of COVID 19 patient after taking vaccine, in addition to types of vaccine taken, period of infection, vaccinated or not, and number of vaccine doses received(19).

Diabetes, respiratory disease, and chronic heart disease are the most significant risk factors, according to our research (Figure 6) Fever, loss of smell and taste, cough and sore throat, and shortness of breath were found as the top five strongest indicators of a COVID-19 infection (Figure 7) Nausea and vomiting have been recorded as a rare symptom.

Figure 6: Risk factor

Demographical and clinical characteristics of participants (n = 930)
Demographical and clinical characteristics of participants (n=930)

Loss of smell and taste, according to Menni et al, should be part of routine COVID-19 testing. We fully agree with this viewpoint, as our data also suggests a high level of predictability. Although chills and fever are more common in COVID-19 patients, a loss of taste and smell in a clinical setting is unique and helps to distinguish COVID-19 from other infectious diseases, as many of them are followed by chills and fever. In the current situation, we believe that loss of taste and smell, especially when combined with the other symptoms, should be treated as a red flag, prompting immediate testing for COVID 19 and isolation of the patient until the virus is identified(20).

Vertigo, painful back, burning sensation of the tongue, thoracic pain, diarrhea, and runny nose were all found in the answers to the open question that asked for additional symptoms. Due to a lack of data and incomplete, a static analysis of these reported symptoms is not yet possible.

Furthermore, the data in this study also shows that only 20.3% of the participants had a chronic disease. Thus, people with these diseases can express them with the word (other) and persons don't have any history of disease express by (healthy person) when analyzing the data. In this study when comparing the data between healthy group (vaccinated and unvaccinated), reported a significant difference between vaccinated person (who receiving two doses vaccine) and unvaccinated persons like sore throat and loss of smell and taste, the P values are 0.012, 0.046, 0.0047, and 0.028, respectively.

While in other group (people with chronic disease) reported a significant difference between vaccinated (who receiving two doses vaccine) and unvaccinated persons like sore throat and loss of smell and taste, the P values are 0.04 and 0.001, respectively. While insignificant difference in symptoms like fever and cough.

Although our collected data shows a significant difference between infected persons (healthy status) who receive two dose vaccines and another group of infected persons (healthy status) who receive one dose vaccine for symptoms such as fever, cough, loss of smell and taste, and sore throat, the P values are 0.012, 0.046, 0.0047, and 0.028, respectively.

In the other group of chronic disease individuals, this study found no statistically significant difference in all symptoms between those who received two doses of vaccination and those who received one dose of vaccine.

Our research has various advantages. First, as far as we know, this study, which examines the severity of COVID symptoms using a one-to-one questionnaire, has the most participants, with over 900 persons participating. Second, the study included a sufficient number of individuals from each risk group, including mild, moderate, and severe. Third, we looked into the vaccinated dosages. Fourth, we utilized a file to determine degrees of sensitivity to symptoms, use a scale of 0 to 4.

Limitations

This study has some limitations, the main limitation of our study is the timing of the survey in 6th of October to 22nd of October, 2021. The patient’s self-declaration is the foundation of this investigation. There might be disparities in how patients perceive, express, and take their symptoms seriously. Another drawback of our study is the self-reporting nature of our data recovery methods. The design prevents confirmation of reported symptoms or test findings. Aside from that, participants are neither invited or
pre-selected, and they may not be representative of the broader population. The usage of a smartphone device may have resulted in a lack of representation among elderly persons. Another possible limitation is the number of participants that had been tested for SARS-CoV-2 infection. A possible correlation between age and gender was not considered in the univariate analysis of symptoms.

**Conclusion**

Our findings show that people should be more aware of COVID-19 and should get vaccinated. This study also shows that a large percentage of COVID-19 patients do not become infected after receiving the vaccine (74%), and that the symptoms of COVID vaccinated people are less severe than those of unvaccinated people. There is no doubt that there are gaps in people’s knowledge of vaccines. As a result, people must be completely vaccinated in order to activate their immune systems and defend themselves against the epidemic[19].

**Ethics**: Approval of the study design was obtained by the Ethics Committee at the University of Basra /Medical college). The committee was very supportive of the project.

**Source of funding**: Self

**Conflict of interest**: Nil

**References**


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