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From the Desk of Editor-in-Chief

I am pleased to present the second issue of the year 2023 of Journal of Punjab Academy of Forensic Medicine & Toxicology. I am thankful to the authors and contributors for the scientific articles and research papers which are being published in this issue. I am also thankful to the editorial team and the members of the Academy for supporting me in this publication. My special thanks to Joint Editor Dr Amandeep Singh for his support and sincere efforts for publication and release of this issue. This is the last issue under present editorial team. Our best wishes to the next team.

The Journal publishes original research papers, review articles, case reports and review of books on Forensic Medicine and Toxicology. The Journal highlights the achievements of the academy and its members. This journal is meant for achieving the aims and goals of the academy to expand the academic activities, spread the knowledge and latest research in the field of Forensic Medicine and Toxicology.

Any suggestions and advice for further improving the standards and quality of the journal will be highly appreciated and may be sent to the new editorial team.

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Editorial

Criminal Procedure (Identification) Act 2022 A Critical Analysis

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ABSTRACT

Introduction: With increasing crime in numbers and sophistication and comparative lag in the identification of the suspects, narrowing down to accused and then filing charge sheet against them have led to low conviction rates in crimes especially the heinous crimes like rape, murder etc. This led to enactment of the new act, Criminal Procedure (Identification) act 2022 repealing the century old "The Identification of Prisoner's Act, 1920" with amendments increasing the scope of the identification aspect of the suspects/accused hoping to get a better conviction. The changes increased the types of parameters taken as "measurements"; and digitization of these identification marks so as to get their comparison from records and subsequent sharing made easier on Pan India level. But despite the noble ideology behind the enactment, individual privacy and a host of fundamental rights infringement was observed which has raised fear and alarm in relation to implementation of this act. A critical analysis has been penned down here based on these questions raised across India.

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INTRODUCTION

Criminal Procedure (Identification) act 2022 was enacted in April 2022 with the aim to authorize for taking measurements of convicts and other persons for the purpose of identification and investigation in criminal matters and to preserve records. This act repealed its predecessor "The Identification of Prisoner's Act, 1920".

The purpose of Identification of people in conflict with law, based on measurements is in the form of "Demonstrative Evidence", which works in four ways.

Firstly, measure-ments taken establish the involvement of the person in the present crime or previous similar crimes.

Secondly, measurements taken from convicted or persons ordered to give security for good behaviour may help looking into possible future crimes.

Thirdly, persons may form database to establish past convictions, ie. identity of the arrested person with a person previously convicted.

Finally, form basis of statistics based on anthropological interests. For example, if by Locard's principle, some physical or biological evidence in the form of fingerprints, blood, semen etc. are left behind at the scene of crime and the same information is already available in data base owing to his/her previous encounters with law, it helps to nail the accused or exonerate the innocent.

The evidence of maintenance of such identification traits on records have been there in history since ages, where in the sole purpose of such records was to decide for quantum of punishment, i.e. if the person caught is a repeat offender (decided based on number of times his personal traits were recorded), the punishment would be severe. These personal traits were maintained as means of identification on official records by means of simple methods as Memorization on one end of spectrum to use of brutal methods of permanent body markings on the offenders.

These Old Techniques used were Branding **(Figure 1)**, Tattooing **(Figure 2)**, Memorization, Written Description, Photographs **(Mug Shots) (Figure 3)**, Bertillon's Method (Anthropometry), Fingerprinting. Bertillon method card was worked out which included basic anthropometric measurements, photographs for the convicts of the prison. **(Figure 4)**. Others were paraffin wax branding, old fractures documentation, Old Scar marks/moles etc. documentation. These techniques evolved over the years. The modern techniques have now been introduced which

Figure 1: Branding

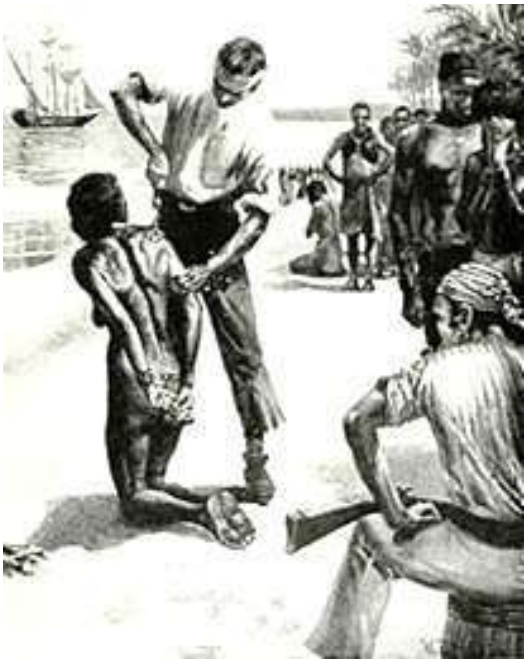
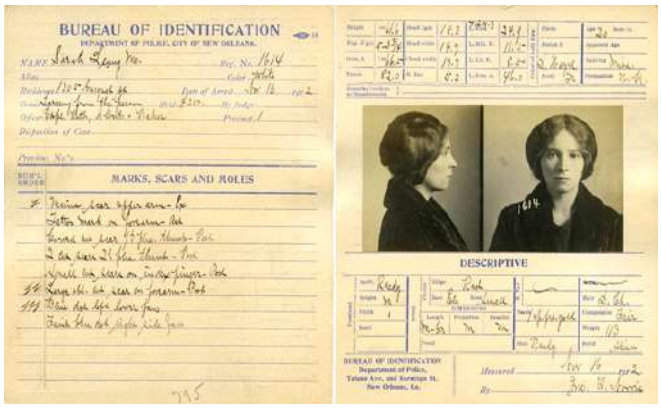


Figure 2: Tattooing

Figure 3: Mug Shots

Figure 4: New Orleans Police Department Bertillon Card Collection



are more individual specific and non invasive. These are Ear Prints, Palm prints, Foot prints, Lip Prints, Palatoscopy or palatal rugoscopy. The techniques that are widely used are are Biometrics based Bone comparisons, DNA profiling, frontal sinus pattern, Iris scans, Skull suture patterns, Vascular grooves, veins on the back of hand etc. New Generation identification techniques that are under experimentation include DNA Phenotyping, Hair Bacteria Assessment, Time-Tracing Fingerprint Technology, Technology using Stable Isotopes of Water for Geo-locating a Suspect or Victim, 3-D Photography Technology, Forensic Palynology, NGI Iris Service etc.

In order to implement the basic techniques of identification in the colonies under the British Rule, The Identification of Prisoner's Act, 1920 was passed in India which authorized the law enforcement agencies to take and store photographs, fingerprints and footprints of then declared convicts or freedom fighters. Provision for their storage and destruction was also made. This Act had 9 sections. This act over the years had state amendments wherein State of Madhya Pradesh added palm impressions along with finger prints in the definition of measurements (1962) while Tamil Nadu added to the definition of Police officers, Finger print experts of Finger Print Bureau (1981). Maharashtra added " ....convicts or made to give security under Dangerous Drugs Act, 1930 and Bombay Prohibition Act, 1949. (1971) etc.

This century old act was revisited by the **Justice Malimath Committee** in their 87th Report of Law Commission of India, 1980 on Criminal Justice system. Hon'ble Supreme Court in their various judgements pointed out that great

progress has been made in the field of Physics, chemistry wherein their use in collection of documentary evidences should be promoted.

It was also observed that as the techniques of committing crimes have evolved, equally newer techniques of their detection were needed. Owing to the restrictions of the measurements to be taken from convicts, the prosecution was not able to make strong case and the overall conviction rates were low.

The National Crime Records Bureau (NCRB) data for the year 2020 showed the conviction rates for heinous crimes like murder to be as low as 44%, Rape 39% and attempt to murder as 24%. The comparative Conviction rates for other countries were on an average 88.6% in England, Canada 64%, USA 93%.

These arguments led to the enactment of this Criminal Procedure (Identification)Act, 2022 which repealed the Act of 1920. This Act was introduced in Lok Sabha on 28th March 2022 and passed on 4th April 2022; then passed in Rajya Sabha on 6th April 2022 and received Assent by President and was implemented Pan India on 18th April 2022.

This act had 10 Sections and Explained:

1) The type of data that may be collected
2) Persons from whom such data may be collected
3) The authority that may authorise for such collection
4) How and where to store data in the central database.

Other Features of the Act are:

The National Crime Records Bureau (NCRB) will be the central agency to maintain the records. It will share the data with law enforcement agencies. Further, states/UTs may notify agencies to collect, preserve, and share data in their respective jurisdictions. The data collected will be retained in digital or electronic form for 75 years. Records will be destroyed in case of persons who are acquitted after all appeals, or released without trial. However, in such cases, a Court or Magistrate may direct the retention of details, after recording reasons in writing. **(Section 4)**

The power to make rules with respect to the implementation of this act was bestowed to both Central and State Government. Rules made by Central/State government have to be passed by both/one houses of Parliament/State Legislature respectively. **(Section 8)**

If any difficulty arose in giving effect to provisions of this act, Central Government may, by order published in Official gazette make such provisions as appears to be necessary for removing the difficulty. **(Section 9)**

The Rules for this Act of 2022 was enacted and notified on 19th September 2022 defining the procedures for performing and implementation of this act on grass root level. Rules are necessary to supplement the primary Act. The salient features of these rules are:

- In relation to from whom the measurements can be taken, the rules specify that for persons who are held under section 144 or 145 of CrPC or under Preventive Detention under section 151 of CrPC, measurements will not be taken unless they have been charged or arrested in connection with any other offence.
- With respect to the officials who are authorized to collect measurements, the rules specify that an authorized user who could be a police officer or a prison officer who has been authorized by NCRB to assess the database, or any person who is skilled in taking such measurements or a registered medical practitioner , or any person authorized in this behalf may take such measurements.
- The Rules further specify that NCRB will issue Standard operating procedures (SOPs) for taking measurements which should include the format for taking measurements, devices to be used, method of handling and then storing these measurements, the digital format into which the data would be converted and then encrypted. Further in order to use the stored data for sharing, NCRB as per their own guidelines will entertain the request sent to it and it will match and provide the report through a secure network.
- Regarding the destruction of the records, the SOPs will be made by NCRB regarding the procedure to be followed on the lines that a nodal officer will be nominated by state or central government or UT administration for taking requests for destruction of records of measurements and he or she in turn after verifying that the data is not connected to any other on going criminal case will then recommend the NCRB to destroy such records.

This Act though need of the hour to fight the ever advancing crimes in terms of numbers and techniques, have raised issues and argumentation where in the balance expected between the social issues and individual privacy as suggested by the Malimath Committee was not maintained and the fine line between the two is meagre or debatable. With respect the rules, as per Supreme court, the rules

formulated cannot alter the scope, provisions or principles of the parent act. But the rules meant for implementation of this act have surely gone beyond the scope of the Act in many instances as discussed later.

Issues have been raised that the Act violates various fundamental rights like the Right to Privacy owing to the battery of measurements collected. The right to privacy is protected under Article 21 and various measurements collected and subjected to analysis are extremely personal data of an individual. Supreme Court in its Puttaswamy Case (2017) verdict stated that the right to privacy of an accused may be challenged but it cannot be violated without providing a legitimate purpose. Right against Self Incrimination is challenged and so are the right to freedom of speech and expression; Right to Purpose Limitation; Right to equality;Right to life and personal Liberty. Further, it is even stated that this might lead to a state of Mass Surveillance and may be abused to target particular groups.

On close analysis, in **Section 2**, the definition of measurement is unclear. Behavioural attributes also are not specified. The word "analysis" used in the context of measurement is vague and undefined. Nature of these measurements taken will not be limited to their requirement in that specific investigation. It is also argued that biometrics technology does not guarantee 100% accuracy and is only 99.76% accurate. Diseases like Cataract can alter Iris scans, Pharyngitis can alter voice recognition. Even UID data showed fingerprints weren't useful as identification in 100% of cases. The Act authorized a police officer of the rank of Head constable to take measurements. The rules also authorized any skilled person in taking measurements (skilled person is not defined though) or registered medical practitioner to collect measurements.

The Rules went beyond the scope of the Act and further authorized the person who has assess to the database to collect measurements which is questionable and can raise a doubt in the integrity of evidence in the court of law. **Section 3** describes that persons who are either Convicted of an offence under any law or Ordered to give Security for good behaviour or maintaining peace or just arrested in connection with an offence punishable under any law or detained under any preventive detention law.will be asked to submit their measurements, if so required. Except for offence against women or a child or for any offence punishable with imprisonment for a period not less than 7 years, arrested person may not be obliged to allow taking of his biological samples. Nothing has been said as to specify the requirement or seeking justification for those requirements. Any person who is convicted of petty theft will be asked to submit his "measurements" like retinal scan, signatures etc. If these crimes are against a man, he may not be asked for biological samples but if the same thief snatches purse of a woman, exception necessitates collection of biological samples too. If any person is arrested in connection to a punishable crime but his crime is still not proved, the collection of measurements like convicted goes against the whole basic notion of our judicial system wherein the suspect is "Innocent until Proven Guilty". Detention of people when they are protesting against the government when detained for their exercising of right to freedom of speech may be asked to give their measurements like criminals. Overall, it is important to note that investigation in all cases do not require collection of personal data and the act has failed to distinguish the kinds of cases where police may require or be allowed to collect such data from accused persons. This act also gives no reason as to why a person accused of petty crime and a terrorist are subjected to same data collection. The rules enacted under this Act though excludes certain persons from collection of these measurements but in doing so, it is altering the grounds specified in the act.

Section 4 authorises National Crime Records Bureau to collect record of measurements and store at National level which will be retained in electronic or digital form for 75 years. The NCRB though was set up to function as repository of information on crime and criminals, does not have any wing which can collect the measurements including biological samples thus the feasibility issue. For this, Central Forensic Science Laboratories and State Forensic Science Laboratories which themselves are limited in manpower and infrastructure would be required to play a substantial role. The rules have also added other officials like experts, medical officers to the category who can collect these measurements, going beyond the scope of the parent act.

The rules regarding the implementation was to be made by Central/State Governments and those rules had to be tabled in Parliament or Legislative assembly for discussion and agreement as such or with modifications as directed in the act. The rules now made have delegated the power to make Standard Operating procedures (SOPs) for manner of collection and storage on NCRB. The Rules specify that

NCRB, through their SOPs, will specify the guidelines and procedure for the functioning of its own. This may violate the principle of separation of roles between the entity who is making the guidelines and the one who has to follow these guidelines. Also, the SOPs made by the NCRB as rules for implementation should have to be laid before Parliament or Legislative assembly for scrutiny. This appears to have not be followed for these rules.

Another issue is the preservation of such huge data in the digital format for 75 years will be an operational challenge and further securing any breaches or leaks of such data will be difficult as still the legal framework for data protection is still in its infancy stage as it was recently passed by both Lok Sabha and Rajya Sabha on 7th and 9th August 2023 and made an Act on 11th August 2023. The data sharing among law enforcement agencies is mentioned but are not clearly defined. The mere preservation of data for 75 years is against the Principle of Purpose Limitation which means even if data collection from an individual is allowed, it needs to be used only for the specific purpose it is collected, for eg. UID.State Government/ Union Territory who are to notify appropriate authority if decide to privatize, it may loose the very purpose as it will be open to illegal usage of this individual data as and when required, wherever required.

Further, the removal of record from database will only by be made after final acquittal or discharge of a person arrested for an offence as per the act. The rules meant for destruction of the records of measurements put the onus on the individual seeking the destruction instead of it being handled like in the Juvenile Justice (Care and Protection of Children) Act, 2015 wherein the Juvenile Justice Board directs the police and the court itself to destroy the data of the juveniles who have been convicted except for ones involved in heinous crimes.

Section 5 gives power to Magistrate to direct any person to give measurements. Though the Law commission report, 1980 pointed out that though Magistrate can make anyone give their measurements as per the Act of 1920 but the ambit was very wide. They recommended that the Magistrate should be made to record his reasons for giving the order. This Act of 2022 did not provide that safe guard.

Section 6 stated that resistance to allow taking measurements will be deemed as offence under Sec.186 IPC which in turn states that Obstructing public servant in discharge of his public function is punishable with imprisonment upto 3 months or fine which may extend to Rs. 500 or both.

Section 8 gave power to both Central Government and State Government to make rules. Police/Prison being the state subject come directly under the purview of state government. But rules formulated by both Central government and state government may have differences seeking jurisdictions or means.

The PRS Legislative Research legislative, in its independent analysis exemplified the instances where this act (then it was still a bill), appeared to be questionable in its approach and these queries yet remain unresolved even after Act was notified and rules made :

Illustration 1: Person W is found guilty of rash and negligent driving (and fined Rs 1,000). He may have his signature collected and stored in a central database for 75 years. The Bill permitted this.

Illustration 2: Person X is arrested for an offence. He refuses to give his fingerprints. He is charged with preventing a public servant from performing his duty (Section 186 of the Indian Penal Code, 1860). His fingerprints are forcibly taken under both cases. He is subsequently discharged from the original case. However, as he is guilty under Section 186 of the Indian Penal Code in the second case, his fingerprints can be stored for 75 years. This implies that anyone who is arrested for any offence and refuses to give measurements can have their data stored for 75 years, even if they are acquitted in the main case.

Illustration 3: Person Y is arrested. The case goes on for 20 years through several appellate levels (this is not unusual). His records will remain in the database for this period. He gets acquitted. He is arrested in another case just before the final acquittal in the first case. The records can be kept in the database until the second case is decided. This process can be continued through a third case and so on.

Illustration 4: Person Z defies Section 144 orders under the Code of Criminal Procedure, 1973 (unlawful assembly) and is arrested. His fingerprints are taken (the Bill does not require a connection between the measurement and the evidence needed for investigation). He is found guilty under Section 188 of the Indian Penal Code (disobeying an order of a public servant) and fined Rs 200. His fingerprints will be in the database for 75 years.

Current Status of the act is that it has been challenged and a PIL has been filed in Delhi High Court and Madras High

Court following which the reply has been sought from Central Government over these issues. The rules designed to further the act have gone beyond the scope of the act which is unconstitutional and can also be challenged in the court of law.

In my opinion, through this Act, legal authorization is granted for collection of samples which could be subjected to new forensic techniques to better crime detection and improve conviction rates, but the ambiguous terms and the increased but vague ambit of the persons who may be subjected to such collections irrespective of the law breached; and rules formulated with contrary sections and monopolized authority figure of NCRB to not only make and then follow what is made by itself without any external monitoring and surveillance, does not help to build confidence in citizens of this country and into the Act.

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Original Research Paper

Wildlife Crime and Technologies Used For Its Prevention

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ABSTRACT

Introduction: Wildlife crime is any illegal act committed causing threat to animal and plant species. Nowadays, such type of crimes became a matter of serious concern for the forest department and other concerned authorities. In this paper we have discussed about the wildlife crime rate of Wildlife division North of West Bengal and the technologies they use to prevent such crimes.

Material and Methods: The data for the study were collected through various techniques such as- census survey, interview, case study and data collected from secondary sources. All those data collected from three divisions were segregated and analyzed.

Result: According to the data collected for last 10 years shows that highest number of illegal trading has been reported among which trading of Rhinoceros horns are mostly in demand as compared to others. Also, 30 and 26 cases of hunting and poaching were reported respectively for past 10 years.

Conclusion: Although various initiatives and protective measures are taken by the forest department by using modern technologies but too delay in wildlife crime cases in court results in disappointment among officials. Early disposal and conviction will definitely give confidence to officials and discourage the offenders from committing such type of crime.

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INTRODUCTION

The term 'wildlife crime' commonly refers to any act that has been committed or is been done which are against the laws that are made by the national or international governing bodies to protect the natural ecosystem so that proper balance will be maintained between the environment and human community which means to properly manage the natural resources and at the same time fulfil the human needs from the same environment.

'Wildlife crime' is an umbrella term which is used for all those illegal activities such as poaching, hunting, and illegal trafficking of animal species whether marine or terrestrial.

POACHING: poaching as per defined by the law is the term used for any kind of illegal hunting or killing of wild animals. The term is also associated with aquatic species and is not only limited to wild animals. Capturing those animal species which are mentioned under Wildlife Protection Act is also considered to be poaching.

In a country like India which is considered the homeland of diversified animal species such as Rhinoceros, different species of reptiles, snakes, elephants, leopards and also a wide variety of bird species are now protected by implementing the Wildlife (Protection) Act 1972. So that all those vulnerable species can be safeguarded from any kind of illegal hunting or captivation. Despite all the implementation of laws, according to the data that are available from the wildlife crime control board from the year 2012-2018 there were several cases of poaching and the maximum poaching is reported in the state of Madhya Pradesh.

WILDLIFE TRAFFICKING: Wildlife trading is any kind of business associated with animals or plants, usually extracted from their natural habitat or raised under certain specific conditions which can be living or dead animals or their various body parts.

Illegal wildlife trafficking is any crime that is related to environment involving the illegal activities such as illegally trading, poaching, capturing or having a collection of endangered animals, wildlife that are protected, derivatives or products of the same.[1]

Nowadays, the cases of illegal wildlife trafficking are rapidly increasing due to the high demand both nationally and internationally for a variety of products around the world such as bushmeat, exotic pets, trinkets and accessories for uses ranging from coats to traditional costumes and various trophies.

All these activities pose a serious threat to the survival of all those animal species which either has been migrated from one place to another or animal living in their habitat.

At the international level wildlife crimes are considered to be any violation or any activity committed that contrasts the treaties made by intergovernmental for example, the Convention on international trade in endangered species of Wildlife Fauna and Flora, the Convention on Conservation of migratory species of Wildlife animals, all these conventions regulate the export and import of wildlife and violation of the export and import guidelines will be considered as wildlife crime.[1]

In other words, wildlife crime can be considered as a type of transnational crime that has no definite borders or boundaries to follow or to restrict and with the development of technology this type of crime is also witnessing its hype at an alarming rate.[3]

The main reason being this type of crime is to gain money as there are huge demands made for exotic animals, ivory and their products which are believed to be used as traditional medicines all these products are of billions of rupees and the networks are very difficult to detect easily that makes one of the major reasons of motivation for the offender to commit such kind of illegal activities that are spoiling or destroying the ecosystem and conservation work from many years.[4-5]

AIMS AND OBJECTIVES

To obtain data from the wildlife divisions of North Bengal in West Bengal through survey method and analyze the following:

- The wildlife crime rate of the division
- Illegal trades reported
- Cases under investigation/ investigated
- The precautionary measures taken and technologies used to prevent such crimes.

MATERIALS AND METHOD

To accomplish the study of the subjective, as stated before, fieldwork was conducted in the wildlife divisions of the North Bengal Forest department for twelve days from 1st September to 12th September 2022 at different divisional and range offices of Gorumara, Jaldapara and Darjeeling. Under the guidance of the Department of Forensic Medicine and Toxicology of Kasturba Medical College, MAHE, Manipal.

For the present study, various techniques were followed to collect the data which are as follows:

CENSUS SURVEY: Through this method, quantitative data on Wildlife crimes from wildlife divisions of the North Bengal Forest Department were collected that includes the number of cases reported, cases under investigation, number of people charge-sheeted, cases under trial and cases deposed and pending. All these data were collected from respected divisional forest offices with prior permission.

INTERVIEW: Our topic of study is "Wildlife Crime and Technologies Used for Prevention" We also used the interview method to collect data and various other information regarding the various measures taken to prevent various wildlife crimes such as hunting and illegal trades along with strategies and protocols followed by the officers and ground staffs for preventing the same.

CASE STUDY: Data were also collected through various case studies by having detailed discussions with officers of the divisional officer who share certain aspects of various cases which they have encountered.

DATA COLLECTED FROM SECONDARY SOURCES: Apart from the observation interview and case-study method, data were also collected through some secondary sources. Several books, journals and other governmental online portals were used to collect relevant information for the preparation of the dissertation.[6-9]

All the data collected were segregated into three divisions and analyzed.

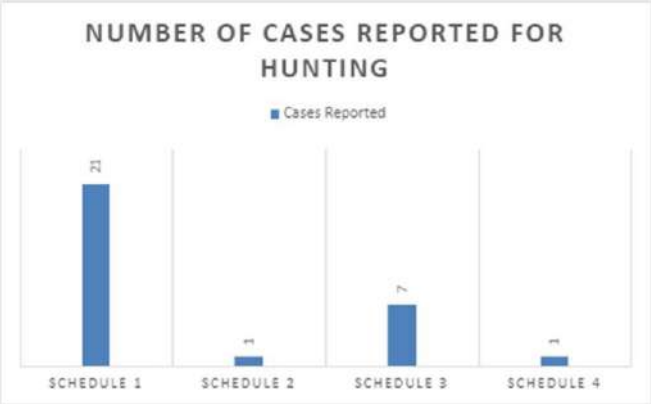
RESULTS

The data collected from the wildlife divisions of the North Bengal Forest Department covers the wildlife crimes that have been reported in the last ten years. The data required for the study has been collected from the divisional office of Gorumara, Jaldapara and Darjeeling Wildlife divisions. The study excludes illegal trading of forest products and mainly focuses on illegal trades, wildlife trafficking, and

hunting/poaching of various wild animals under the wildlife protection act 1972.

The above-mentioned figure (**Figure 1**) shows the number of cases that have been reported in the past ten years for the hunting of wild animals under different schedules that are mentioned in the wildlife protection act 1972.[10]

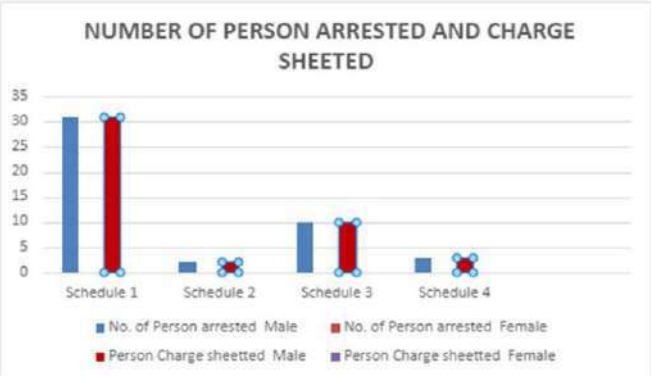
Figure 1: Number of Cases Reported for Hunting



In this figure 30 cases in total have been reported among which 21 cases were reported under schedule 1, only 1 case was reported under schedule 2, 7 cases were reported under schedule 3 and 1 case under schedule 4.

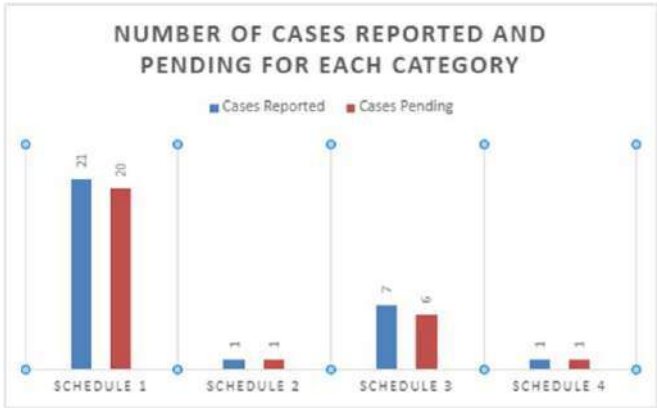
The above bar graph (**Figure 2**) shows the number of persons who have been arrested and charge-sheeted for hunting wild animals of different schedules thereby violating the laws that are mentioned in the Wildlife Protection Act 1972.[11] In the above graph, the number of male and female persons arrested under different schedules is mentioned. It shows that under Schedule 1 total of 31 males was arrested whereas under Schedule 2 only 2 male persons were arrested and charge-sheeted. The number of persons who were arrested under schedule 3 and 4 were 10 and 3 males respectively. As per the official reports received no female persons were arrested under hunting cases for the past ten years.

Figure 2: Number of Person Arrested and Charge Sheeted



The graph (**Figure 3**) shown above is the comparison between the number of cases reported and the cases that are still pending or in court trials. In Schedule 1 out of the total 21 cases that were reported among that 20 cases are pending or under trial. The same was observed in the case of schedule 3 where out of 7 cases that were reported 6 cases are still pending whereas in the case of schedule 2 and 4, both have pending or cases under trial.[12]

Figure 3: Number of Cases Reported and Pending for each Category.



According to Wildlife Protection Act 1972, illegal trading is defined as any kind of selling or exchanging of plant and wild animal products which includes both living and dead animals, prohibited by law. This study included the illegal trading associated only with wild animal products such as elephant tasks, snake skin, rhino horn, tiger skin, leopard skin and leopard bones. Out of these, there are also various other types of illegal trading of wild animal products such as pangolin scales, and red pandas which are also emerging as a serious concern.

As per the report received from the officials of the wildlife division of North Bengal, the graph above shows the cases reported and the cases that are pending court trial. In this bar chart (**Figure 4**), it is evident that illegal trading of Rhino horns is the major area of concern with 20 cases reported during the period of the last ten years out of which 14 cases are still under trial. Similarly, the bar chart shows 4 cases were reported for the elephant task with 1 case under trial whereas 2 cases were reported for tiger skin and leopard bones out of which 1 and 2 cases are still under trial respectively. The number of cases reported for leopard skin and snake skin were 2 and 1 respectively all of which are under trial.[13]

The above bar chart (**Figure 5**) shows the number of persons arrested and charge-sheeted in the illegal trading of elephant tusks, snake skin, turtle shells, rhino horn, tiger

Figure 4: Number of Case Reported and Pending for Illegal Trading.

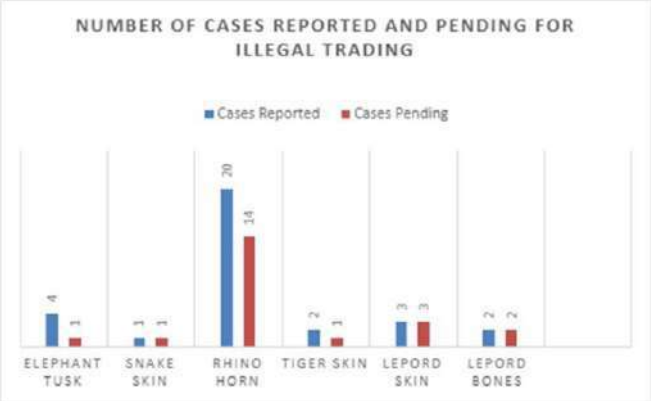
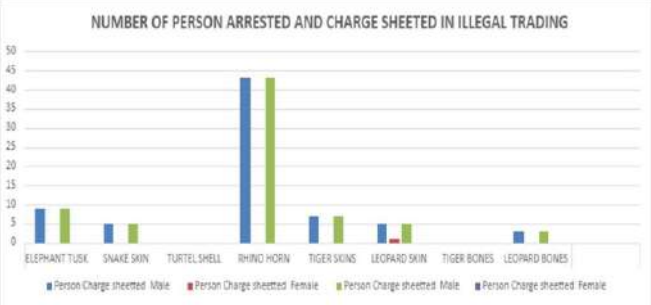


Figure 5: Number of Person Arrested and Charge Sheeted in Illegal Trading.



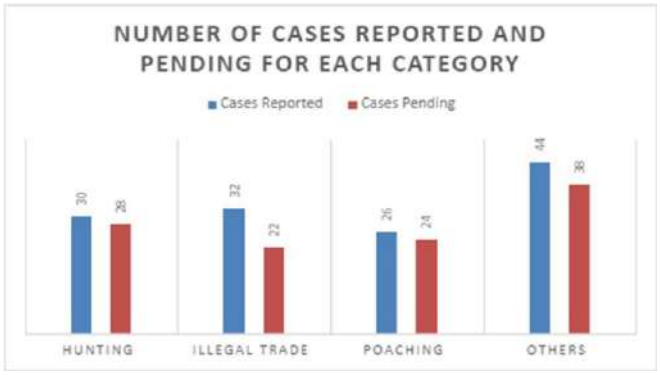
skin, leopard skin and leopard bones. As earlier stated, the illegal trading of rhino horns became a major concern due to its increase in numbers, a total number of 43 persons were arrested for the same whereas 9 persons were arrested for elephant task trading and 7 were arrested for tiger skins. For the illegal trading of snakeskin and leopard bones 5 and 3 persons were arrested whereas in the case of the illegal trading of leopard skin, a total number of 6 persons were arrested out of which one was a female offender.[14]

While comparing, (**Figure 6-7**) it has been found that 32 cases have been reported of illegal trading with 30 cases of hunting and 26 cases of poaching over the past 10 years in these three divisions. The bar chart also shows 44 cases related to some other types of cases that have been reported too in the same period. With 32 cases, illegal trading in these areas becomes a major reason for concern as this type of illegal trading of wild animals is not only a threat to the environment but also may lead to a serious threat to national security.[15-16]

Depending upon the cases reported in each category, the number of cases that are pending or under trial has been shown in the bar chart that tells out of 32 cases reported for illegal trading, 22 cases are pending similarly in cases of

Figure 6: Number of Cases Reported.

Figure 7: Number of Cases Reported and Pending for each Category.



hunting and poaching 28 and 24 cases are under trial concerning 30 and 26 cases reported respectively. This chart also gives an idea about the conviction rate in such types of crimes that are reported in the wildlife division of the northern part of West Bengal.[17]

DISCUSSION

The study done by Maneesha Mishra and Arpita Mitra on "Technology and wildlife crime: an appraisal in India" covered the overall wildlife crime scenario of India and how technological assistance is/ will become a boon for combating such crimes. Through the study, they gave certain recommendations on how the use of technologies will become beneficial to combat wildlife crime and concluded that India being a developing country till now had managed to develop various technical assistance and preventive measures to detect and prevent various kinds of wildlife crime which may lead to serious threat to national security.[5]

In India, there are 28 states and 8 union territories out of which West Bengal, situated on the eastern part of the country is considered to be one of the popular states and also home to various forests and wildlife. The wilderness of Bengal is home to various endangered species such as the Great one-horned Rhinoceros, Asian Elephant, Gaur, Red panda, Crocodile, the famous Royal Bengal tiger, Leopard,

Golden cat, Fishing cat etc.[18-19]

This study on **WILDLIFE CRIME AND TECHNOLOGIES USED FOR PREVENTION** covers the wildlife divisions of the northern part of the state that includes Gorumara division, Jaldapara division and Darjeeling division as the area of study to understand the type and range of wildlife crime reported in particular divisions, their conviction rates and what all preventive measures are been taken and challenges have been faced to combat such type of wildlife crimes.

GORUMARA WILDLIFE DIVISION

Gorumara is one of the famous national parks of North Bengal which is situated in the Terai region of the Himalayan foothills and is popular for Indian Rhinoceros (**Figure 7**). Gorumara was a reserve forest since 1895. It was declared a wildlife Sanctuary in 1949 and finally declared an Indian National Park on 31st January 1994. The total area of the Gorumara National Park is almost 80 sq kilometres with its extended part which is the Ramshai extension of about 351.5 sq km, Bamundenga extension and Tandus extension of about 320.48 and 175.01 sq km. Wild animals that are majorly found in this region are horn Rhinoceros, Indian elephant, Monkey, Leopard, Spotted dear, Barking dear, Hog deer, Sambhar, Indian goat and among the reptiles we can find King cobra, Rock python and Banded krait. As per the official report that has been received from the Divisional Forest office of Gorumara division, it has been observed that there is an increase in poaching cases of one-horned rhinos especially for their horns due to its increased demand in the international market especially in a country like China. Some of the poaching cases of one-horned rhinos that were reported in this division were – the rhino poaching case in the year 2014, in the year 2017 there were two cases reported on the same and the latest case was in the year 2018 poaching case.[20]

The main reason behind this demand and illegal trading of rhino horns is that the countries like Vietnam, China and several Western countries have a strong belief that rhino horns (**Figure 8.1** and **Figure 8.2**) can be used as a traditional medicine to cure any snake bites, headaches, hallucinations, vomiting, food poisoning and Devil possession etc.

Another major reason why horned Rhinoceros are under threat is that people in China and Western countries believe that the horns of rhinos can be used as an aphrodisiac is untrue and is misled by the Western media which led people to strongly believe in the same.[21]

Figure 8: Office of Range Officer, Gorumara South Range

Figure 8.1: Shows The Horns Of Rhinoceros

Figure 8.2: Shows The Horns Of Rhinoceros

As per the recent census done by the officials of Gorumara wildlife division in the year 2022, only 55 one-horned Rhinos are present in the Gorumara forest.

Along with one-horned rhinoceros, there are other animals and reptiles also which are under threat such as Leopard, Deer, Indian elephants and Snakes.

In the years 2020 and 2021 there were two dealing and detention cases where leopard skins were seized by the forest officials from the nearby market and tea garden areas.

In the year 2021, there was also a seizure of the skin of a barking deer which the forest officials after getting intel from their sources had found the skin of a barking deer inside a bag that had been hidden inside the forest. Along with this almost 11 spotted deer horns (**Figure 8.3**) were captured by the officers at Khuniya in the year 2020.[22]

Due to the increase in such types of poaching and illegal trades of these animals, it became very necessary to take

Figure 8.3: Spotted Deer Horns.

Figure 8.4: Checking of Vehicles Passing Between Gorumara Forest Area

Figure 8.4: Checking Of Vehicles Passing Between Gorumara Forest Area

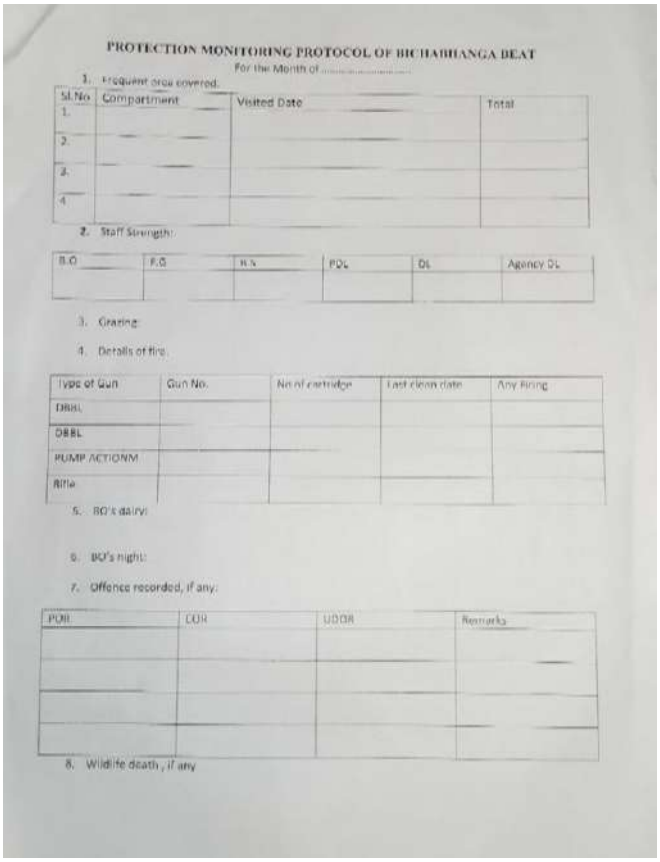
certain precautionary measures from the department side to protect and prevent such types of illegal activities. Therefore, several protective measures with the help of various technical assistance which are discussed below have been taken.

Preventive measures that are taken by the department are-

NAKA CHECKING- Naka checking is done at midnight or any time specifically at night time and checking of vehicles passing between the forest area is done. Naka checking is surprise checking done by the officials to check whether there is any suspicious activity happening inside or around the forest area and if while checking any such vehicles or person is caught doing any kind of activities that are prohibited under the Wildlife Protection Act 1972 then those are detained (**Figure 8.4**).

PMP (Protection Monitoring Protocol) meetings are held regularly in which reports are required to be submitted which are known to be the PMP forms that are strictly maintained and are submitted to the division office with GPS track record every month.[23] According to the report at the PMP meeting, a map is prepared to spot the area that requires more protection and accordingly

Figure 8.5: Shows The Format For Protection Monitoring Protocol



PROTECTION MONITORING PROTOCOL OF BICHABHANGA BEAT

For the Month of .....

1. Frequent area covered:

Sl.No	Compartment	Visited Date	Total
1.			
2.			
3.			
4.			

2. Staff Strength:

B.O	F.G	H.G	PDL	DL	Agency DL

3. Grazing:

4. Details of fire:

Type of Gun	Gun No.	No of cartridge	Last clean date	Any Firing
DBBL				
DBBL				
PUMP ACTIONM				
Rifle				

5. BO's dairy:

6. BO's night:

7. Offence recorded, if any:

POR	COR	UCOR	Remarks

8. Wildlife death , if any

necessary measures are taken (**Figure 8.5** and **Figure 8.6**).

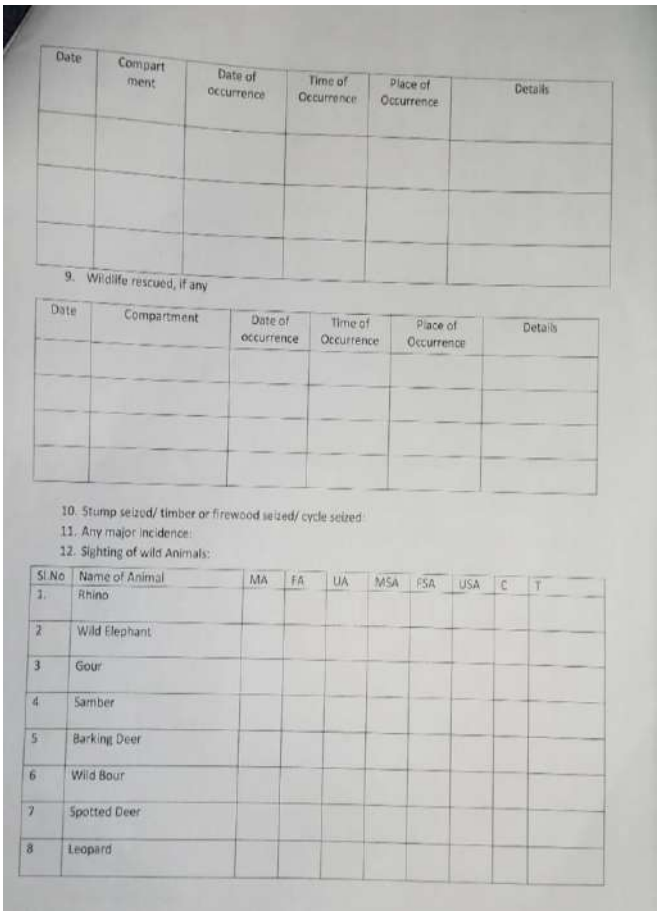
Scanning is done every month at all beats of the division thoroughly with many elephants for full patrolling of the forest and while doing the same special priority is given to one horn Rhinoceros by two shift monitoring done in the Gorumara national park (**Figure 8.7**).

Different names are also given to different Rhinoceros depending upon their features and behaviour, which are maintained and photos are pasted in the record book and checking whether the same animal is visible. If not visible for more than 7 days then the suspect increases.

While discussing all these measures that are taken by the division, the officers of the same division said that- "Preventive measures within the forest area are not enough and protection can be increased by interacting and building good relationships with the villagers and localities or local bodies".

To achieve these various other strategies are also made into action. Some of these strategies are the establishment of JFMC under poor villagers adjoining the national park

Figure 8.6: Shows the Format for Protection Monitoring Protocol



Date	Compartment	Date of occurrence	Time of Occurrence	Place of Occurrence	Details

9. Wildlife rescued, if any

Date	Compartment	Date of occurrence	Time of Occurrence	Place of Occurrence	Details

10. Stump seized/ timber or firewood seized/ cycle seized:
11. Any major incidence:
12. Sighting of wild Animals:

Sl.No	Name of Animal	MA	FA	UA	MSA	PSA	USA	C	T
1.	Rhino								
2	Wild Elephant								
3	Gour								
4	Samber								
5	Barking Deer								
6	Wild Bour								
7	Spotted Deer								
8	Leopard								

Figure 8.7: Scanning Done at Gorumara Forest.

are made involvement in various activities such as plantation, nursery preparation, cleaning, patrolling, and weeds removal for grazing land along with this wildlife management is properly maintained and villagers are made involved so that they do not get involved in any kind of illegal activities while providing them with monetary help, involving in eco-tourism, cultural activities and providing 40 per cent share given annually to the villagers for development.[24]

Along with all these preventive measures, the division also enhanced its techniques by taking the support of various

Figure 9: Office of the Divisional Forest Officer, Jaldapara Wildlife Division

Figure 9.1: Representational Image of Rhinoceros Poaching

Figure 10: West Range Office of Mahananda Wildlife Sanctuary, Darjeeling Wildlife Division, Sukna

modern technological assistance some of which are discussed below.

GPS TRACKER: As discussed above, this GPS tracker is used by the officials to keep a record of the animals that are present inside the forest and the same report of the record is submitted to the divisional office at the time of the PMT meeting. GPS Tracker is considered to be a part of smart patrolling for the detection and prevention of crimes. The tracker is used to track the path of the animals.[23]

TRAP CAMERA: Trap cameras are installed in various areas of the forest to monitor the movement of animals and illegal activities. A trap camera is a type of camera that gets triggered by itself if there is any activity near the area

where the camera is installed for example the presence of any human or animal with the help of a motion sensor.

IR CAMERAS and **DRONE MONITORING** is also used as a part of smart patrolling in this division.[25]

SENSORS are also been installed around the boundaries of the forest area to avoid Human-animal conflict which is also a major issue that is faced by the department as many times several incidents where wild animals sometimes enter the village area adjacent to the forest or the national park and destroys the field and houses of the villagers which not only affects the tribal habitat but also due to the conflict animals get harmed or injured. By installing sensors at the boundaries, the officials and the villagers get alert when any wild animal crosses the boundary of the forest.[23]

JALDAPARA WILDLIFE DIVISION

Jaldapara is situated in the Alipurduar District of the northern part of West Bengal near the banks of the Torsa River.[25] It was earlier known to be a reserve forest in 1895 in the British era (**Figure 9**) Later in the year 1949, the same was declared a wildlife sanctuary for its population of one-horned Rhinoceros. But as discussed above, one-horned Indian Rhinoceros are now becoming one of the endangered species therefore, the Jaldapara Wildlife Sanctuary which is not only considered to be one of the homes of horned Rhinoceros but also some other endangered species in the year 2014 gained the recognition of 'National Park' by the Union Forest and Environment Ministry to increase the protection of all the endangered species residing there.[17]

As per the data that were received from the Divisional Forest Office of Jaldapara it has been observed that for the past 10 years, there were almost 23 poaching cases that have been reported for which 31 persons were arrested having 21 cases still under trial with 1 conviction and 1 case has been disposed of. Along with poaching cases, there are also 19 cases of illegal trading of rhinoceros horns, 3 cases of elephant tusks and 2 cases of illegal trading of tiger and leopard skin along with 1 case reported for leopard bone. For the illegal trading of rhinoceros horns a total of 42 persons has been arrested with 13 cases that are still under trial with 5 conviction and 1 case has been disposed of which makes it one of the highest cases that are being reported and also the highest number of the person arrested for the same as compared to other illegal trading cases of elephant tusks, tiger and leopard bones and skin. Apart from all cases that have been mentioned above, various 44 other types of cases are reported in the division as per the official data.[18]

To prevent all these types of illegal activities various measures are taken by the department some of which are already been mentioned above. But the primary measures that the officials rely on are patrolling and checking regularly in and around the forest area. They also depend upon their informants to get definite information about any kind of suspicious activities taking place or any kind of illegal trading that is going to take place and depending upon that information further actions are been taken by the officials.

One of the recent cases that has been reported in this division is the poaching case of a one-horned Indian rhino.

The incident took place at the Chilapata forest range where the authorities have recovered 24 bullets, two riffles and 3 silencers which were allegedly used in the poaching incident. The body of the rhinoceros was recovered by the departmental staff deep inside the forest on 4th April and found that the horn of the dead animal was missing along with bullet marks on the body (**Figure 9.1**)

The officials arrested one person related to the case on 4th April 2021 and also arrested three persons from Manipur, the next day of the poaching had taken place.[26]

Such types of incidents that are taking place inside a National Park where patrolling takes place regularly and with the utmost sincerity, many a time rises a concern that more advanced preventive measures need to be incorporated so that such types of incidents can be avoided priorly and for that reason, various technological assistance will be a great help both in case of prevention and investigation of the same.

DARJEELING WILDLIFE DIVISION

Darjeeling Wildlife Division comprises two wildlife sanctuaries and one national park that are – Mahananda Wildlife Sanctuary, Senchal Wildlife Sanctuary and Singalia National Park. Mahananda Wildlife Sanctuary is situated in the Darjeeling district of West Bengal, between the Teesta and Mahananda rivers on the foothills of the Himalayas and comes under Darjeeling Wildlife Division (**Figure 10**). The sanctuary got its status in the year 1959, covering an area of about 159 kilometers per square to protect the famous Royal Bengal tiger and Indian bison which were also an endangered species. Some of the animals that are likely to be found in this area are the Himalayan Black Bear, Porcupine, Serow, clouded leopard, and Binturong (some

of the rarest animals). Some others are Indian Bison, elephant, chital, barking deer, sambar, rhesus monkey etc.[27-28]

Similarly, the Senchal Wildlife Sanctuary is one of the oldest wildlife sanctuaries in India covering an area of 38.6 kilometers square situated in the Darjeeling District of West Bengal. The animals that are found in this sanctuary are barking deer, wild boar, Himalayan black bear, leopard, rhesus monkey, Assam macaque and Himalayan flying squirrel.[29]

Singalia National Park, situated in the Darjeeling district was first declared as a wildlife sanctuary in the year 1986 and later in the year 1992 was declared a National Park. The national parks cover an area of about 78.6 km square. It has various kinds of mammals such as red panda, leopard, barking deer, pangolin, yellow-throated marten, wild boar, Himalayan black bear, clouded leopard, serow and takin. Tigers are also rarely found inside the national park.[29-30]

The data provided by the Darjeeling division regarding the wildlife crime rate of the division shows that for the past ten years, three cases of hunting have been reported under Schedule 1 and Schedule 3 including one case of elephant and two cases of Spotted deer in which a total number of 6 persons have been arrested and all the cases of hunting are under trial. Similarly, two cases of poaching were reported in the years 2021 and 2022 of wild boar and some other animals under Schedule 3 respectively. A total number of 3 persons were arrested for poaching cases and both the cases are under trial. No illegal trading cases were reported in the division.

SUMMARY AND CONCLUSION

In this era where technology has become an integral part of every individual's lifestyle, it also has both pros and cons i.e., technologies on the one hand can be used for the betterment of society and on the other hand the same technologies can be used by the criminals to commit any kind of illegal activities or crimes. Similarly, technology has a huge role to play in the field of wildlife in India. As discussed earlier, wildlife crimes such as poaching, and illegal trading of animal products have become a matter of huge concern for the country. The poachers and illegal traders of animal products also take the help of various technologies to succeed in their mission. On the other hand, the Wildlife Department of India is now also taking various initiatives to combat such types of crimes. Along with the traditional practice of patrolling by the departmental authorities at regular intervals, the wildlife division of the forest department also started using various technological tools to detect any kind of unusual activities in and around the protected or restricted areas. For example, in the wildlife divisions of the Northern part of West Bengal, devices like GPS Tracker, Trap Cameras, and IR Cameras are installed to have a continuous record of all activities going on inside the forest area. Whereas Sensors are also installed to avoid human-animal conflicts. Apart from all this Drone monitoring is also been introduced as a part of smart patrolling. While all these preventive measures are taken by the wildlife division but a lot of challenges are faced by the officials and more advanced preventive measures along with proper training for the staff should be provided.

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Original Research Paper

Blood pH As Indicator Of Time Since Death - A Postmortem Study

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ABSTRACT

Introduction: Time since death is one of the most important factors connected with the investigation of a case. Determination of the TSD (time since death) is one of the primary goals of medico-legal autopsies. Hospital records, witness statements, police - investigation reports, and other sources of information can all aid in determining the time of death, but it can be difficult to predict when such data is unavailable, particularly in cases of unwitnessed death. Estimating the time since death with blood pH is one solution which is critical in guiding law enforcement in establishing a timeline, determining the location of the victim's final action, and excluding or narrowing down the number of suspects involved.

Results: The study shows a highly significant negative correlation between time and the person's pH score. From the statistical analysis and from the graph, it is evident that there is a substantial decrease in the blood pH levels with the rise in the time since death.

Conclusions: The study comprised 20 cases brought to the hospital. Blood was analysed for pH levels using a meter. Results have been presented in comparison to findings obtained in other studies done on dead individuals. In this study, it is found that there is a highly significant negative correlation between time and the person's pH score.So, it can be used as a tool to estimate time of death such as from the crime scene as well as from the corpse which can be a valuable investigating tool.

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INTRODUCTION

One of the primary goals of medico-legal autopsies are to determine the TSD (time since death). Hospital records, witness statements, police - investigation reports, and other sources of information can all aid in determining the time of death, but it can be difficult to predict when such data is unavailable, particularly in cases of unwitnessed death. Estimating the time since death is critical in guiding law enforcement in establishing a timeline, determining the location of the victim's final action, and excluding or narrowing down the number of suspects involved.[1] Thanatochemistry; "chemistry of death" refers to the transformations that the human corpse's chemical composition undergoes as soon as death occurs. It was one of the primitive methods used to estimate the time of death because it depends on various intrinsic and extrinsic factors.

Hence biological fluids and their chemical constituents analysed by researchers. Besides these, various techniques used to determine PMI (Post-mortem interval) by using the immediate, early and late changes occurring post-mortem. But in the case of extensive putrefaction cases, it becomes unreliable and this will limit its usage because of extensive chemical changes like fermentation and sulphation. Biochemical changes in the early postmortem period begin around 3 hours after death and can last up to 72 hours.[2] The first biochemical change that occurs soon after the post-mortem is a decrease in the concentration of oxygen due to the absence of circulation, which results in anaerobic metabolism in the absence of the citric acid cycle. At the same time, anaerobic glycolysis takes place. This causes lactic acid to build up and the concentration of NADH to rise. The level of formic acid will also increase. The pH drops postmortem mostly due to the formation of lactic and formic acids, followed by cell death and subsequent changes.

Several methods have been proposed for determining the time since death, but none is shown to be specific and

reliable. Furthermore, regardless of the fact that biochemical changes have been used to establish time since death in medicolegal cases, it has yet to be evaluated for their reliability to be used as prospective evidence in a court of law.[3,19,25] Various bodily fluids have been researched in this field such as blood, pericardial fluid, Cerebrospinal fluid, vitreous humour, etc. Even though studies conducted on different bodily fluids, always blood remains the preferred medium for analysis of time since death, despite the fact that it is the most difficult method for analysis as many analytes in blood show, various degrees of post-mortem changes.[4-5]

As per earlier studies, many studies carried out on cardiac blood,[9-10] femoral blood, liver acidity,[8] the estimation of time since death by the age of fly pupae.[11] post-mortem drug concentrations[12-14] degradation of DNA in tissues of drowned rats,[15] decomposition in exposed cadavers,[16] vitreous potassium level,[17,20,23] thanatochemistry,[18] the potential use of fungi community,[21] entomology-based methods,[13,22,26] histopathologic evaluation of post-mortem autolytic changes,[24] Cerebrospinal fluid,[33] etc. In that, all showed some correlation with postmortem time interval, but they depend upon various factors, including age, sex, gender, environmental temperature, seasonal variation, cause of death and co-morbidities of the deceased.[6-7]

Blood pH as a source to estimate the time since a death has been researched extensively in the past, and it has been demonstrated that the value falls as the post-mortem time gap increases. As a result, an attempt made by carrying out a study on femoral blood pH levels with time intervals to determine the PMI and hence time since death was estimated.

MATERIALS AND METHOD

This was a comparative Analytical study. Purposive sampling was carried out. Brought death cases, where the time of death is not known were excluded from this study. Only the cases of Possible death cases from the hospital, where the time of death is known with the exact time of death given by law enforcement agencies were included in the study. Femoral blood samples were collected from 20 corpses. These samples were collected at the hospital soon after the death occur.

Femoral blood was collected in a standard sampling tube (red vacutainer) using a 2ml plastic disposable syringe from each subject and was preserved. On average, approximately 14ml of femoral blood was collected in 7 tubes. The vials were kept at the average room temperature without adding any anticoagulants. A detailed proforma was filled out for each case to collect the relevant information such as name, age, sex, cause of death, exact death time, sampling time, and the respective pH value.

At the completion of the sampling, the samples were taken for laboratory analysis by using a pH meter. The blood pH level was checked at an interval of 12 hours for 3 days (0 hours,12 hours,24 hours,36 hours, 48 hours, 60 hours, and 72 hours) in 7 vials, with the help of a pH meter. The pH meter automatically calculates the pH of each blood sample.

RESULTS

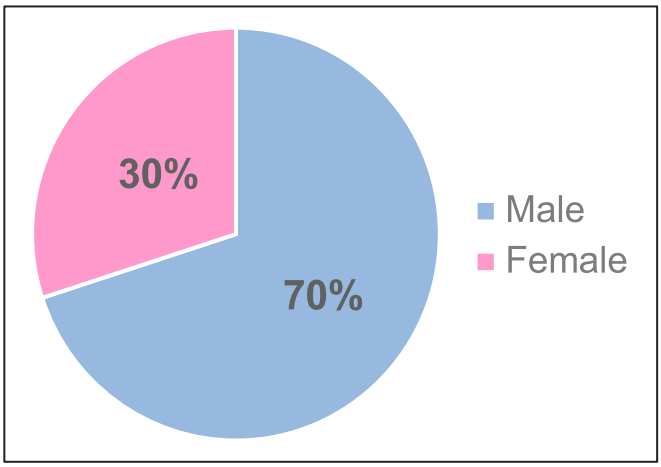
The collected data was analysed using the IBM SPSS statistical software. Regression and correlation analysis was used. P- value ≤ 0.05 was considered statistically significant. Mean, SD (Standard Deviation), frequency and percentages were calculated.

The results are presented in tables and graphs. Then a scatter plot was utilized to depict the relationship between PMI and pH value of femoral blood. The study shows a highly significant negative correlation between time and the person's pH score. The linear regression equation was obtained from the pH value of blood versus time since death as,

pH = 6.863 - 0.005 * time

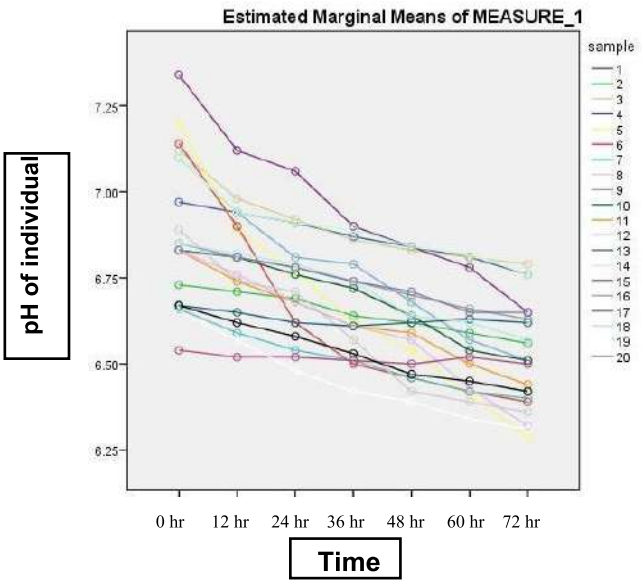
Data analysis revealed that majority of cases were males 70 % and females were only 30%.

Diagram 1: Distribution of gender



From the statistical analysis and from the graph, it is evident that there is a substantial decrease in the blood pH levels with the rise in the time since death. The mean Pearson correlation of pH with time was found to be -0.992.

Graph 1: The pH of individual vs time



Graph 2: The Mean pH of individual vs Time

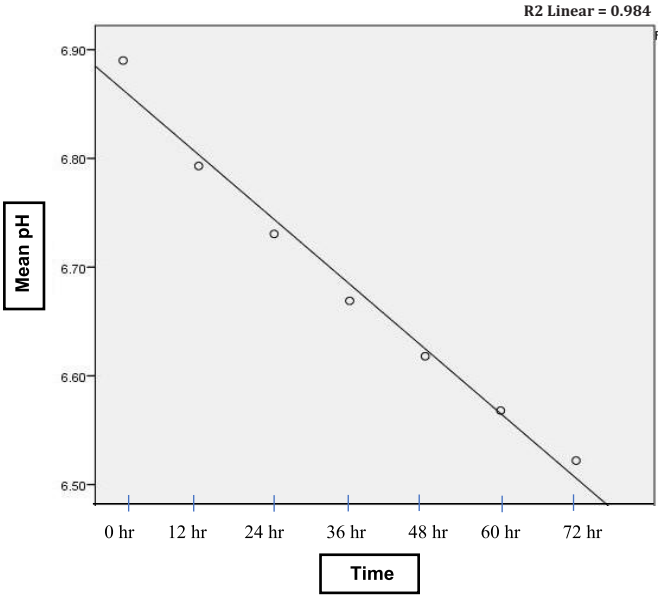


Table 1: Patient's Pearson Correlation of pH with Time

Sample	Pearson Correlation of pH with Time
1	-0.997
2	-0.994
3	-0.938
4	-0.982
5	-0.981
6	-0.919
7	-0.990
8	-0.978
9	-0.987
10	-0.982
11	-0.994
12	-0.989
13	-0.693
14	-0.942
15	-0.773
16	-0.999
17	-0.985
18	-0.981
19	-0.987
20	-0.997

DISCUSSION

In Forensic Medicine, determining the time of death is essential since it may make a significant contribution to criminal investigations. One of the most crucial forensic problems is figuring out when someone died since so many endogenous and environmental variables may have a big impact on how the body decomposes. Hence to determine the time since death is a challenge faced by forensic pathologists.

The purpose of this study was to find any correlation between the blood pH level and the time since death from the cases brought to the hospital. The known time of death was noted as stated in the police inquest form or records in the hospital and the change in the blood ph level was observed with the help of a ph meter. Previous studies show a negative correlation between blood pH and time since death. In the study conducted by William R. Sawyer,[9] et al, in 1998 to determine PMI in rats with cardiac blood, the cardiac blood pH was considerably lower. They also conducted a pilot investigation with 11 human patients, finding a substantial negative connection between

Table 2: Coefficient

Model	Unstandardized Coefficient		Standard Coefficient	t	Sig.	95.0% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
(Constant)	6.863	-0.012		567.881	0.000	6.832	6.894
Time	-0.005	-0.000	-0.992	-17.757	0.000	-0.006	-0.004

postmortem interval and cardiac blood pH.

Our study conducted by taking blood from cadavers immediately after death and preserving the blood in the red vial at room temperature. There can be a difference in values obtained if the blood collected at '0' hours is stored and analysed at different intervals when compared to a sample of blood collected from the corpse at a different interval after death.

The main differences in blood pH between when it is kept outside and when analysed just after collecting from the dead body at a particular time are; First, there is no glucose reserve in blood to provide energy for anaerobic metabolism in a test tube, therefore no considerable lactate accumulation occurs to reduce blood pH. Second, autolysis does not occur in erythrocytes (red blood cells) because they lack lysosomes, which prevents the production of acidic cellular metabolites, including carbon dioxide, hydrogen ions, formic acid, and lactic acid from lysing cells to significantly lower pH as it would happen in a corpse. Gradual cell lysis occurs in the blood stored in tubes because cell membrane proteins are not maintained or regenerated. Hence a slow decrease is seen in blood pH in tubes compared to blood from a corpse.[19,27]

The most noticeable biochemical change that takes place right away following the post-mortem is an increase in anaerobic metabolism due to the absence of the citric acid cycle as a result of a reduction in oxygen concentration brought on by the lack of circulation. Anaerobic glycolysis occurs at the same time, causing lactic acid to build up and the concentration of NADH to rise.

Formic acid concentrations will rise as well. The pH decline after death is mostly caused by the build-up of lactic and formic acids, followed by cell death19. In our study, we took 20 samples and analysed them to estimate the time of death of persons. A general linear regression was the method used to analyse the data collected. As per the information we collected from the previous study, it is very true that there is a significant negative linear correlation between blood pH and the time interval.

The study since it showed a negative linear correlation between blood pH and time interval, which can be used by the pathologist as a very handy method in determining the time since death. The range of blood pH obtained by analysing our samples can be used to predict the range of time since death with an accuracy of a few hours. So, we strongly believe that the pH obtained from the blood collected from a corpse can be contributory data, which can aid in the determination of time since death.

Similarly, pH from the blood obtained at a crime scene can suggest the time since deposition which can provide very valuable clues about the offender or victim, thereby helping the enforcement authorities in solving the crime. But it is also necessary to note that the abovementioned conditions can affect the blood pH of one and that is one of the limitations of this study.

Also, there are many factors which will contribute to estimating the time since death such as the cause of death, the age of the person, sex of the person etc. A good number of sample sizes which include these parameters can give some more clarity to this analysis. We consider the sample with only one exclusion criteria that brought dead cases are not taken and hence the results even though showing a significant negative regression, its accuracy is still in the border.

CONCLUSIONS

The study comprised 20 cases brought to the hospital. Blood was analysed for ph levels using a meter. Results have been presented in comparison to findings obtained in other studies done on dead individuals. In this study, it is found that there is a highly significant negative correlation between time and the person's pH score.

To conclude our study, it was evident that the blood pH level decreases with an increase in the postmortem time interval. So, it can be used as a tool to estimate time of death such as from the crime scene as well as from the corpse which can be a valuable investigating tool.

List of Abbreviation

Abbreviation	Full Form
PMI	Post - Mortem Interva
DNA	Deoxyribonucleic Acid
ATP	Adenosine Triphosphate
TSD	Time Since Death
CSF	Cerebrospinal Fluid
LDS	Lactate Dehydrogenase
ADP	Adenosine Diphosphate
TBS	Total Body Score
SD	Standard Deviation

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Original Research Paper

Correlation of Time Since Death and Cause of Death with Blood Glucose and Insulin Level in Postmortem Cases

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ABSTRACT

Introduction: Estimating the time since death is one of the prime objectives of conducting medico-legal autopsies. Biochemical changes commence with the early postmortem changes which starts as early as around 3 hours post-mortem and last upto 72 hours. Blood glucose has been extensively studied in the past, and it has been shown that the value decreases with increase in postmortem time interval (PMI), but the values were too erratic and with not much of forensic value. Insulin, on the other hand, has shown significant correlation. Therefore, an attempt has been made by carrying out a study of blood glucose and insulin level and to see their correlation with cause of death and time since death.

Aims and Objective: The aim of this study was to analyse the quantitative changes of postmortem blood glucose and insulin levels independently and together and correlate it with time since death and the causes of death in brought dead cases.

Materials and methods: The study constituted of 76 brought dead cases from 2018 to 2021, brought to the mortuary of Kasturba Medical College, MAHE, Manipal, where the time of death was known and was not hospitalised earlier. Blood sample was collected at the time of autopsy and sent to Biochemistry lab for estimating blood glucose and insulin level, using Hexokinase Enzymatic reference method [Roche Cobas 6000 (c 501), Cobas 8000 (c702/c502)] and electrochemiluminescence immunoassay method [Roche Cobas 6000 (e601), Cobas 8000 (e602)] respectively.

Results: Correlation between postmortem blood glucose and insulin level, with time since death and cause of death were analysed. We found that there is moderate negative correlation between blood glucose level and time since death and a weak negative correlation between PMI with insulin, but the correlation is not significant ($p>0.05$). In our study, where the majority of cases consisted of traumatic head injury, myocardial infarction and asphyxia, we found that there is significant difference in distribution of insulin among different causes of death with p value less than 0.05.

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INTRODUCTION

The time of death can be known from hospital records, from witness statements, police investigation reports, etcetera, but at times it is difficult to predict when such records are not available, especially in cases of un-witnessed deaths. Estimating the time since death is essential to help the law enforcers to establish a timeline, whereabouts as to the last activity of the victim and also help to rule out or to narrow down the number of suspects involved.[1]

Various methods have been experimented to arrive at the time of death, exploiting the immediate, early and late changes that occur postmortem, and one of them being the use of biological fluids and their chemical constituents i.e., Thanato-chemistry, which is a crude method owing to the fact that it is dependent on various intrinsic as well as extrinsic factors. It becomes increasingly less reliable in cases with advanced putrefaction, making their use limited in such cases. Biochemical changes commence with the early postmortem changes which starts as early as around 3 hours postmortem and last upto 72 hour.[2] Early postmortem interval (PMI) commences with cell death and subsequent changes that occurs which is exhibited as

elevated or depressed levels of different biochemical markers. Several methods have been proposed to estimate the time since death, but none of them proved to be of any practical relevance. And furthermore, inspite of all the research available, it has not been so far screened for its reliability so as to use in the court of law as potential evidence, though it has been used for establishing time since death in medico-legal cases.[3]

As per earlier studies carried out on serum potassium, sodium, chloride, calcium, phosphatases, lactate etc showed some correlation with postmortem time interval, but they depend upon various factors including age, sex, gender, environmental temperature, seasonal variation, cause of death and co-morbidities of the deceased. Blood glucose has been extensively studied in the past, and it has been shown that the value decreases with increase in postmortem time interval, but the values were too erratic and with not much of forensic value. Insulin, on the other hand, has shown significant correlation.[2-4]

Meurs et al,[3] 2 in 2019 investigated the significance of 388 postmortem biochemical markers (PBM) in various body fluids, based on the literature available and scored it according to Daubert and Frye criteria, ranging from 0 that is not investigated to 17 that is well investigated. The highest score was of 12 for potassium. Apart from they also found that sodium, urea, chloride, magnesium, hypoxanthine and cardiac troponin T were sufficiently investigated. 6 other biomarkers were found to be suitably researched but they were not found suitable for use practically. 18 other markers were poorly investigated and rest 364 of them did not have enough information. In their study glucose was scored at 8 and insulin at 3. Glucose in vitreous humor, cerebrospinal fluid and pericardial fluid was found to be not significant. Blood insulin was found to be significant upto 24 hours postmortem.

Majority of the previous studies in the literature has studied the correlation of insulin and glucose with PMI independently. Therefore, an attempt was made by carrying out a study of blood glucose and insulin level together and to see their correlation with time since death.

MATERIALS AND METHODS

A prospective study to correlate the post-mortem time interval by analysis of blood glucose and insulin levels were carried out on bodies brought for medico-legal post-mortem examination conducted in the mortuary of Kasturba Medical College, MAHE, Manipal, between 2018 and 2021, where 76 cases were studied, after institutional ethical clearance. Only sudden death cases which are brought dead, with no prior hospitalization and whose time of death is known were included in the study. Decomposed bodies were excluded.

Following details of the deceased in each case were obtained from police inquest papers, brought dead intimation obtained from hospital, interviewing relatives, first responders and police.

1. Age, sex, gender
2. Previous medical or surgical history
3. Time of death, time interval, place of death
4. Preservation in cold chamber

After selecting the cases for study, postmortem examination was performed. After external examination of the body, blood was collected using a 5ml plastic disposable syringe with a wide bore cannula (24 gauge) from either of the following sites – femoral vein (blood drawn at 1cm medial to the mid-point of inguinal ligament between pubic symphysis and anterior superior iliac spine), external jugular vein (blood drawn at midpoint between angle of mandible and midclavicular line) or inferior vena cava directly at the lumbar region, before the beginning of dissection in each case. 2ml and 3ml of blood was taken in EDTA sodium fluoride tube and standard sampling tube and sent to Biochemistry Lab, Kasturba Medical College, Manipal, for glucose using Hexokinase Enzymatic reference method and insulin estimation using Elecsys Insulin assay method (electrochemiluminescence immunoassay method) respectively.

The specimens were immediately analysed after collection. If any delay is expected then the sample can be stored at 2-8^0 C for 24 hours, where it will be stable. Reagents for the determination of the same were obtained from Roche Diagnostics.

Limits and ranges: The glucose concentration in plasma is measured in the range of 0.11–41.6 mmol/L (2–750 mg/dL).0.11 mmol/L (2 mg/dL) was the lowest detection limit. The measurement range for the insulin present in the serum: 0.200–1000 µU/mL or 1.39–6945 pmol/L. Lowest detection limit: 0.200 µU/mL (1.39 pmol/L).

RESULTS

Out of the 76 cases studied ranging from 13 years to 85 years, 68 were males and 8 were females (as shown in **Table 1)**. The mean age of study participants were 47.07 years with a SD (Standard deviation) of 16.608 years.

Among the brought dead cases, 46 cases had no significant

Table I: Distribution According to Age and Gender of Participants

S. No.	Age (in years)	Gender	
1	11-20	Male	2
		Female	1
2	21-30	Male	9
		Female	0
3	31-40	Male	20
		Female	2
4	41-50	Male	10
		Female	1
5	51-60	Male	15
		Female	0
6	61-70	Male	9
		Female	2
7	71-80	Male	2
		Female	1
8	>81 Years	Male	1
		Female	1

past history, while 10 out of them had known history of alcohol consumption, 7 of them were known case of coronary artery disease and 3 cases with known case of diabetes mellitus, as shown in **Table 2**.

Out of 76 cases, 64 cases were preserved in mortuary cold chamber prior to collection of blood sample, as shown in **Table 3**.

Table 2: Frequency and Percentage of Past History of Deceased

Past History	Number of cases	%
Alcohol Consumption	10	13.2
Coronary Artery Disease	7	9.2
Bronchial Asthma	2	2.6
Liver Disease	2	2.6
Migraine	2	2.6
Hypertension, Diabetes Mellitus"	2	2.6
Diabetes Mellitus	1	1.3
Hypertension	1	1.3
Seizure Disorder	1	1.3
Pregnant	1	1.3
Pulmonary Tuberculosis	1	1.3
No Significant Past History	46	60.5
Total	76	100

Table 3: Showing Cases Preserved In Cold Chamber

Cold Storage	Frequency	Percentage
Yes	64	84.2
No	12	15.8
Total	76	100

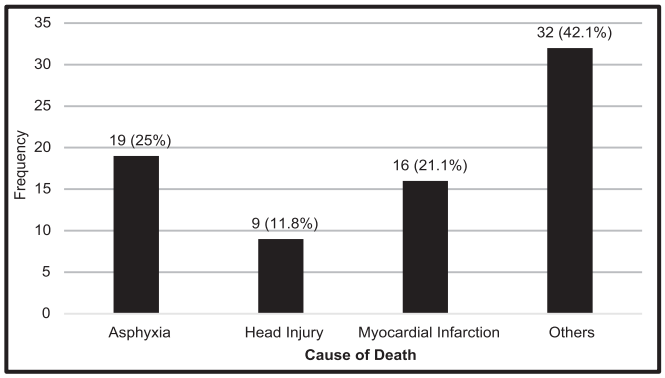
Distribution of glucose and insulin levels: The distribution of glucose level in the study participants ranges from 2 to 552 mg/dl. The mean glucose level of study participants was 89.82 mg/dl with a SD of 130.547 mg/dl. The distribution of insulin level in the study participants ranges from 0.2 to 429 µU/mL. The mean insulin level of study participants was 8.982 µU/mL with a SD of 49.172 µU/mL. (as shown in **Table 4**)

Table No. 4 Distribution According to PMI

PMI	Value (in minutes)
Minimum	30.000
Maximum	3030.000
Mean	916.510
Standard deviation	564.514

Out of 76 cases, in 19 cases the causes of death were due to asphyxia as a result of hanging,"16 cases due to myocardial infarction, 9 due to head injury, and others consisting of varying causes of death such as electrocution, organophosphorus and carbon monoxide poisoning, spinal cord injury, haemorrhage secondary to major artery transection, liver disease and other natural diseases, as depicted in **Diagram 1**.

Diagram 1: Distribution of cases as per cause of death



Correlation between PMI with glucose and insulin: In this study it is found that there is weak negative correlation between PMI with glucose and insulin and the correlation is not significant (p>0.05), as shown in **Table 5**.

Table No. 5: Correlation Between PMI with Glucose and Insulin

Variable	Pearson Correlation	p-value
Glucose	-0.128	0.271
Insulin	-0.147	0.206

Comparison of glucose and insulin among cold storage, history of alcohol intake and cause of death: Comparison of glucose and insulin among cold storage and history of alcohol intake was done using Mann-Whitney U test. It is found that there is no significant difference in distribution

of glucose and insulin among cold storage (p>0.05). There is significant difference found in insulin among history of alcohol (p<0.05), but the difference is not significant among glucose and history of alcohol (p>0.05). Comparison of glucose and insulin among cause of death was done using Kruskal-Wallis test. It is found that there is no significant difference in distribution of glucose among different causes of death (p>0.05), but there is significant difference in distribution of insulin among different causes of death (p<0.05). (as shown in **Table 6**)

Table 6: Comparison of Glucose and Insulin Among Cold Storage, History of Alcohol Intake and Cause of Death

Variables	p-value
Glucose and Cold Storage	0.441
Insulin and Cold Storage	0.402
Glucose and History of Alcohol	0.180
Insulin and History of Alcohol	**0.005**
Glucose and Cause of Death	0.309
Insulin and Cause of Death	

DISCUSSION

Postmortem blood Glucose : Previous studies conducted by various investigators state that blood glucose level tends to fall post-mortem with increasing time interval, and this is explained by the fact it happens due to continuing glycolysis by the surviving cells8.

In our study we found that there is a weak negative correlation between blood glucose level and time since death, which was not significant. The rate of fall could not be assessed since only one sample was collected from each case. The study conducted by Dhiraj Trivedi and K Sankara Narayana[9] was partly in accordance with our study, that there was moderate negative correlation between blood glucose level and time since death, but it did not show any specific pattern of fall.

We observed in our study that there is no significant difference in distribution of glucose among different causes of death. Observations were made by Tonge10 and later by Hill,[11] showed there is correlation between glucose level and certain cause of death. Postmortem hyperglycemia was observed in cases of asphyxia, carbon-monoxide poisoning, increased intracranial pressure, coronary artery disease and electrocution. However, we observed elevated levels of glucose in some cases where cause of death was due to asphyxia, myocardial infarction and head injury, but the findings were isolated. This could be due to the fact that the samples collected were collected across varying time interval and not immediately after death and hence needs to be further explored with a fixed time interval.

Postmortem blood Insulin: It was determined by earlier investigations that there is still uncertainty surrounding the interpretation of insulin levels from postmortem bodily fluid samples, and that it has low diagnostic value because of ongoing autolysis and putrefaction processes.[12]

In our study we observed that the insulin levels were not corresponding to the glucose levels. Muschoff et al[13] suggested that blood insulin level will be corresponding to that of blood glucose level. Though that holds true in an apparently healthy individual during life, however after death it is not the case, as observed by our study, due to the reason that during life insulin is produced in response to blood glucose level, while after death the level is altered with postmortem changes that occur.

Hess et al[14] suggested that within 48 hours of death, total insulin degradation occurs. Sachil Kumar and Anoop K Verma4 suggested that the insulin level fall below the limit of detection by 27 hours postmortem. However, in our study all samples, except 5 cases, were collected within 27 hours. Out of the 5 cases, in 3 cases insulin values were 0.2 µU/mL, which is the lowest detectable range, while in rest two cases insulin level were 4.68 µU/mL and 14.48 µU/mL at 29 hours 5 minutes and 50 hour 30 minutes postmortem respectively.

In our study we found that there is weak negative correlation between PMI with insulin but the correlation is not significant (p>0.05). This was partly in consistent with the study conducted by Sachil Kumar and Anoop K Verma4 where the insulin level correlated with postmortem time interval with high significance with p value<0.001. The weak correlation in our study maybe due to the fact that the samples were not collected at fixed time interval and collected at varying time intervals, giving weak correlation.

In our study, where the majority of cases were due to traumatic head injury, myocardial infarction and asphyxia, we found that there was a significant difference in distribution of insulin among different causes of death with p value less than 0.05. However, we could not find other studies correlating between cause of death and insulin level, apart from cases studied for raised blood insulin level in cases where death is caused by insulin overdose.[7,15]

In the current study there were 10 cases where there was

history of alcohol intake prior to death, whose time since death were distributed between 5 hours 30 minutes to 21 hours postmortem, and their postmortem blood level varied from least detectable value, that is 0.2 µU/mL to 1.2 µU/mL, showing significant difference found in insulin among history of alcohol intake with p value less than 0.05. However further studies are required in this regard for a more definite conclusion.

CONCLUSION

To conclude from our study, it was evident that blood glucose and blood insulin level decreases with increase in postmortem time interval, however it only had a weak negative corelation. We also found out that the postmortem peripheral blood insulin value at a given period of time is greatly influenced by the cause of death of the individual and also intake of alcohol prior to death. Further studies with larger sample size considering the postmortem time interval, cause of death, history of alcohol intake, and other factors can be undertaken. However, from a practical point of view, it is obvious from the studies that the blood glucose and insulin level prior to death of the individual has to be known and the rate of fall till the time of collection of postmortem samples can be used to estimate the time since death.

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Original Research Paper

Comparison of Left and Right Handwriting of Ambidextrous Writers

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ABSTRACT

Introduction: Ambidextrous writers are very rare in a population and comprises less than 1% ambidextrous people in the world. Handwriting is one of the significant parameters, which can aid in identifying the handedness of a person. Since there has not been much research done on the handwriting of ambidextrous writers, more of the studies are required to explore this aspect of handwriting examination in forensic science.

Material and Methods: Present descriptive study was done on the ambidextrous writers who can efficiently write with both the hands. The current study is a pilot study to compare the handwriting samples of ambidextrous writers. The handwriting samples from left and right hand were collected from ambidextrous writers. Quantitative data for slant, alignment, size of letters, spacing was extracted from the handwriting samples with the help of Digimizer software, online protractor and ruler. The data analysis was done using SPSS 23.

Result: There is no significant variations in size, alignment, spacing and slant of left and right handwriting of Ambidextrous writers.

Conclusion: Based on the analysis, the study could not find significant variation between the parameters of handwriting from left and right hand of ambidextrous writers. The extended study can be helpful to the investigators when they are collecting the requested handwriting samples and hence to the forensic document examiners when they are examining the handwriting.

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INTRODUCTION

Handedness identification in forensic handwriting analysis could play a pivotal role in classifying a writer of the suspected text. Handedness is the hand preference in performing a task. A writer can be right-handed, left-handed or ambidextrous and their percentage in a population is approx. 90 % for right handed and rest 10 % for left hand preference or ambidextrous, ambidextrous writers being less than 1%.[1] Since the left handed and ambidextrous people are less in number hence, their handwriting must be studied for forensic analysis. If a handwriting examiner can classify the writer into left handed or right-handed based on handwriting features, the investigation would become easier for the investigating officers.[2-3] Some handwriting characteristics like right to left horizontal strokes, clockwise direction of strokes in rounded letters etc. are attributed to left hand writers and vice versa for right hand writers.[4] Ambidextrous writers are people who can equally write with both hands and their handwriting features must be studied. Handedness has been studied for the cognitive aspects in psychology or neurosciences and less in forensic handwriting analysis. Previous research concentrated on the left and right handed participants in handwriting analysis especially the disguised writing for handedness determination.[5] There are not much literature related to ambidextrous writers in general and forensic science in particular. Few of the some recent studies have been done for ambidextrous writers. In one of the studies, the authors studied the handwriting features of ambidextrous writers using image processing. The study focussed on CNN based classifiers to extract handwriting features from the images of handwriting.[6] But such types of image processing and various classifiers are yet to reach to forensic experts and

more of the work need to be done for their infallible induction into the handwriting examination. In another study, the authors worked on handwriting of ambidextrous writers of Veena Vadini School and studied few handwriting features. They found no fundamental differences among the handwriting of both the hands of ambidextrous writers.[7]

Some previous studies considered the training of non-dominant hand of writers to understand the difference or similarity in handwriting of the two hands. Participants in one study were trained for around a month and the progress and handwriting of non-dominant hand was compared with dominant hand based on parameters like slant, spacing, size, legibility etc.[8] Dziedzic in 2011 examined the development of left handed writing features of a right handed writer by providing training in writing to his left hand for a period of 3 years from 2007-2010. The legibility, speed, line quality, letters zones, inverted elements, horizontal strokes etc. were analyzed and it was found that the effectively trained hand could be used to produce a good disguise.[9] It can also produce significant differences in certain parameters like speed when written from the non-dominant hand.[1] Though there could be improvement in handwriting, especially legibility, if practiced from a non- dominant hand over a period, it is not necessary that the person will completely shift the hand preference ''.[10]

Huber & Headrick in their book 'Handwriting Identification: Facts and Fundamentals' mentioned ambidexterity as the earlier sinisters / left handed writers when converted to right hand writers and suggested no significant differences in their handwriting except the fluency and movements.[11] However, some studies specifically considered the ambidextrous writers who can write with both the hands with practice and found no significant differences in the handwriting samples of two writings from the same person. Major characters taken into consideration were h, m, n for size and shape.[7] With the development of technologies like image processing, artificial intelligence & machine learning, the computer systems are trained to identify or classify the handwriting into various demographic groups like sex, handedness etc. giving automatic handwriting identification.[1,4] Nonetheless, handwriting of ambidextrous writers should be explored more.

The main aim of the study is to find any significant variation in handwriting from left and right hand of ambidextrous writers. This would help in understanding role of handedness in handwriting analysis can also aid the investigating officers in collecting requested/specimen handwriting samples from suspects. Requested handwriting samples from both the hands of suspects can be taken in case of purported disguise. If the dominant hand of suspect is not fit for writing, then in such cases he/she can give handwriting samples from other hand. Therefore, it is important to understand the handwriting features of ambidextrous writers. However, despite of less population of ambidextrous writers, few schools in India train their students to adopt handwriting from both the hands. Such schools are Veena Vadini School, Madhya Pradesh[12] and Shriram Kannada Convent School, Kapnoor, Kalaburagi, Karnataka.[13] For the current study, participants from Shriram Kannada Convent School were considered. For this study, the null hypothesis was that 'there is no significant variation between the handwritings of the left hand and right hand of ambidextrous writer' and alternative hypothesis vice-versa. The hypothesis was tested using statistical tests in SPSS 23 as explained in further sections.

MATERIAL AND METHODS

Present quantitative study with descriptive study design was carried out as a pilot study on ambidextrous writers from November 2022 - January 2023 on 30 participants (Male and Female) (60 handwriting samples). The total characters available for analysis were 148. The participants were the students of Shriram Kannada Convent School, Karnataka as these students are being taught to write with both the hands and are ambidextrous writers. To obtain their handwritings, participants were asked to copy a paragraph on a plain white sheet. The paragraph given to copy included 'London Letters' (which consists of all the roman alphabets) and numerals 0 to 9. The participants were asked to sit in comfortable position and copy the text in paragraph with their left and right hand.

The handwriting samples were scanned to extract data. The data for slant, alignment, size, spacing were extracted using tools like 'Digimizer', an Image Analysis Software, online ruler and online protractor[14-16] Digimizer allows manual as well as automatic measurements of object characteristics from the Image as shown in **Figure I**. The scanned image was uploaded in the software and various tools available in digimizer allowed easy measurements. The measurements are shown in **Figure I**, **II, III**. The data collected were transferred in MS Excel.

Figure I: Working Window of Digimizer Software for Angle Measurement.

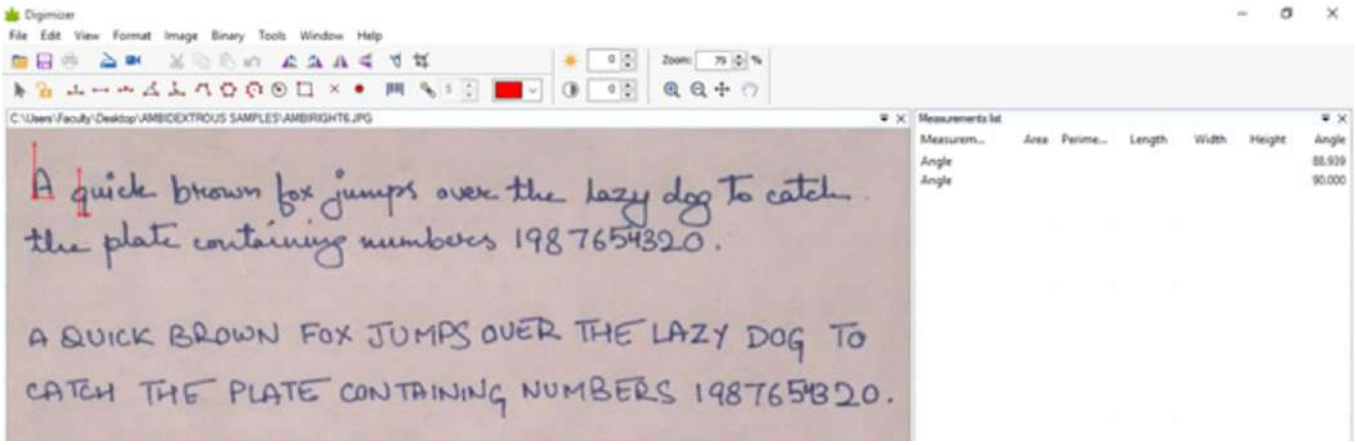


Figure II: Working Window of Digimizer Software For Spacing and Size Measurement.

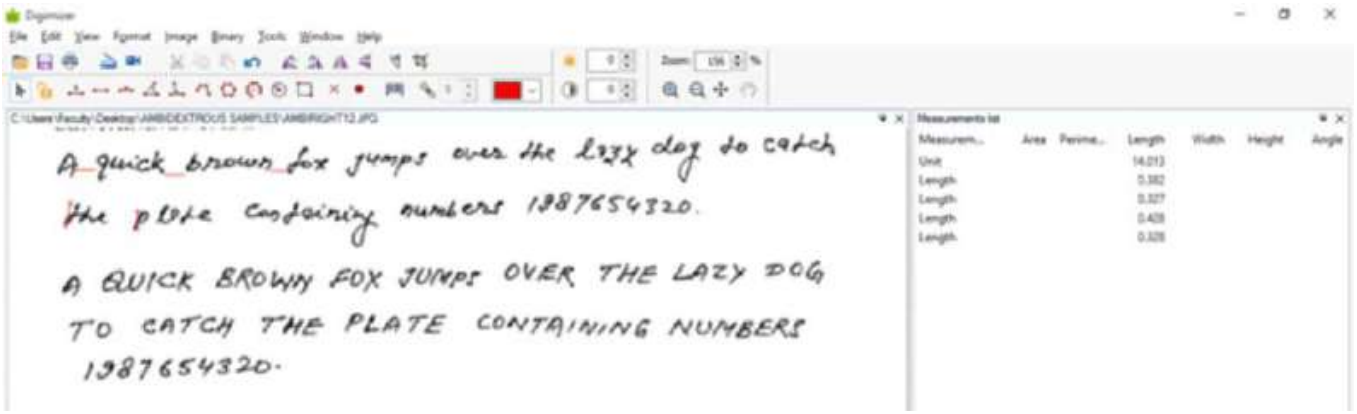
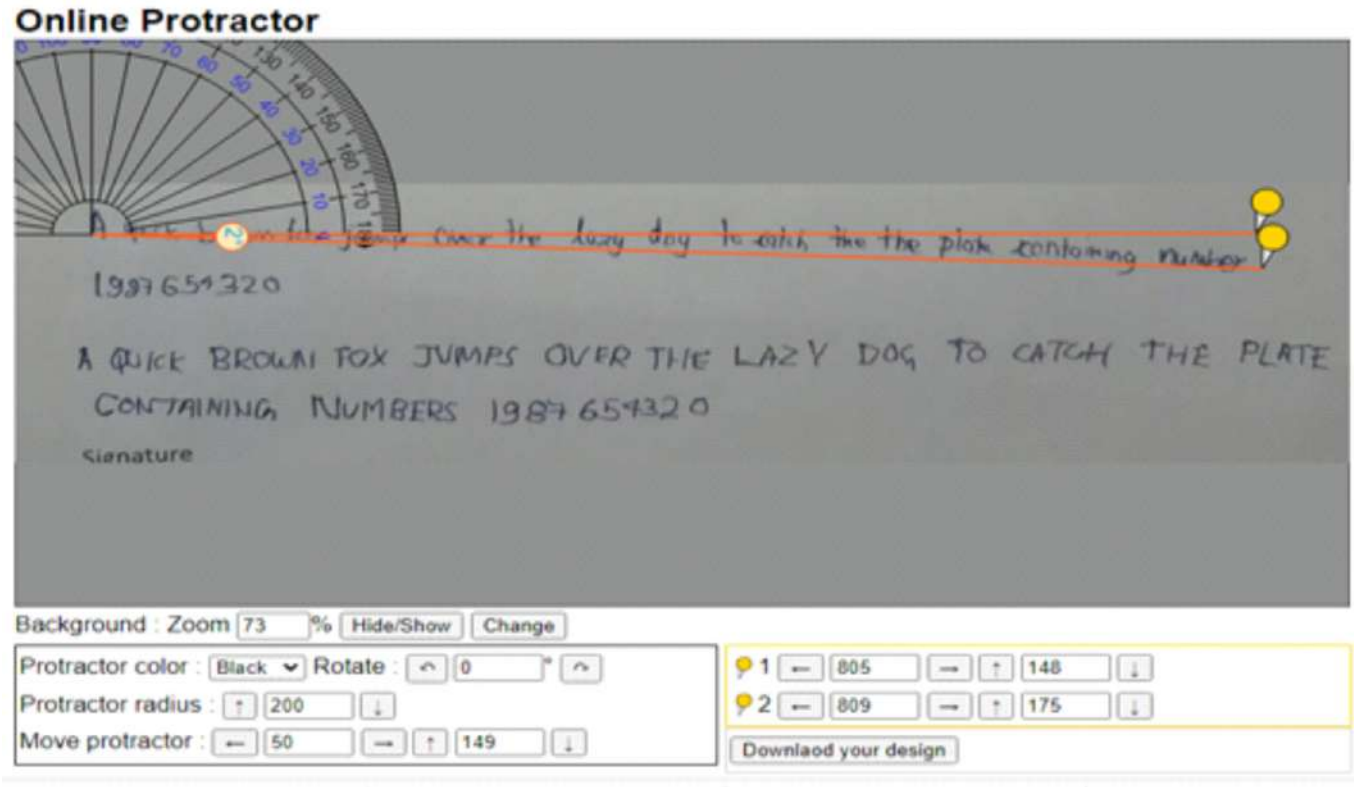


Figure III. Angle Measurement For Alignment of Handwriting From the Baseline using Online Protractor.



The variables chosen for the study was handwriting parameters which are dependent variable and handedness (left and right hand of ambidextrous writers) as independent variable. No external treatment was given to the participants. For data analysis, SPSS 23 was used. Paired sample t-test was performed to test the hypothesis.

RESULTS

The results are based on the analyzing the handwriting samples of ambidextrous writers from Shriram Kannada Convent School, Kapnoor, Kalaburagi, Karnataka as mentioned aforesaid. This included male and female students in the study. These participants were not given any specific treatment rather they were given comfortable environment to write. The handwriting parameters and their hypothesis test results are discussed. To test the hypothesis, the confidence level considered was 95%.

For Slant:

Null hypothesis considered was that 'there is no significant variation in the slant of left and right handwriting of ambidextrous writers'. The alternative hypothesis was considered as 'there is significant variation in the slant of left and right handwriting of ambidextrous writers'.

The data was found normally distributed as shown in **Figures IV a** & **IV b**. Therefore, Paired sample t-test was applied on the data to test the hypothesis. The result showed no significant difference between the mean of handwriting slant of right hand (M=89.03, SD= 8.068) and left hand (M=88.54, SD=6.387). The results show the p (probability value) value > alpha 0.05 (level of significance) which means that one fails to reject null hypothesis. This infers that there is no significant variation

Figure IV: (a) Normal Q-Q Plot of Right Hand Letters Slant

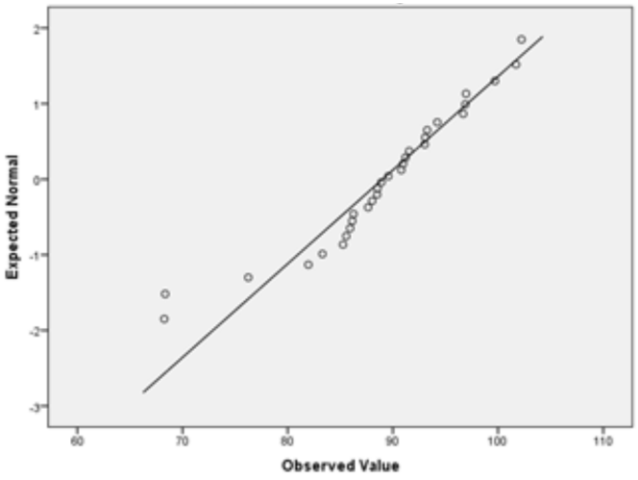


Figure IV: (b) Normal Q-Q Plot of Left Hand Letters Slant

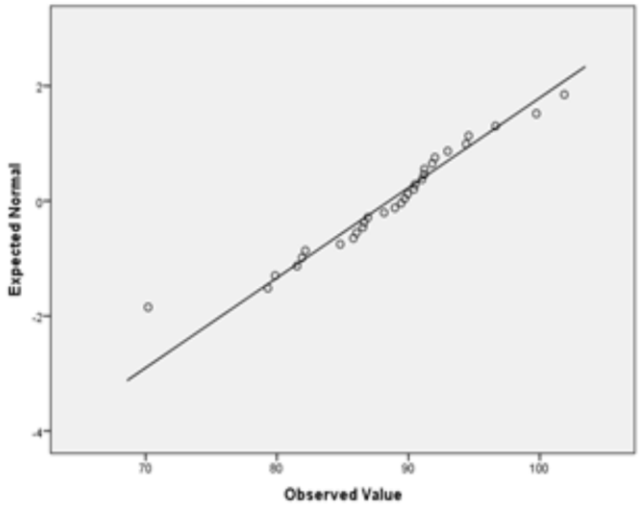


Figure IV: (c) Normal Q-Q Plot of Right Hand Letters Size

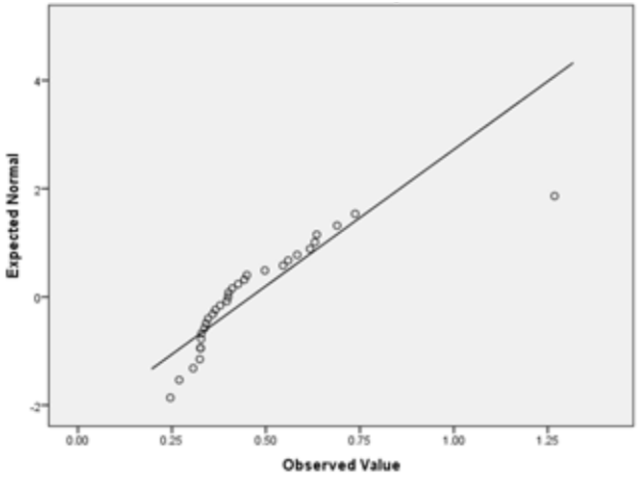


Figure IV: (d) Normal Q-Q Plot of Left Hand Letters Size

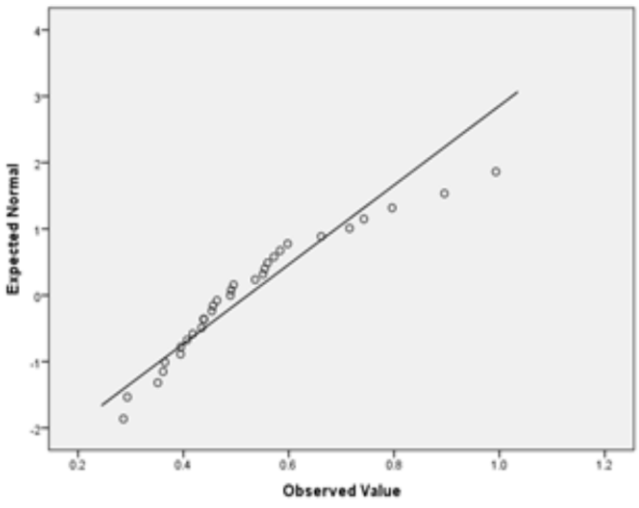


Figure IV: (e) Normal Q-Q Plot of Alignment of Writing with Reference to Baseline in Right Hand Writing.

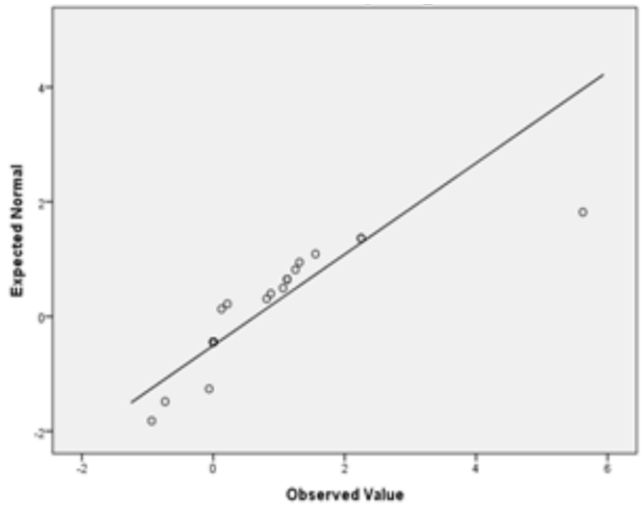


Figure IV: (f) Normal Q-Q Plot of Alignment of Writing with Reference to Baseline in Left Hand Writing.

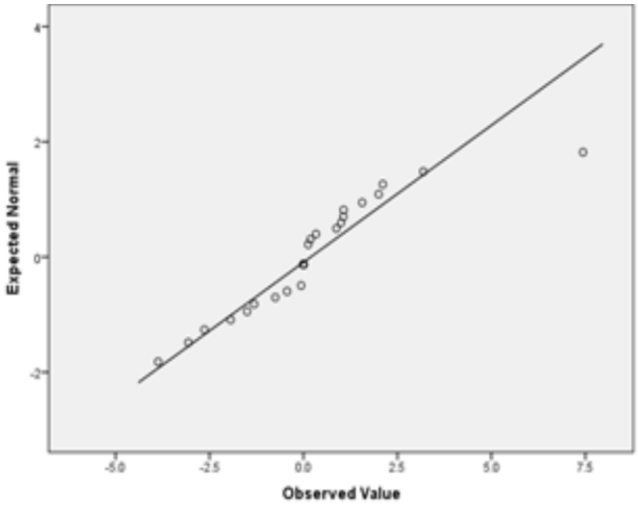


Figure IV: (g) Normal Q-Q Plot of Letter Spacing in Right Hand.

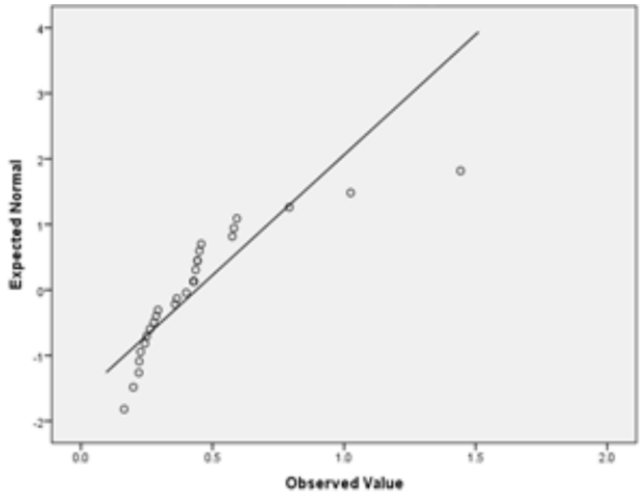


Figure IV: (h) Normal Q-Q Plot of Letter Spacing in Left Hand.

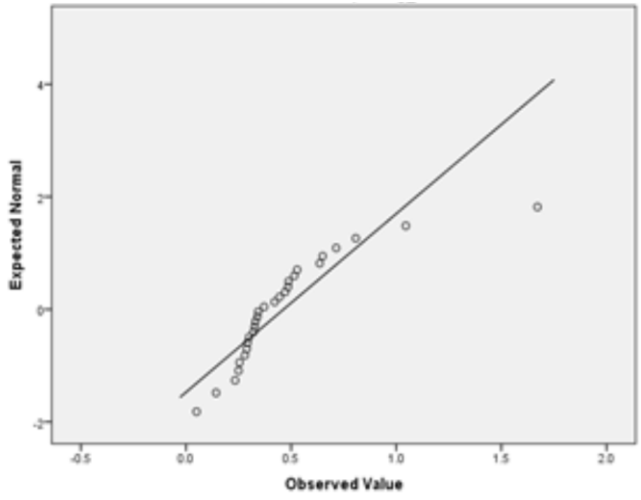


Table I: Statistical Analysis for Significant Variation in Letters Size Writing of Ambidextrous Writers

			Paired Differences							
	Mean	Std. Deviation	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference		t	df	Sig. (2-tailed)
						Lower	Upper			
Size Right Hand	0.46	0.198	-0.062	0.164	0.029	-0.122	-0.002	-2.106	30	0.051
Size Left hand	0.52	0.167								

Table II: Statistical Analysis for Significant Variation in Letter Slant in Writing of Ambidextrous Writers

			Paired Differences							
	Mean	Std. Deviation	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference		t	df	Sig. (2-tailed)
						Lower	Upper			
Slant Right Hand	89.03	8.068	0.484	5.929	1.083	-1.731	2.698	0.447	29	0.658
Slant Left hand	88.54	6.387								

Table III. Statistical Analysis For Significant Variation in Alignment of Writing of Ambidextrous Writers

			Paired Differences							
	Mean	Std. Deviation	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference		t	df	Sig. (2-tailed)
						Lower	Upper			
Alignment Right Hand	0.64	1.257	0.445	2.125	0.402	-0.379	1.269	1.109	27	0.277
Alignment Left hand	0.19	2.102								

Table IV. Statistical Analysis for Significant Variation in Word Spacing of Ambidextrous Writers

			Paired Differences							
	Mean	Std. Deviation	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference		t	df	Sig. (2-tailed)
						Lower	Upper			
Spacing Right Hand	0.44	0.272	-0.026	0.156	0.029	-0.086	0.035	-0.870	27	0.392
Spacing Left hand	0.46	0.315								

in the handwriting slant of ambidextrous writers.

For Size:

Null hypothesis considered was 'there is no significant variation in the size of left and right handwriting of ambidextrous writers'. The alternative hypothesis considered was 'there is significant variation in the size of left and right handwriting of ambidextrous writers'.

The data for size was normally distributed as shown in **Figure IV c** & **IV d** and paired sample t test was also applied here. The size of letters also showed no significant variation between the mean of right hand (M=.46, SD=.198) and left hand (M=.52, SD=.167) as depicted in **Table I**. The p value found to be > alpha value (0.051). Therefore, here also one fails to reject our null hypothesis.

Alignment/ Spacing

Null hypothesis considered was 'there is no significant variation in the alignment/spacing of left and right handwriting of ambidextrous writers'. The alternative hypothesis considered was 'there is significant variation in the alignment/spacing of left and right handwriting of ambidextrous writers'.

Alignment and spacing were also found normally distributed and shown in **Figure IV e** to **IV h**. After applying t test, alignment and spacing also showed no significant variation between right and left handwriting of ambidextrous writers. P value found to be greater than the alpha value of 0.05, therefore one fails to reject null hypothesis again. Hence, in each case, significant variations between handwriting of ambidextrous writers written from right and left hand were not found. The results of hypothesis tests are shown in **Table I** to **Table IV**.

DISCUSSION

With the main aim to find significant variation in the handwriting of ambidextrous, the results show no significant variation in the writing parameters examined in this study. The result is also consistent with the previous studies which say that handwriting of a person does not change fundamentally even if hand is changed. This can be helpful in document examination while examining the handwriting or collection of specimen writings as stated earlier also. However, handwriting parameters like slant, alignment, spacing, size etc. together with other parameters speed, direction, letter formation etc. can be included in the extended research for more robust results. This is a pilot study and extended research required on more number of samples. This study can also be extended to compare handwriting of ambidextrous writers in other scripts also like kannada script, urdu script, gurumukhi etc.[18] Other aspects of this study could include effect of drugs, alcohol, and other external pressure on handwriting of ambidextrous writers.

CONCLUSION

Ambidextrous writers are rarely found and therefore it is important to understand the handwriting features of such people for forensic examination. The absence of significant variation in the handwriting parameters discussed in the study signifies that handwritings of either hand of ambidextrous writes can be used in handwriting sample collection and examination. However, extended research on handwriting of ambidextrous writers with other parameters as well as on more samples can be done in this area.

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Original Research Paper

Cheiloscopic Study in Personal Identification of Ante Mortem and Post Mortem Individuals

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ABSTRACT

Introduction: The aim of this study is to find out the difference in lip print pattern among live and dead individuals.

Materials and Methods: A total number of 200 individuals (100 live and 100 dead individuals) were taken for the study. The live individuals were between 18-25 years of age and for dead individuals the lip prints were taken between 24-48 hours of death from the dead body. The lip prints from live individuals have been taken by traditional lipstick paper method described in the literature while for dead individuals it was taken by DIGITAL METHOD focussing camera on the lips.

Results: The lip prints for live and dead individuals were studied by Suzuki & Tsuchihashi classification. In live individuals the lip patterns were Type I, I', II, III, IV and V while in dead subjects the same lip patterns of Type I, I', II, III, IV and V were found with significant difference.

Conclusion: The study of lip prints for dead individuals has been for first time in the field of forensic odontology and this study is first of its kind in the academic literature. However, there is need for further study to be done in different parts of the world for different dead individuals lip print patterns.

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INTRODUCTION

A matter of great importance in any crime investigation is identification. Since the availability of DNA and fingerprints methods are time tested yet these evidences are not always available at the site of investigation. In these circumstances it is of prime importance to go for application of different and less known technique like identification of a person based on characteristic arrangement of lines appearing on red part of the lips which is called Cheiloscopy. The scarcity of the use of this method in these days of advanced scientific crime detection methods is just the lack of acknowledgement to the need of forensic dental services.

Apart from this there are studies which were done on this technique from the past to recent days had focused on lip prints of living individuals. Occasionally some studies just guide that may be the pattern of lip prints must be same in deceased persons as in living persons. In point of this view this study is conducted to compare the pattern of lip prints in identification process between living and deceased persons.[1]

MATERIALS AND METHODS

A total number of 200 samples (100 samples from live individuals and 100 samples from dead individuals) were taken. Live individuals between 17 years to 28 years with no history of any surgery or trauma or any other medical conditions related to oral cavity and lips were included. Leaving sample was taken from students of Chandra Dental College and Hospital, Barabanki (KGMU).

The lip prints from live subjects have been obtained by traditional lipstick method and the individuals have been asked to wash their lips and then a thin layer of glycerine has been applied so that on application of lipstick it will leave a better impression on paper. Then the individuals were made to apply a single coat of lipstick on the upper and lower lips and made to sit straight on the dental chair. A

small paper of 11 x 11 cm has been folded from middle and placed between the lips touching the commissures and the individual has been asked to press gently on the paper till the philtrum area of the upper lip and the border where the skin and lip meet each other. The paper has been removed gently and the impression obtained has been scanned for the type of lip prints. The lip prints were edited by the ADOBE photoshop software and then were studied for the types of lip prints obtained.

The sample collection from dead individual's: After clearance from the CMO (Chief Medical Officer), ethical committee and mortuary staff, the lip prints from dead individuals were obtained by digital method i.e., by taking the closeup photograph by digital camera SONY CYBERSHOT. The photographs were edited by the ADOBE photoshop software and then lip prints were studied for the type of lip prints. Dead samples were taken from the mortuary of KGMU.

RESULTS

The examination of lip print patterns among female and male in live study subjects revealed that the most predominant pattern in the entire study population was Type II (98). This was followed, in order, by Type I (92), Type I' (71), Type III (51), Type V (8) and Type IV (4). In females Type II (42) lip pattern was most commonly found followed by Type I (41). Equal frequency distribution of lip pattern was found in Type I'(28) and Type III among live female subjects. In males, Type II (56) lip pattern was predominant, followed by Type I (51) and Type I' (43). So, the most common lip pattern for females and male live study subjects was is Type II. **(Table 1)**

The frequency distribution of different lip patterns among female and male in dead study subjects was Type I (82). This was followed, in order, by Type III (48), Type I' (45), Type II (42), Type IV (14) and Type V (6). In female Type I(6) lip pattern was most commonly found followed by Type III (3) and Type I' (3) out of total 8 dead female subjects. In males, Type I (76) lip pattern was predominant, followed by Type III (45) and Type I' (42) out of total 92 dead male subjects. Present study found that the Type I lip pattern was most common lip pattern in dead males as well as female study subjects. **(Table 2)**.

Table 1: Frequencies Distribution of Different Lip Patterns Among Female and Male in Living Study Subjects.

Lip Patterns		Female (n=44)	Male (n=56)	Total
Type I	n	41.0	51.0	92.0
	%	44.6	55.4	100.0
Type I'	n	28.0	43.0	71.0
	%	39.4	60.6	100.0
Type II	**n**	**42.0**	**56.0**	**98.0**
	%	**42.9**	**57.1**	**100.0**
Type III	n	28.0	23.0	51.0
	%	54.9	45.1	100.0
Type IV	n	3.0	1.0	4.0
	%	75.0	25.0	100.0
Type V	n	4.0	4.0	8.0
	%	50.0	50.0	100.0

Table 2: Frequencies Distribution of Different Lip Patterns Among Female and Male in Dead Study Subjects.

Lip Patterns		Female (n=8)	Male (n=92)	Total
Type I	**n**	**6.0**	**76.0**	**82.0**
	%	**7.3**	**92.7**	**100.0**
Type I'	n	3.0	42.0	45.0
	%	6.7	93.3	100.0
Type II	n	1.0	41.0	42.0
	%	2.4	97.6	100.0
Type III	n	3.0	45.0	48.0
	%	6.2	93.8	100.0
Type IV	n	1.0	13.0	14.0
	%	7.1	92.9	100.0
Type V	n	1.0	5.0	6.0
	%	16.7	83.3	100.0

DISCUSSION

This is the first study of its kind which was conducted to compare the lip print pattern between the live and dead individuals. This study was performed with the intention that the lip prints in dead individuals give different patterns as compared to live individuals. The lip prints in live individuals have been taken by traditional lipstick paper method and was analyzed using Suzuki and Tsuchihashi Classification. Saraswati et al[1] in their study found that the most common lip print pattern among males and females was Type III and IV. In females they found the pattern of Type III and Type II while in males it was Type III and Type IV which is partially consistent with our study. Ahmed SA[2] et al found that Type IV pattern was the most frequently represented pattern and pattern of Type I and II was prevalent in males and females which is similar to our findings in live male and female subjects.

Our study was consistent with the study of N Ghimere[3] et al who also found the predominant Type II pattern in females and second predominant pattern of Type I in males and females both. Similarly, Srishti Aditi[4] et al done the study on lip prints in association with different skeletal

malocclusions and found that the predominant pattern was Type II which is consistent with our study as we have also found Type II as the predominant pattern however in our study skeletal malocclusion is not been considered. Deepa Jatti[5] et al also found Type II pattern as a predominant pattern in both males and females which is similar to our results of Type II pattern in both males and females.

Negi A[6] et al showed that males have lip pattern of Type II followed by Type I and IV while females have lip pattern of Type I followed by Type IV and II which is partial consistent with our study as in our results in males also showed the pattern of Type II followed by Type I which proves that lip prints are unique to an individual and can be used for determination of sex.

Shilpa Jain[7] et al found that Type II and I pattern was predominant in females while in males it was Type IV, III and V which is consistent with our results in females where we have found predominant pattern of Type II and I which proves that lip prints can be used for sex identification and individual identification as they remain stable and unique over time.

Ishaq N[8] et al found that the most common pattern in females was Type II which is prevalent in our study as we have also found Type II pattern most in females. Our study is consistent with Murkey P N[9] et al who found prevalent pattern of Type II followed by I', I, IV and III and we found prevalent pattern of Type II followed by I, I', III, V, IV. Bharati S[10] et al found that Type I as the predominant pattern followed by Type II in females and Type IV as predominant pattern in males while our study showed predominant pattern of Type II in both males and females proving the peculiarity of lip patterns in the identification process.

Almuhaiza M[11] et al found Type II (branched) as the predominant pattern in both males and females as our study also showed the similar Type II pattern in both males and females. Bai JK[12] found that Type II lip pattern were predominant in both males and females followed by Type I, I', III, IV and Type V which strongly correlates with this study as the lip pattern in found in males and females is Type II, I, I', III, IV and Type V. A highly significant correlation with this study and the study done by MA Eldomaity[13] et al has been found as both the studies show the predominant pattern of Type II followed by Type I, III, IV and V.

Simovic[14] et al found that females are having commonest pattern of Type II followed by Type I, III, IV and V while males had shown the pattern of Type III followed by Type II, IV, V and I which partially correlates with this study as in females the pattern was Type II, I, I', III, IV and V. Similarly, Ranjan V[15] also found Type II as the common pattern in males while females showed Type I pattern which partially correlates with this study as in males the common pattern was Type II. On contrary, the most prevalent pattern found by Ishaq N[16] et al was Type I in both male and female and least common was Type IV which is similar in this study as in this study Type IV was the least lip pattern found. Pelin C[17] et al found that Type II was the most predominant pattern followed by Type IV and I in both males and females which is consistent with the findings of Type II in both males and females which shows that lip prints give good predictability in determining sex. Apart from above studies our results show strong correlation with the studies of Borase P[18] et al, Karki R K[19] et al, Vildana K[20] et al, Karn A[21] et al, Gugulothu RN[22] et al, Kaushal B[23] et al, Shokri H[24] et al, Dey A[25] et al, Dr Huda Abdul Wadood Mohammed Omer[26], Pathmashri VP[27] et al, Rekha VR[28] et al, Bano AM[29], Sumathy G[30] et al and Kesarwani P[31] et al.

Since this study has been done in both live and dead subjects therefore there are no previous studies relating the lip prints in dead subjects. However, this study showed the same type of lip print patterns according to the Suzuki and Tsuchihashi Classification described in living individuals which proved the fact that lip prints do not change in the life of the individuals even after death.

CONCLUSION

This is the first study of its kind in the academic literature till now to be performed with the lip prints analysis between live and dead individuals. It was proved that lip prints do not change from the birth to after death however, this study will help in personal identification of a person even after the person is deceased. However, a large sample study needs to be done in different parts of countries so that in future there will be no difficulty of identification in dead individuals.

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Figure 1: Live Male Lip Prints

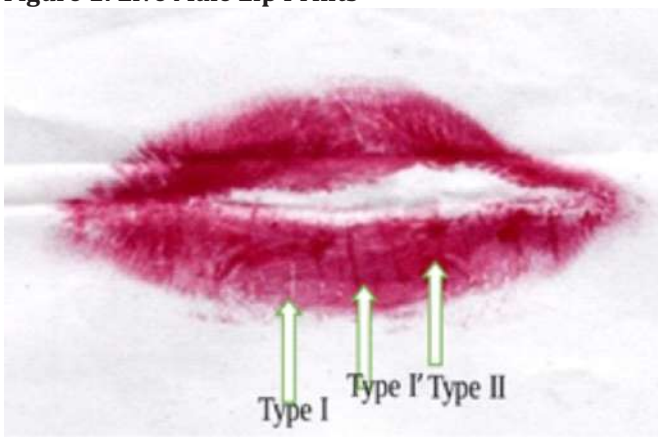

Figure 2: Live Female Lip Prints

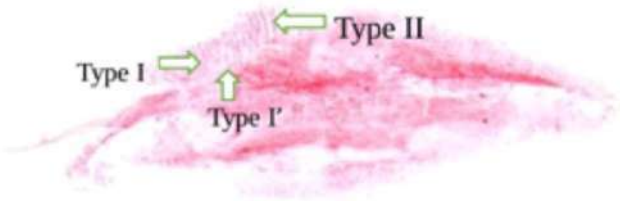

Figure 3: Dead Male Lip Prints

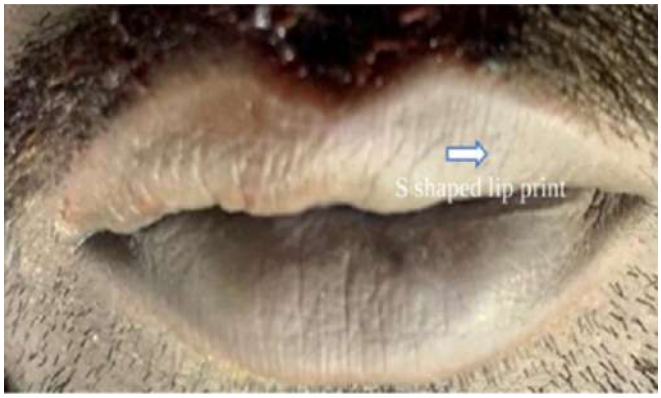

Figure 4: Dead Female Lip Prints

Table 3: Lip Print Classification for Dead Individuals, 2022

S.No.	Pattern Name	Pattern Shape
1	Hebrew Accent Pashta	֙
2	Hebrew Accent Geresh	֜
3	Hebrew Accent Tipeha	֖
4	Hebrew Accent Merkha	֥
5	Vatn	⫸
6	Stomp	⫷
7	Dagger	†
8	Aquaris	≋
9	Libra	≎
10	Greater than	>
11	Lesser than	<
12	Identical to	≡
13	Equal to	=
14	Horizontal Bifurcated	>-----
15	Integral	∫
16	Double integral	∬
17	Right triangle	◺
18	Reverse Solidus	\
19	Curly logical	⋎
20	Very much less than	<<<
21	Very much greater than	>>>
22	Wavy line	~
23	Small asterisk	*
24	Lambda	λ
25	Perpendicular	⊥
26	Nu	ν
27	Xi	ξ
28	Psi	Ψ
29	Gamma	γ
30	Capital Chi	X
31	Water	≈
32	Air	≋
33	Peak	⋀
34	Inverted dome	□
35	Triple dome	≡

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Original Research Paper

A Study on Estimation of Stature From The Measurement of The Percutaneous Length of Tibia in an Adult Tamil Population

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ABSTRACT :

Introduction: Stature of an individual is one the most important data of personal identification. Both in living and as well as in the deceased the stature estimation is of great significance. Mutilated remains or bone fragments have always remained a challenge for the Forensic anthropologists.

Material and Methods: This cross-sectional study was conducted on 200 subjects of ethnic Tamil origin (100 males and 100 females). The percutaneous length of the tibia was measured, and the height of the individual was measured from vertex to heel in the Frankfurt's plane. For the tibial length Measurement, was taken with a spreading caliper from the medial most point on the upper border of medial condyle of the tibia to the tip of medial malleolus of the tibia.

Results: The mean length of the right and left tibia in males as found in this study were 35.11 cm and 35.93 cm, and in females were 28.81 cm and 28.76 cm respectively. The results of the student's t test between male and female stature showed a t value of 23.824, with p value of 0.033 which is statistically significant. A regression formula was developed separately for males and females based on the correlation between stature and percutaneous length of tibia.

Conclusion: This study has worked on establishing a relationship between the percutaneous length of tibia and stature among adult males and females of ethnic Tamil origin and has also successfully derived a regression formula for males and females separately.

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INTRODUCTION

Stature is an important data of human identification which helps a great deal in separately identifying both males and females. However, there exists considerable problem in the process of stature estimation in practical aspects of Forensic anthropology. Mutilated remains or bone fragments have always remained a challenge for the Forensic anthropologists. In the process of establishment of the identity of a person estimation of stature is regarded as a very valuable data in the field of Forensic anthropology.[1] The principle which is used in stature estimation when fragmented remains are found is based on the theory that a very strong correlation exists between the stature and individual bone of a human.[2] In Forensic anthropology the importance of limb bones has been underscored by various authors.[3-7] Among the long bones again lower limb bones have been more useful than upper limb bones in estimation of stature in humans.[8] Various factors affect the stature of an individual such as race, age, sex, diet etc. In humans the height taken in the standing position is very much in relation with length of the lower limb and hence the importance of the length of lower limb bones such as the femur, tibia, and fibula and why these bones are most commonly useful in predictive equations which are used in stature estimation of approximate stature.[9]

Even though the method of estimation of stature from the measurement of long bones have been developed, however the fact remains that, for every different ethnic population there is a need to develop a specific formula so that it represents for each population representing a different

ethnic group and which is equally medico legally significant.[10-15]

Traditionally previous studies have shown that there exist significant differences in the proportion of limb length in different ethnic population across the world and this is largely due to influence of hereditary, ethnic, environmental, and dietary factors which affects the overall stature of an individual.[16] Among the lower limb bones the tibia is most often chosen because the medial end of the tibial tuberosity and the medial malleolus are both subcutaneous and therefore it is easy to obtain measurement.

This present study was done with the aim of establishing correlation between the percutaneous length of the tibia and stature and to develop regression formulae which should be applicable to both adult males and females of ethnic Tamil origin.

MATERIALS AND METHODS

This cross-sectional study was done in the Ammapettai area of Kancheepuram district of Tamil Nadu after obtaining permission from the institute ethical committee of Shri Sathya Sai Medical College and research institute on 200 study subjects of ethnic Tamil origin comprised of 100 males and 100 females from the age group between 18-25 years. Since the maximum height of an individual is attained by this age. Those subjects who were having any significant disease, orthopedic deformity such as achondroplasia, metabolic or developmental disorders which could have affected the general or bony growth were excluded.

The Stature of the subject was measured in a standing position on a standard Stadiometer with both feet in close contact with each other with the trunk straight along the vertical board, and the head adjusted in Frankfurt plane. The measurement was taken in centimeters by bringing the horizontal sliding bar to the vertex.

For measuring the tibial length (PCTL) subject was asked to stand and keep her/his foot on a stool to maintain the angle between the flexor surface of leg and that of the thigh at 90 degrees. Afterwards two points were marked by skin marking pencil. Measurement were taken with a spreading caliper from the medial most point on the upper border of medial condyle of the tibia to the tip of medial malleolus of both the right and left tibia. All the collected data was tabulated, analyzed, and subjected to statistical calculations using SPSS 23 software. Descriptive statistics was used to obtain mean, standard deviation, standard of error. Students t test was done for tibial length in both males and females. A regression formula was developed separately for males and females based on the correlation between stature and percutaneous length of tibia.

RESULTS

The data analysis of this study on 200 healthy adults (100 males and 100 females) shows that the mean stature in males was 168.34 cm, mean length of the right and left tibia were 35.11 cm and 35.93 cm, and with standard deviation of 1.82 (for stature), 1.53 (for right tibia), 1.51 (for left tibia and standard error of 0.18 (for stature), 0.15 (for right tibia), 0.15 (for left tibia) respectively as shown in **Table 1**.

In females the mean stature was 159.41 and the mean length of the right and left tibia were 28.81 cm and 28.76 cm, and with standard deviation of 3.35 (for stature), 2.16 (for right tibia), 2.18 (for left tibia) and standard error of 0.33 (for stature), 0.21(for right tibia), 0.21 (for left tibia) respectively as shown in **Table 2**.

Table 1: Descriptive Statistics for Male Stature, Age, Right and Left Side of Tibia Length

Tibia	Male (n=100)			
	Stature	Age	Rt TL	Lt TL
Range	9.00cm	4.00cm	7.20cm	7.00cm
Mean	**168.34cm**	19.17cm	35.11cm	34.93cm
Std. deviation	1.82	1.00	1.53	1.51
Std. Error	0.18	0.10	0.15	0.15
CV	1.08%	5.22%	4.36%	4.32%

Table 2: Descriptive Statistics for Female Stature, Age, Right and Left Side of Tibia Length

Tibia	Female (n=100)			
	Stature	Age	Rt TL	Lt TL
Range	13.00cm	3.00cm	8.80cm	9.00cm
Mean	159.41cm	19.33cm	28.81cm	28.76cm
Std. Deviation	3.35	1.02	2.16	2.18
Std. Error	0.33	0.10	0.21	0.21
CV	2.10%	5.28%	7.50%	7.58%

The results of the student's t test between male and female stature showed a t value of 23.824, with p value of 0.033 which is statistically significant as shown in **Table 3**.

Table 3: Student T-Test Between Stature of Male and Female

Statistics for Stature	Student T-Test Value
T - Value	t = 23.824; df=198
P -Value	0.033
P - Value Summary	Significant
Avg. Mean (Stature) Male & Female	163.88 cm & 159.41

Table 4: Student T- Test Between Tibia Length of Right and Left For Male

Statistics for Stature	Test Value
t- value	t=0.855; df= 198
p- value	0.021
p- value summary	significant
Avg. mean (rt+lt)	35.02 cm

Table 5: Student T- Test Between Tibia Length of Right and Left For Female

Statistics for Stature	Test Value
t- value	t=0.175; df=198
p- value	0.043
p- value summary	significant
Avg. mean (rt+lt)	28.79 cm

Table 6: Formulation of Multiple regression equation for calculating the stature from age, tibia length in male both right & lift side

Multiple Regression Statistics	Male		
	Age	Rt tibia	Lt tibia
Intercept	130.12		
Regression co-efficient	0.765	1.345	0.841
Correlation co-efficient (r)	0.874		
Co-efficient of determination (R2)	0.764		
Std. error of estimate	0.899		
p-value	0.041	0.000	0.003
p- value summary	NS	S	S
Multiple regression formula	$X= 130.12+0.765X_1+0.998X_2+0.841X3$		

Table 7: Formulation of Multiple Regression Equation for Calculating The Stature from Age, Tibia Length in Female Both Right & Lift Side

Multiple Regression Statistics	Female		
	Age	Rt tibia	Lt tibia
Intercept	116.86		
Regression co-efficient	0.977	1.345	0.713
Correlation co-efficient (r)	0.893		
Co-efficient of determination (R2)	0.797		
Std. error of estimate	1.534		
p-value	0.001	0.000	0.021
p- value summary	S	S	S
Multiple regression formula	$Y= 116.86+0.977X_1+1.345X_2+0.713X3$		

Figure 1: Showing Relation Between Length of Right Tibia and Stature in Males

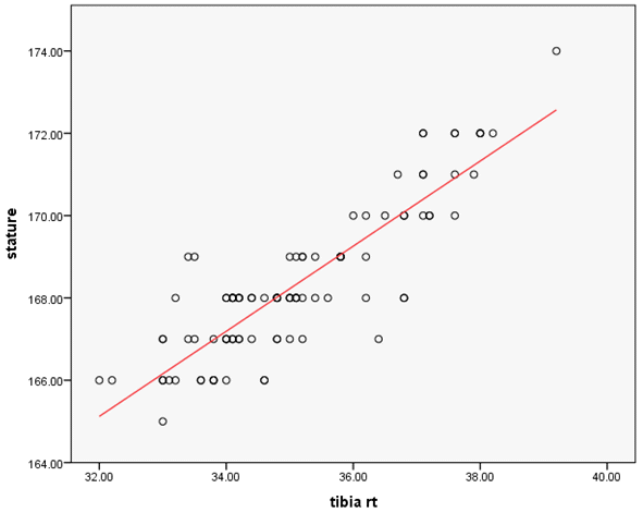
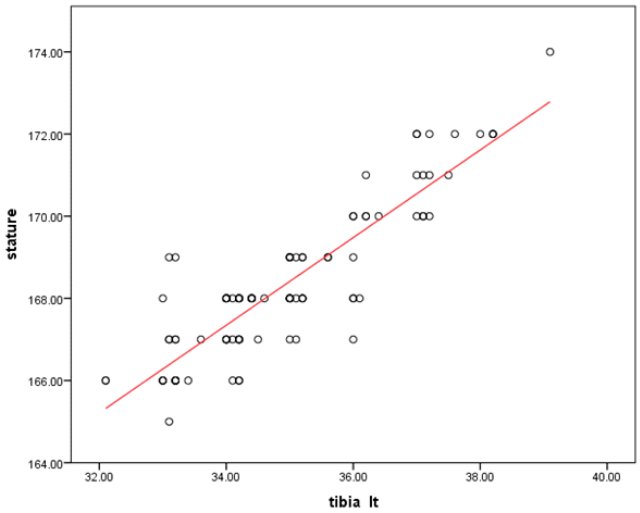


Figure 2: Showing Relation Between Length of Left Tibia and Stature in Females



The student's t test value for comparison between the right and left tibia in males showed a p value of 0.021 in males a p value of 0.043 in females which were statistically significant, as shown in **Table 4-5**. The correlation coefficient (r) for males was 0.874, with regression coefficient value of 0.765 (for age) and 0.998 (for right tibia) and 0.841 (for left tibia) respectively as shown in **Table 6**. The correlation coefficient (r) for females was 0.893, with 0.1345 (for right tibia) and 0.713 (for left tibia) as shown in **Table 7**. In accordance with the findings of this study scatter plots were drawn to show the correlation between the stature and the length of the tibia bone, as shown in **Figure 1-2** respectively.

Multiple Regression Formulae were Developed Separately for Both Males and Females Which are:

Multiple regression formula for males X= 130.12 + 0.765X1

+ 0.998X2 + 0.841X3

Multiple regression formula for females Y= 116.86 + 0.977X1 + 1.345X2 + 0.713X3

DISCUSSION

Among long bones of the lower limb tibia is a useful bone and has been used in various geographical regions and on various ethnic groups. The credit for developing regression equation for stature estimation from measurement of long bones goes to Rolet.[17] In this study the correlation between tibial length and the stature of males and females have been established by developing regression formula for people of ethnic Tamil origin. The estimated mean height of males and females were 168.34 cms and 159.41 cms respectively which is like the findings of the previous studies such as those of Kavyashree A.N, who found the mean stature of males and females as 170.88 cms and 158.87 cms respectively and those of Chavan et al who found 167.89 cms and 151.41 cms mean height of males and females.[18-19]

The mean percutaneous length of tibia in males and females as found in this study are like the results of earlier studies.[20] In this present study the findings reveal that the correlation coefficients (r) of height and PCTL for male and female were 0.874 and 0.893 respectively which were statistically significant and similar correlation was shown in previous studies.[21-22] The results of this study differed with the findings of previous authors who did their study on foreign population.[23-24] In the year 1929 Stevenson observed and opined that regression formula developed for one population will not be useful for another population and hence there is need to develop specific formula for a specific type of population.[25]

The regression formulas derived in this study is meant for a particular ethnic group as included in the study and there are already various studies on various ethnic groups which are specific for both the population and the gender.[26] This study become more useful for the development of a population specific regression formula as every population by virtue of their genetics will vary considerably in their size and stature.[27-28]

CONCLUSIONS

Stature is a very crucial data for personal identification. There has been a series of studies on different populations across the world on establishment of correlation between stature and long bones. This study has worked on establishing a relationship between the percutaneous length of tibia and stature among adult males and females of ethnic Tamil origin and has also successfully derived a regression formula for males and females separately. The findings of this study prove that there is strong positive relationship between the length of the tibia and the stature of the individual and equally in males and females. This would be very useful in anthropometric procedures and in situations where there is recovery of fragmented remains of lower limbs and especially in the Tamil population.

"List of abbreviations".

PCTL: Per Cutaneous Tibial Length

CMS: Centimeters

SPSS: Statistical Package for Social Sciences

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Original Research Paper

Estimation of Age by The Study of Closure of Ectocranial and Endocranial Sutures on Autopsy

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ABSTRACT

Introduction: Identification refers to the process of determining the individuality of a person and it is of great significance in the field of law enforcement. An integral part of identification of a deceased and unknown individual is age estimation. In cases with bodies found in mutilated or decomposed state or when only fragments of remains are discovered, age estimation is of utmost importance and helps to aid in identification of the individual. Even though age estimation from cranial sutures is frequently used for age estimation along with other methods or in the absence of alternate evidence, there are few studies on the reliability of cranial sutures reported in India. Therefore, this study was done to observe the utility in the usage of cranial sutures as a method of age estimation in the population of Faridkot and adjoining areas.

Aims and Objectives: The purpose of the study was to analyze the sequence and pattern of cranial suture (sagittal, coronal and lambdoid) closure on the ectocranial and endocranial surfaces along with bilateral and gender variations. We also studied the correlation between suture closure and age of the individual.

Materials and Methods: A sample of 200 autopsy cases which satisfied the criteria was included in the study. The skulls were cleaned and sutures were examined. The amount of suture closure was noted based on the Acsadi Nemeskeri scale bilaterally.

Results: There was no bilateral variation in the amount of suture closure. Males showed early suture closure than females. Endocranial sutures were found to close early and uniformly when compared to ectocranial sutures. Positive correlation was found between age and suture closure till the age of 45 years.

Conclusion: The results showed that cranial suture closure on the endocranial surface can be used as an adjunctive tool in age estimation along with other methods to a certain amount of accuracy.

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INTRODUCTION

Identification refers to the process of determining the individuality of a person and it is of great significance in the field of law enforcement. It helps to solve medico legal cases which could be either civil or criminal. The identification of an individual is done by utilizing various biological and physical variables. Some of these parameters are malformations, tattoo marks, birth marks, occupational marks, personal identification features such as clothing, intelligence, speech, habits, age, sex, race, stature, anthropometric measurements, foot prints, lip prints, finger prints, DNA profiling, DNA fingerprinting etc.[1]

An integral part of identification of a deceased and unknown individual is age estimation especially in cases with bodies found in mutilated or decomposed state or when only fragments of remains are discovered.[2] Age estimation is done with the help of macroscopic and microscopic methods. The macroscopic methods of examination of age include estimation of dental development and eruption, degenerative stages of pelvic articular surfaces, stages of fusion of the epiphysis of long

bones and fusion of cranial sutures and microscopic examination includes histological analysis of long bones.[1]

The part of the skeleton which is most preserved and resists putrefaction is the skull which makes it particularly important in age estimation. The complete fusion of the sutures of the skull takes place at a later age after the eruption of all the teeth and fusion of epiphysis after the age of 21 years. This particular characteristic of skull sutures makes it highly reliable for age estimation in adults.[3]

The correlation between cranium and age estimation has evoked interest since its inception. Twenty two bones constitute the skull which acts as a protective covering for the brain. Base of the skull develops by endochondral ossification while most of the remaining bones of the skull develop by intramembranous ossification. Skull is formed by a number of cranial bones which are attached to the each other by a saw tooth or zipper-like articulation known a suture. These cranial sutures are immovable joints of the skull.[3] These joints remain patent allowing the growth of the brain in intrauterine life and extrauterine life. They also facilitate the passage of fetus during vaginal delivery by non traumatic compression of fetal skull bones.[4]

There are ongoing debates about the credibility of age estimation from cranial sutures considering their high intra sutural variability throughout its length.[5] Cranial synostosis is the term used to denote premature ossification before 2 years of age.[4] Another feature associated with abnormal development of the frontal sinus is the persistent presence of metopic suture which is observed in 0 to 7.4% in different populations.[6]

The first scoring system for suture closure was given by Todd and Lyon with a scoring of zero to four which denotes no closure to complete closure. The scores for each landmark on the sutures were noted and the average was calculated to determine the trend of suture closure in the individual. It was observed that ectocranial sutures showed high unreliability when used for age estimation while endocranial sutures were found to be more active from 26 to 30 years. Acsadi and Nemeskeri observed that there is a uniform trend of rapid closure early on followed by a slower and gradual closure with advancing age. Miendl and Lovejoy's recent study showed that cranial suture when combined with other age indicators can be a reliable method for forensic purposes.[7]

Our study involved three sutures namely coronal, sagittal and lambdoid which are graded on the basis of the stage of suture closure using the Acsadi -Nemeskeri scale. This study was planned considering the lack of research on this subject in the north Indian population. The study was conducted in the Department of Forensic Medicine and Toxicology, Guru Gobind Singh Medical College and Hospital, Faridkot, Punjab which included 200 deceased individuals above the age of 25 years. This study also helped in assessing the reliability of cranial sutures for age estimation in the Faridkot and adjoining areas population.

MATERIALS AND METHODS

The study was conducted in the Mortuary under Department of Forensic Medicine and Toxicology, G.G.S. Medical College and Hospital, Faridkot, Punjab on 200 deceased individuals who were brought for medico-legal postmortem examinations during time period of 18 months using purposive (non-probability) sampling. It was an observational study. Written informed consent was taken from the relatives of deceased for the study. Age of the deceased was confirmed by documentary evidences which include identification cards like aadhar card, ration card, birth certificate, hospital records etc

Inclusion Criteria

1) The cases of known age coming for medico-legal postmortem examination
2) Subjects of more than 25 years of age were taken.

Exclusion Criteria

1) Unknown, unclaimed bodies where exact age cannot be confirmed.
2) Diseased, operatively intervened or fractured skull which may interfere with the study of suture closure

DURING POSTMORTEM EXAMINATION

Data collection tools: Statistical method was applied after manual collection of data.

An incision from mastoid on one side to the mastoid on the other side was given to reflect the scalp to study the coronal, sagittal and lambdoid sutures by applying Acsadi-Nemeskeri scale ectocranially. The same scoring system was used endocranially after removing the calvaria with the help of an autopsy saw. The calvarium was cleaned of soft tissues to make the sutures prominent so as to observe the obliteration of sutures both ectocranially and endocranially. Photographs were taken in all cases. Ectocranially, the different sections were distinguished by differences in the character of the suture. Endocranially the sutures did not show these differences in character of the suture so they were divided into sections of equal

length.

The scale for suture closure according to the Acsadi-Nemeskeri complex method included the following scores:

0 Open. There is still little space left between edges of adjoining bones

1 Incipient closure, Clearly visible as a continuous often zigzagging line

2 Closure in process. Line thinner, less zigzags, interrupted by complete closure

3 Advanced closure. Only pits indicate where the suture is located

4 Closed. Even location cannot be recognized

Mean suture closure stages were calculated for the three main sutures by adding the scored closure stages of the different sections and dividing the result by number of sections which compose the particular suture.

To estimate the relation between obliteration of sutures and age, appropriate statistical tools was used (student's t test, spearman correlation coefficient).

RESULTS

The sample for the study consisted of 200 cases which were brought to the Mortuary of Guru Gobind Singh Medical College and Hospital, Faridkot for postmortem examination. Out of them 175 cases were male and 25 were females. The age of the cases ranged from 25 years to 76 years. Maximum number of cases was observed to be in the age group of 25 to 35 years. There were least number of cases in the age group between 46 and 55 years. The mean ± SD age of the study sample was 39.1±14.04 years as shown in **Table 1**.

Table 1: Descriptive statistics for Age of study subjects

Age Group (Years)	Frequency	Percent
25-35	100	50.0
36-45	41	20.5
46-55	22	11.0
> 55	37	18.5
Total	**200**	**100.0**
Mean + SD	39.1+14.04	

When the sagittal suture closure stages of the ectocranial and endocranial sites were compared, significant difference was observed between them. Suture closure was observed to start first on the endocranial surface with a mean of 3.00 while the mean of the ectocranial sagittal closure was 2.36.

The mean value of the stage of suture closure was found to be 2.38 in ectocranial right and left sides while it was 3.0 and 3.06 on the endocranial surfaces of the right and left sides respectively which indicated the difference in extent of fusion as shown in **Table 2**.

Table 2: Comparison between ectocranial and endocranial coronal suture closure of left and right sides

	Mean	N	Std. Deviation	T value	P value
Ecto RC	2.38	200	.79	-16.607	<0.01*
Endo RC	3.0	200	.86		
Ecto LC	2.38	200	.79	-17.594	<0.01*
Endo LC	3.06	200	.83		

When the comparison between endocranial and ectocranial suture closure was done in lambdoid suture on the left and right sides, there was again statistically significant difference that was observed between the two surfaces as shown in **Table 3**.

Table 3: Comparison between ectocranial and endocranial lambdoid suture closure of left and right sides.

	Mean	N	Std. Deviation	T value	P value
Ecto RC	2.02	200	.77	-13.67	<0.01*
Endo RC	2.52	200	.84		
Ecto LC	2.02	200	.77	-13.654	<0.01*
Endo LC	2.52	200	.83		

No significant difference was observed in the extent of suture closure between the right and left sides in sagittal, coronoid or lambdoid sutures. Therefore, there was no bilateral variation in any of these sutures.

On comparison of the amount of suture closure in males and females, statistically significant difference was observed. Males were found to have a greater amount of suture closure compared to females of the same age. This pattern was found similar in sagittal, coronal and lambdoid sutures. This gender variation was found to be more significantly expressed on the endocranial surfaces compared to the ectocranium as shown in **Table 4**.

In the study, the sagittal suture has been divided into pars bregmatica (S1), pars vertices (S2), pars obelica (S3) and pars lambdica (S4) to estimate the stage of closure of the suture ectocranially and endocranially. On comparison of these four subdivisions ectocranially, it was found that within the sagittal suture, at the age of 25 to 35 years,

Table 4: Comparison between males and females for each side of ectocranial & Endocranial suture closure.

	Mean	N	Std. Deviation	T value	P value
Ecto RC	Male	2.44	0.78	2.526	.012*
	Female	2.01	0.78		
Endo RC	Male	3.11	0.82	2.878	.004*
	Female	2.59	0.99		
Ecto LC	Male	2.43	0.78	2.589	.010*
	Female	2.00	0.79		
Endo LC	Male	3.12	0.79	2.966	.003*
	Female	2.60	1.00		
Ecto RL	Male	2.06	0.77	2.006	.046*
	Female	1.73	0.73		
Endo RL	Male	2.59	0.82	3.323	.001*
	Female	2.01	0.75		
Ecto LL	Male	2.06	0.77	1.980	.049*
	Female	1.73	0.73		
Endo LL	Male	2.59	0.82	3.289	.001*
	Female	2.01	0.77		
Ecto S	Male	2.42	0.82	2.944	.004*
	Female	1.91	0.72		
Endo S	Male	3.07	0.86	3.126	.002*
	Female	2.49	0.90		

fusion was observed to start in S1 (1.940) followed by the other divisons S2, S4 and S3 respectively. In the age group of more than 55 years, S1 appeared to fuse slower with a mean of 3.027 compared to the others with mean of 3.162

On comparing the extent of fusion of S1, S2, S3 and S4 divisions of the sagittal suture endocranially, in the age group of 25 to 35 years, the suture closure was found to start at S2 with an average of 2.510 followed by S3 with an average of 2.470, S1 and S4 with average of 2.450 each respectively. Similar sequence of closure was seen in almost all age groups except above the age of 55 years when suture closure was found to be nearly complete in S4 with a mean of 3.757 followed by S2, S3 and S1 with mean of 3.730, 3.703 and 3.649 respectively.

The coronal suture was also divided into three different parts for convenient evaluation of the suture closure stages both on the ectocranial and endocranial sides namely C1, C2 and C3. Ectocranially, C1 was observed to fuse early with a mean of 2.030 followed by C2 (2.010) and C3 (1.990). Complete closure was not seen in the coronal suture ectocranially with the maximum closure being observed in C1 with a mean value of 3.02

It was observed that there was positive correlation between age and amount of suture closure in all the sutures ectocranially and endocranially in all the groups. This correlation was found to be statistically significant in the age groups of 25 to 35 years and 36 to 45 years. It was also significant in the ectocranial coronal suture closure on the left and right sides in the age group of 46 to 55 years. There was no such statistical significance observed in the remaining sutures in the 46 to 55 age category or in cases with an age of more than 55 years as shown in **Table 5**.

When comparison was made between sagittal, coronal and lambdoid sutures regarding the closure timings, it was found that coronal and sagittal sutures were found to close first with a mean value of 3.0 followed by lambdoid suture with a mean value of 2.52 endocranially while on the ectocranial surface, coronal suture closure occurred first

Table 5: Spearman Correlation Between Age, Ectocranial And Endocranial – Left & Right Coronal, Lambdoid And Sagittal Suture Closure Among Various Age Groups.

Age Group (Years)	Variables	Ecto RC	Endo RC	Ecto LC	Endo LC	Ecto RL	Endo RL	Ecto LL	Endo LL	Ecto S	Endo S
25-35	Correlation Coefficient	.237*	.357*	.232*	.380*	.378*	.322*	.378*	.321*	.303*	.344*
	Sig. (2-tailed)	.018	.000	.020	.000	.000	.001	.000	.001	.002	.000
36-45	Correlation Coefficient	.452*	.396*	.452*	.381*	.320*	.372*	.320*	.354*	.414*	. 388
	Sig. (2-tailed)	.003	.010	.003	.014	.041	.017	.041	.023	.007	.012
46-55	Correlation Coefficient	.508*	.282	.508*	.367	.145	.017	.041	.023	.007	.388*
	Sig. (2-tailed)	.016	.203	.016	.093	.519	.941	.709	.585	.072	.074
> 55	Correlation Coefficient	.359	.134	.383	.116	.399	.304	.431	.303	.277	.085
	Sig. (2-tailed)	.290	.428	.079	.495	.145	.067	.088	.068	.097	.616

*Correlation is significant at the 0.05 level (2-tailed)

with an average closure stage of 2.38 followed by sagittal (2.36) and lambdoid (2.02).

DISCUSSION

The human body is a constantly changing entity which undergoes progressive changes during development which can be assessed scientifically.[8] Chronology of the changes associated with aging may present with some physiological variations among individuals. But in the absence of other parameters, these changes can be utilized to estimate the age of the deceased in forensic medicine.[9] The areas of human skeleton which are commonly used for age estimation include pubic symphysis, cranial sutures, sternum and articular surface of the ileum. Cranial sutures were one of the first areas of the human skeleton to be used for age estimation. They remain patent during growth and development to allow the expansion of developing brain.[10]

The maximum number of cases was observed in the age group of 25 to 35 years contributing to 50% of the total sample considered for the study. Among the cases, 87.5% were males while the rest were females which show a greater male predominance in the number of autopsy cases of the Faridkot region. Similar observation was also made in the study by Kalsi et al in 2019.[11]

When bilateral variations were studied for coronal and lambdoid sutures, it was found that there was no statistically significant difference between the right and left sides in either of the cranial sutures. This was similar to the findings in the study done by Upreti et al.[12] This was true on the ectocranial as well as the endocranial surfaces. Another study done by Ramanan et al also reported the absence of bilateral variations.[13]

The suture closure stages of males and females were also compared and it was found that males showed an early union of the sutures in comparison to females of the same age. This pattern was observed in sagittal, coronal and lambdoid sutures. It was in accordance with the findings of the study conducted by Parsons et al[14], Sahni et al[15] and Parmar et al[16] On the other hand, the studies conducted by Kumar et al[17] and Bhengra et al[1] showed earlier fusion of the sutures in females. Another study done by Perizonius et al[11] reported no significant difference in suture closure between males and females.

Another parameter included in the study was the extent of suture closure on endocranial and ectocranial surfaces and their comparison. While the sagittal suture was considered as a whole, the endocranial surface was found to obliterate first with a mean of 3.0 while ectocranial surface suture closure was at 2.36. This finding of faster closure at the endocranial surface in comparison to the ectocranial surface was also similar in the coronal and lambdoid sutures. In the coronal suture, the endocranial union had an average score of 3.0 while the ectocranial union had a mean score of 2.38. While studying the lambdoid suture, the scores for ectocranial and endocranial sutures were 2.02 and 2.52 respectively. This finding was in accordance with the studies done by Kalsi et al[11], Modi et al[18] and Bhengra et al[1] This finding has been uniform in all the studies reported and could imply that endocranial sutures is a better parameter to be utilized in age estimation considering its early fusion.

The results of our present study along with other similar studies point to the unpredictable nature of the sequence of suture closure which makes it difficult to derive a general formula to correlate the age of a person with a particular score of suture closure.

In a study conducted by Oza et al in 2019, the author reported a significant correlation between age and cranial suture closure till the age of 40.[19] This is in accordance with the findings of our study with the exception that our results showed correlation till 45 years of age. In another study done by Ramanan et al, similar significant correlation was found till the age of 50 years and not in the later years.[20]

Thus it can be concluded that there is a definite positive correlation between age and cranial suture closure till the age of 45 to 50 years.

In general, when the various cranial sutures included in the study were compared, it was found that sagittal and coronal sutures closed almost at the same time followed by lambdoid suture on the ectocranial surface while the suture closure sequence was found to be coronal, sagittal and lambdoid sutures respectively on the endocranial surface. Kumar et al in their study also showed similar results with suture closure in the order of coronal, sagittal and lambdoid respectively.[7] Similar finding on the endocranial surface was also reported by Upreti et al in their study done in the year 2019.[12]

A definite correlation was found in our study between the age and stage of suture closure till a certain age. The age derived would be in decades which limit the accuracy of the method. Therefore, it can be concluded that endocranial suture closure could be used as an adjunctive tool in age estimation along with other methods with a fair amount of accuracy.

CONCLUSION

In the present study it was concluded that the endocranial suture closure is a more reliable parameter to estimate the age of an individual in comparison to the ectocranium. Ectocranially, it was found that maximum suture closure in sagittal suture was observed in the age group of more than 55 years while it was in the age group of 46 to 55 years for coronal suture and lambdoid suture.

Ectocranially, the sequence of closure in the sagittal suture in all age groups except more than 55 years, showed pars bregmatica (S1) to close first followed by pars vertices (S2), pars lambdica (S4) and pars obelica (S3) respectively. In coronal suture, pars bregmatica (C1) fused earlier followed by pars complicata (C2) and pars pterica (C3). In Lambdoid suture, fusion was found to occur first in pars lambdica (L1), followed by pars intermedia (L2) and pars asterica (L3). Endocranially, in sagittal suture, it was found that closure was first observed in S2, S3, followed by S4 and S1which showed similar fusion. In coronal suture, C3 was found to close first, followed by C2 and C1. In Lambdoid suture, L3 showed fusion first followed by L2 and L1. Ectocranially, the pattern of suture closure was found to occur first in coronal followed by sagittal and then lambdoid sutures. Endocranially, it was found that coronal and sagittal sutures closed first followed by lambdoid suture. Suture closure was found to occur early in males in comparison to females in all the sutures both ectocranially and endocranially. There was positive correlation between age and suture closure till the age of 45 years after which no significant correlation was observed.

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IMAGE 1 : Skull of a 31 Year Old Male Showing Score of 2 on Ectocranial Sagittal Suture. (PMR/AV/2021/25)

IMAGE 2 : Skull of a 70 Year Old Male Showing Score of 4 on Ectocranial Sagittal Suture. (PMR/AV/2022/80)

IMAGE 3 : Skull of a 40 Year Old Female Showing Score of 3 on Endocranial Coronal, Sagittal and Lambdoid Sutures. (PMR/AV/2021/59

IMAGE 4: Metopism is Seen in 30 Year Old Male Skull. (PMR/AV/2020/49)

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Original Research Paper

Monitoring of Cadmium and Lead Levels in Ayurvedic Medicines Marketed in Delhi- NCR

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ABSTRACT

Introduction: As per Ayurveda, several Herbo-metallic preparations termed as Bhasma are in clinical use since the 8th century AD. The quality and safety of these traditional ayurvedic medicines have become a serious health concern as a large number of the population uses them.

Aim and Objective: The present study was done to detect the lead and cadmium levels in Randomly selected Ayurvedic Bhasma samples by Atomic Absorption Spectroscopy (AAS). Twelve (12) different types of ayurvedic bhasma were collected in a total of forty-four (44) samples were examined.

Result: In the Analytical study it was observed that in many samples lead (Pb) and Cadmium (Cd) was present, also the concentration of lead was higher than that of cadmium. Among them38.6% (17/44) samples have lead (Pb) & 9% (4/44) samples have Cadmium (Cd) in higher concentration which is above the permissible limits.

Conclusion: The study shows that lead (Pb) and Cadmium (Cd) are detected in many samples in higher concentrations which is above the permissible limits and may cause toxicity. Rasa shastra medicine uses heavy metals as part of their preparation as they claim, after properly detoxifying the harmful effects of metal, however, proper quality checks should be ensured of products and strict regulations for local manufacturers should be there in preparation of these bhasma and should be tested for heavy metals concentration and making sure they are within permissible limits.

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INTRODUCTION

Ayurveda is a historic Indian medical system that is widely practiced throughout the Indian subcontinent, among South Asian ethnic groupings, and all over the world. Ayurvedic medicines are used by 80% of Indians, according to estimates. Additionally, its use is growing in popularity in Western nations as well.[1] According to a World Health Organization (WHO) report, many individuals in underdeveloped nations still use herbal remedies to cure a variety of illnesses. Over-the-counter (OTC) health foods, pharmaceuticals, and nutraceuticals derived from plants or various natural sources are gaining popularity in developed countries. These metals contribute to environmental pollution from sources like leaded gasoline, industrial waste, and acid rain, which leach metal ions from the soil into lakes and rivers.[2] Ayurveda, Siddha, and Unani-Tibb are the three main medical systems used in India. These systems make use of medicines made from plants, animals, and minerals that are derived naturally.

However, recent reports of excessive levels of heavy metals in herbal or Ayurvedic remedies have sparked controversy and much worry. One of five Ayurvedic herbal therapeutic remedies (Ayurvedic formulations) made in South Asia, contains potentially hazardous quantities of mercury, Lead, and/or arsenic.[3] Lead, mercury, and arsenic have been found in US- and Indian-manufactured Ayurvedic medications that are being marketed online, according to a

different investigation. Researchers have also observed that herbal medicines used in China and the Indian subcontinent contain more heavy metals than herbal medicines used in other parts of the world and that long-term use of these medicines may be hazardous.[4] According to analytical research, 20% of Ayurvedic goods marketed in Boston and 30% to 65% of those sold outside the United States contain heavy metals beyond the permitted level. The Chicago, Houston and Canadian markets most recently reported Ayurvedic medication containing harmful metals.[5] Ayurvedic practitioners typically create their medications, which are typically based on botanical ingredients.[6] It is a fundamental premise of Ayurveda that anything can be utilized as a medication, only herbal extracts, or in the combination of herbal extracts with animal, vegetable, and mineral products.[7] The heavy metals found in Ayurvedic goods are typically introduced voluntarily rather than as pollutants. The "Vishagarva-jrodhika Tantra" is a specific area of Ayurveda that deals with toxicity and certain Ayurvedic scriptures recognize that these heavy metals and possibly other ingredients of Ayurvedic remedies may be related to severe toxic effects.[8] The metals in Ayurvedic goods are frequently "detoxified" using specific processes that are frequently advised. The usage of "mineral herbs" or other herbal items like tamarind, as well as heating and chilling processes for products containing buttermilk, cow's urine, and sesame oil, are among these techniques.[9] There have been reports of products containing up to 84% heavy metal concentration, so while these methods may affect heavy metal bioavailability, it is unlikely that they will have a substantial enough impact to "detoxify" them.[10] Evidence from numerous nations suggests that dangerous heavy metals and unreported prescription pharmaceuticals may be included in Asian herbal medicines, which could pose a major health risk.[1]

A 58-year-old lady had abdominal pain, anemia, impaired liver function, and a high blood lead level. It was discovered that the patient had been using the Ayurvedic drug Jambrulin before going to the doctor. High quantities of lead were discovered during the medication's chemical examination. This incident emphasizes the need for greater awareness of the danger of toxicities connected with the use of some Ayurvedic medications, which may contain potentially harmful quantities of heavy metals.[2]

To describe a cluster of lead and mercury toxicity cases that occurred in 2011 within an Ayurvedic community of practitioners, Following the discovery of the index case, heavy metals screening was made available to Ayurvedic practitioners. The usage of Ayurvedic supplements has been linked to the greatest cluster of lead and mercury toxicity ever documented in the US. The metal is "purified-out" during the traditional manufacture of bhasmas through repeated chilling and heating cycles as well as the use of particular "mineral herbs." However, due to poor quality control that permits contamination, adulteration, or ineffective purification in current formulations, the concentration of heavy metals may be high.[3] In a study at AIIMS Delhi, there were 78 formulations in total, of which 56 were herbal, 19 were Herbo metallic, and 3 were metallic. Lead was above the limit in herbal formulations at 19.6% (11/56), mercury at 5.3% (3/56), arsenic at 5.3% (3/56), and cadmium at 21.4% (12/56), All metal compositions contained heavy metals over the WHO standard.[4]

It has been observed that the presence of heavy metals is detected in Ayurvedic medicines which may produce toxicity in the body. In present study we are collecting the samples of Ayurvedic Medicines (Bhasma) after doing a thorough literature review, for detection of Lead & Cadmium in the samples.

MATERIAL & METHODS

The study has been conducted at Division of Forensic Science, Galgotias University ,greater Noida. The Instrumental Analysis has been done at Department of Forensic Medicine &Toxicology, AIIMS, New Delhi.

All Ayurvedic medicine samples were randomly selected from local markets of Delhi -NCR and also sold via the Internet. The Product name, manufacturer details, date of manu-facturing, expiry date, batch number, texture, the color of the powder, and the number of ingredients were all taken into consideration &recorded for each sample.

Total forty-four (44) Ayurvedic Medicines (Bhasma) samples have been collected, which are of twelve different types. Each sample has been assigned a unique code.

For the digestion of samples open digestion and microwave digestion were used.

Microwave Digestion: The Vessels of Digester (volumetric flasks) were thoroughly cleaned by leaching them in (1:1) nitric acid for 15-20 minutes, then rinsing them with Milli-Q water. 0.5g of finely powdered sample was put into the Teflon sample vessel. To each sample, 7ml concentrated HNO_3 (65%) and 1ml H_20_2 (30%) were added. If a violent reaction occurred, it was cooled before putting the cap on the vessel. The vessel was capped and

torqued. On Completion of digestion, samples were cool down and transferred to a graduated test tube.

Open Digestion Method: 0.5g of sample was taken in a beaker and added 7ml of Conc HNO_3 and then 1ml of Hydrogen peroxide was added and kept the mixture for 24-36 hrs. The sample was heated at 60^0 C for 10 min and filtered with Whatman filter paper (125mm) and the filtrate was collected and made up to 50ml.

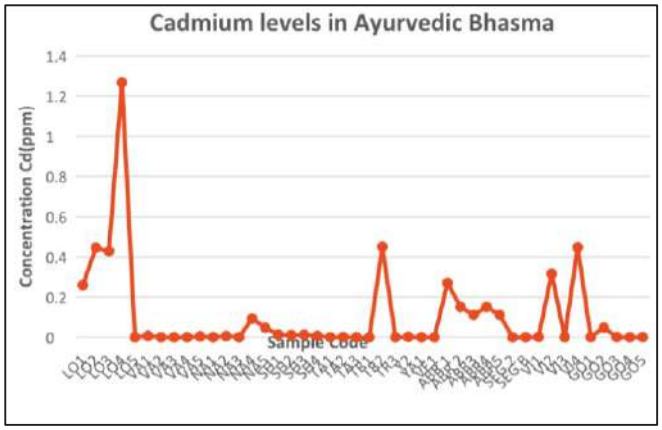
Atomic Absorption Spectroscopy (AAS9000 Jiangsu Skyray JL/TR-YF-035 with flame and graphite atomic absorption spectrophotometer with Hydride Generator HG600S). As per metal and flame ignition, the wavelength, slit width, lamp current, and energy heat transmission (EHT) were all set. Readings of the standard and sample were taken after the instrument was reset to zero. The solution was capillary fed directly from the bottles into the nebulizer, converted into an aerosol, and directed into a spray chamber to remove large droplets. A hollow cathode lamp emitted a narrow spectral line that was used to excite the free atoms formed in the flame.

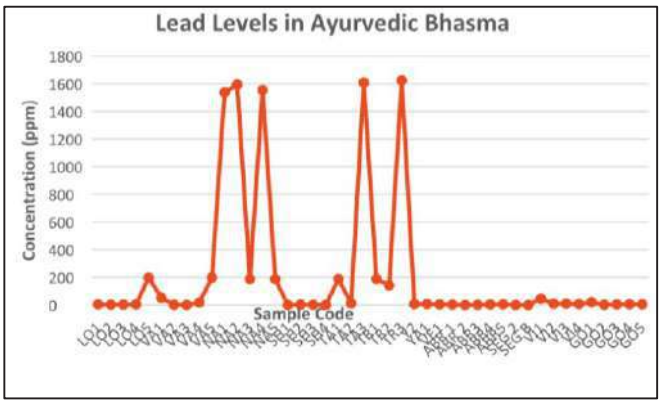
Calibration and Quality Control: The number of Standards were prepared for covering the working range: **0.05 to 0.4 mg/L (or 50-400ppb) for lead (Pb), 0.025 to 1mg/L or (25-1000ppb) for cadmium (Cd)**, working standards in volumetric flasks were stored and they were freshly prepared weekly and analyzed alongside the standard blank and the sample. Calibration graphs were plotted for absorbance v/s concentration (µg/L) were prepared. Frequently Standards were aspirated after an interval of every 10 samples to check for Instrument drift. The concentration of heavy metals in samples was calculated using standard calibration curves. The calibration graphs were used to calculate the corresponding concentrations (µg /L) of heavy metals in the samples.

RESULTS

A total of forty-four 44 Ayurvedic Medicines of Rasa-sastra were screened for heavy metal contents. **As per Ayurvedic Pharmacopoeia the permissible limit for Lead is 10ppm and for Cadmium its 0.3ppm.**[11,4]

The study showed that in 44 Rasa shastra or Ayurvedic bhasma 65.9% (29/44) samples detected cadmium among them 9% (4/44) samples have cadmium above the permissible limit and 88.6% (39/44) samples detected lead and 38.6% (17/44) samples have lead above the permissible limit. In 34% (15/44) samples Cadmium was not detected, and in 11% (4/44) samples lead was not detected.





DISCUSSION

In the present study, we randomly selected commonly easily available ayurvedic preparations from the local market of Delhi-NCR and via the internet also. A large amount of ayurvedic Medicines is available for the treatment of various disorders like diarrhea, fever, and thyroid. Kidney stones, Arthritis, Blood pressure, Diabetes, etc. The ayurvedic Medicines were available in the form of fine powder. They were analyzed by Atomic Absorption Spectrophotometer (AAS) for the detection of Cadmium & Lead in the sample. It has been observed that there is a variation in the concentration of lead and cadmium in each type of sample. Among Lead (Pb) & Cadmium (Cd) lead was present in a much higher concentration than that cadmium. The presence of these Metal in Ayurvedic Medicine may be due to contamination during manufacturing process or deliberately addition for therapeutic use.

CONCLUSION

It is important to determine the levels of heavy metals in ayurvedic medicines as their popularity is increasing worldwide, due to lesser side effects, but it's very essential

to make sure the standard quality parameters are followed. But in recent times it has become a matter of great concern because of the negligence of some standard guidelines for preparation and the presence of heavy metals in these preparations. Some Rasa shastra medicine uses heavy metals as part of their preparation as they claim after properly detoxifying the harmful effects of metal is lost, however, a proper quality check should be ensured of product and should be tested for heavy metals concentration and making sure they are within permissible limits. Also, more pharmacological & Toxicological studies are required for the proper assessment of these Ayurvedic Bhasma and strict protocols & Guidelines should be ensured for manufacturers.

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Original Research Paper

Estimation of Stature from Foot Length in Sub - Adult North Indian Population

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ABSTRACT

Introduction: Stature, or height of a person is an important marker of identification which, in turn, is the linchpin of forensic investigations. The stature may be gauged from the measurements of several organs, the feet being one of these.

Materials and Methods: The present study endeavors to establish a correlation between the mean foot length and height of sub-adults belonging to North Indian region with the aid of a linear regression equation. The regression formula has been propounded separately for right foot length and left foot length, treating all the subjects as a single set.

Results: Such an investigation is anticipated to help identify the victims of natural and man-made disasters from fragmentary and dismembered human remains.

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INTRODUCTION

The height of an individual is one of the most important parameters for ascertaining his or her identity.[1] This is especially true in cases pertaining to forensic medical examinations or anthropological investigations.[2] However, in mass disaster episodes - whether of natural or man-made origin-very often only mutilated human remains are recovered, thus obviating the possibility of directly measuring the height of the deceased. Under these circumstances, the investigators have to resort to an indirect yardstick to ascertain the stature of the victim.[3] One way to achieve this end is to measure the long bone and extrapolate the data.[4] The methodology entails pre-treatment of long bone, which itself is a time-consuming and complicated process.[5] Alternately, the foot length may be measured and correlated with the stature of the individual.

Experience has shown that the measurement of foot length is not only easy and simple, but its reliability in predicting the stature is comparable to to that achieved by long bone anthropometry.[6] Moreover, the ossification in foot occurs prior to the mineralization of long bone. This means that during adolescence, stature is more accurately predicted from foot measurements than from long bone measurements.[7]

In this communication we report the results of a cross-sectional survey to determine the interrelationship between foot length and stature of sub-adults residing in North India. The outcome of this study reveals that the stature and foot length cognate with each other.

MATERIALS AND METHOD

The present study was conducted at Amity University, Manesar (Haryana) on 171 subjects (100 male and 71 female), aged between 17-25 years. Although all the sub-adult student participants belonged to North India, there was considerable variations amongst them in terms of religion, caste, language and culture.

For measuring the height, each student was made to stand bare feet against a vertical metallic plate graduated in centimeters. Care was taken to ensure that the subject stood in upright position, with heel and occiput touching the metallic plate. A thin strip of cardboard was placed on the vertex of the head and horizontally extended to the metallic plate. Height was measured as the distance between the heel and the point at which the horizontal cardboard strip touched the measuring device.

The length of the foot was measured (in cm) from the

outermost margin at the back of the heel to the longest toe with the aid of vernier caliper. The part of nail protruding past the toe was not included in the measurement.

The height of each participant was calculated from his/her left and right foot lengths with the help of regression equations. It was compared with the recorded stature.

RESULTS

The statistical data on foot length and height of 171 sub-adult subjects (100 males and 71 females) was analyzed using the JASP 0.9.0.0 software. As shown in **Table 1**, the right foot length of male subjects varied between a minimum of 23.50 cm to a maximum of 29.20 cm.

The corresponding figures for the left foot length were 23.50 cm and 30.00 cm, respectively. Likewise, for female subjects, the minimum measurement for right foot length was 21.50 cm, while the maximum measurement was 27.50 cm. The left foot length for females was in the 21.0 to 27.0 cm range.

Table 1: Sex Wise Distribution of Foot Measurement Data

Parameter	Right Foot		Left Foot	
	Male	Female	Male	Female
Sample Size	100	71	100	71
Minimum Length (cm)	23.50	21.50	23.50	21.00
Maximum Length (cm)	29.20	27.50	30.00	27.00
Mean Length (cm)	26.44	23.83	26.43	23.72
Standard Deviation	1.317	1.094	1.271	1.044

The average length of right and left foot of male subjects was 26.44 cm and 26.43 cm, the standard deviation being 1.317 and 1.271, respectively. The mean for right and left foot lengths of female subjects measured 23.83 cm and 23.72 cm, with a standard deviation of 1.094 and 1.044, respectively.

Table 2 lists the sex wise distribution of stature of the subjects. For males, the height varied between 159.5 cm to 187.0 cm, the average value being 174.3 cm, with a standard deviation of 5.949. For females, the height range of 138.0-169.0 cm had a mean value of 156.9 cm and a standard deviation of 6.568.

The combined data pertaining to foot length and stature measurements has been displayed in **Table 3**. The mean value of right and left foot length comes out to be 25.35 cm and 25.30 cm, with a standard deviation of 1.781 and 1.785, respectively.

The mean value of stature of all subjects is 167.1, the standard deviation being 10.570.

Table 2: Sex Wise Distribution of Stature Data

Parameter	Stature	
	Male	Female
Minimum Height (cm)	159.5	138.0
Maximum Height (cm)	187.0	169.0
Mean Height (cm)	174.3	156.9
Standard Deviation	5.949	6.568

Table 3: Combined Data of Foot Length and Stature of all Subjects

Parameter	Stature	Right Foot Length	Left Foot Length
Sample Size	171.0	171.00	171.0
Minimum (cm)	138.0	21.50	21.0
Maximum (cm)	187.0	29.20	30.0
Mean (cm)	167.1	25.35	25.30
Standard Deviation	10.570	1.781	1.785

DISCUSSION

In line with the findings of other authors,[10-1] males exhibited a higher mean value of right foot length, left foot length and stature than females. This may be attributed to the early maturity of girls as compared to boys which, in turn, happens because the union of ossification centers in females occurs at a younger age than in males. Vidya et al[12] has reported that the left foot is slightly lengthier than the right foot in both the genders. In our study, the average right foot length was greater than its left foot counterpart, the difference being more pronounced in females than in males. However, since Grivas et al[13] has suggested that right and left foot lengths can predict the stature independently of each other, we have proffered separate regression equations for each feet. These equations are as follows.

$Y = 36.724 + 5.141a$

$Y = 35.756 + 5.190b$

Y is the height of the subject; a is the right foot length; and b is the left foot length.

There is a profound correlation between height and right foot length ($r = 0.877$, $p < 0.001$), as well as between height and left foot length ($r = 0.876$, $p < 0.001$). This indicates that foot measurements bear a biological association with stature.[14] Statistically high and positive values of correlation coefficients obtained for the right foot length ($r = +0.877$) and the left foot length ($r = +0.876$) suggest that either of the foot may be used to predict the stature by the regression formula. This implies that the stature of an unknown person, irrespective of the gender, may be gauged from foot measurements with an appreciably high

degree of reliability and accuracy.

CONCLUSION

The results of the present study show that there exists a positive and significant correlation between stature and foot length. The dimensions of left or right feet can be used for estimating the height of North Indian sub-adults of either gender by linear regression analysis.

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Original Research Paper

A Statistical Comparison Between Handwritings of Visually Impaired and Normal Sighted Writers

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ABSTRACT

Introduction: This communication reports a comparison of handwriting characteristics of visually challenged persons vis-a-vis that of uninstructed, functionally illiterate persons with normal eye sight.

Materials and Methods: This study involves a comparison of handwriting characteristics in 80 samples, half of which were collected from trained, but visually impaired persons and the remaining half from untrained, but normal sighted persons.

Results: The similarities and differences in the handwriting characteristics of the two groups have been analyzed on the basis of the null hypothesis and t test results.

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INTRODUCTION

Forensic handwriting comparison is a key component of the criminal justice system since it connects a questioned document with its author. Handwriting is a learned neuromuscular activity which, being dynamic in nature, is affected by a range of factors.[1] The factors, in turn, are controlled by a host of physical and mental parameters. The physical parameters include eye-hand coordination and writing posture, while the mental parameters include concentration and dexterity.[2]

Handwriting characteristics may be classified into two broad types: Style and personal.[3] The style characteristics, such as cursive or non-cursive calligraphy, evolve mainly as a result of the model education system.[4] The personal characteristics, on the other hand, are changes brought about in the style form, both consciously and unconsciously. These are a function of letter form, line form and formatting.[5]

In the present study, we took handwriting samples from two sets of persons. The first set comprised of visually challenged persons (including those with extremely low vision) who had received some level of formal training in a model institute, while the second set comprised of semi-literate school dropouts. The style characteristics of both the groups are anticipated to be comparable. Dexterity, a component of personal characteristics, is not expected to vary much. However, the visually impaired candidates face the problem of eye-hand coordination, while the semi-literate candidates suffer due to lower ability to focus and concentrate on a topic. In this context it is pertinent to gauge the similarities and differences in the handwriting characteristics of the two groups.[6]

MATERIALS AND METHOD

A total of 80 handwriting samples were collected from females and males of different age groups and varied educational qualifications. All were residents of different districts of Delhi. Of these 40 samples were obtained from persons who were visually impaired or had very poor eyesight, but had received some formal education in institutes meant for physically challenged people. The remaining 40 samples were obtained from persons who had normal eyesight but were only functionally literate and school dropouts.

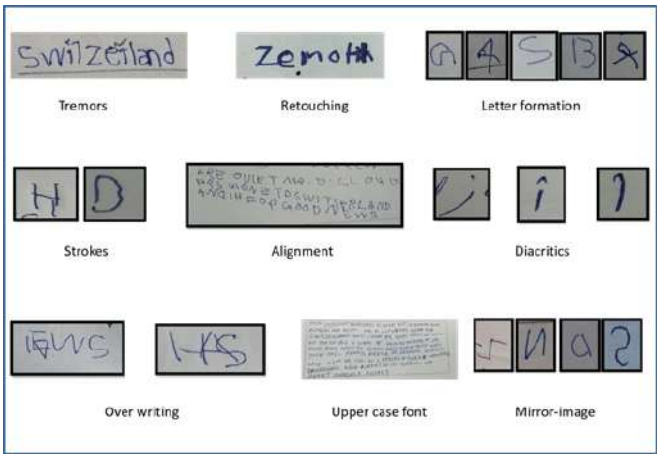
Each participant was provided with A-4 sheets from the same ream and ball pens of same company. A script containing several proper nouns, numerals and

punctuation marks was provided to them. Each person was asked to reproduce the script in her/his handwriting in duplicate. The inter- and intra- group comparisons of handwriting samples was carried out with the aid of Null hypothesis t test, by using a JASP software.

RESULTS AND DISCUSSION

The data pertaining to the handwriting characteristics of 80 samples has been analyzed both qualitatively and statistically. As is usually the case, visual impairment had profound influence on handwriting characteristics.[7] It has been observed that the handwriting of visually challenged persons depicted letters of larger size, as well as greater unevenness in spacing between lines and words as compared to the samples written by semi-literate persons. Moreover, the persons with low or blurred vision found it difficult to make a distinction between letter casings [8]. Representative examples of personal characteristics seen in both sets of handwritings are shown in **Figure 1**.

Figure 1: Representative personal characteristics in handwriting samples



A comparison of the two sets of handwritings are displayed in **Table 1**. While 95% of handwriting samples collected from low-vision persons indicated evidence of tremors in letter formation, only 27.5% samples obtained from normal sighted persons revealed this personal characteristic. The zig-zag and/or wavy strokes, especially in curved letters were indicative of tremors. This, in turn, may be attributed to meager eye-hand coordination in the participants from visually challenged category.[9] A substantially large difference in the handwriting characteristics amongst the visually impaired and normal sighted subjects was also observed in manifestation of distorted strokes in the calligraphic scripts. Broken strokes were found in 77.5% of samples obtained from visually impaired category; the corresponding figure for normal sighted category was just 10%. The low level of neuro-muscular coordination in persons with impaired vision is responsible for improper depiction of letters.[10] Retouchings, in form of spelling corrections and obliterations, were observed in 55% samples of visually handicapped persons, and 65% samples of normal sighted persons. The relatively comparable results may be explained on the basis of the fact that handwriting is taught from a model system[4] and both the groups had only preliminary access to this institution.

It has been reported in literature that visually impaired persons prefer to use capital letters while scripting a document, and that their calligraphy is endowed with copious over writing and reverse letter (mirror-image) characters.[11] Our study confirmed these findings. While 50% of visually impaired participants preferred to use upper case font, 7.5% and 27.5% samples from this group showed over writing and mirror-image characteristics. Not a single handwriting sample collected from the semi-literate group showed capital casing, over writing or

Table 1: Qualitative Assessment and Statistical Analysis of Handwriting Samples

Handwriting Characteristics Arising Due To:	Qualitative Assessment (Number of Samples)		Statistical Analysis		
	Visually Impaired (40)	Normal Sighted (40)	p value	t value	Mean Difference (Standard Deviation)
Tremors	38	11	<0.001	-9.000	-0.675 (0.075)
Retouching	22	26	0.352	0.941	0.100 (0.106)
Letter Formation	23	10	0.008	-2.816	-0.325 (0.115)
Strokes	31	04	<0.001	-9.000	-0.675 (0.075)
Alignment	30	07	<0.001	-5.718	-0.575 (0.101)
Diacritics	15	07	0.058	-1.951	-0.200 (0.103)
Over Writing	03	Zero	0.323	-1.000	-0.075 (0.050)
Upper Case Font	20	Zero	<0.001	-5.421	-0.500 (0.088)
Mirror-images	11	Zero	<0.001	-3.606	-0.275 (0.069)

mirror-image characteristics.

The statistical analysis of the characteristics in handwriting samples was carried out with the aid of Null hypothesis[12] and t test.[13] The results are incorporated in **Table 1**. The factor P in Null hypothesis refers to the probability of similarity, which means that grater the value of P, more is the similarity between the two sets. Conversely, the decreasing values of P suggest progressively less similarity or more dissimilarity. Below a threshold value of 0.05, there is so much variations between the components of two sets that Null hypothesis loses its validity. In our study this situation arises with reference to handwriting characteristic attributed to tremors, strokes, alignment, upper case font and mirror images, and marginally in context of diacritics. It implies that there will be such vide variations in the two sets of handwritings that a forensic document expert will be easily able to discern the identity of the author on the basis of these characteristics. The smallest difference has been observed in case of characteristics arising due to retouching and over writing. As expected, the values of t test are inversely proportional to the P values. The higher t values for tremor and strokes related characteristics suggest wide variations, while smaller t values for retouching and over writing imply relatively less variations in the handwriting samples of two groups.

CONCLUSIONS

The personal characters in the hand writing samples of visually impaired persons have been compared with those of semi-literate persons. The two sets of calligraphy show large variations with reference to characteristics related to tremors and strokes. The occurrence of retouching and over writing is less varied in the two sets.

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Original Research Paper

Mandibular Ramus: An Indicator For Sex Determination – A Digital Radiographic Study

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ABSTRACT

Introduction: The identification of skeletal remains is of paramount importance in medico-legal investigations. Identification of sex from skeletal remains is an important tool in forensic science. Mandibular ramus can be used for sex determination either on dry mandible or through orthopantomogram (OPG). Presence of a dense layer of compact bone makes it very durable and well preserved than many other bones. Mandibular ramus can be used to differentiate between sexes and it also expresses strong univariate sexual dimorphism.

Aim and Objectives: The aim of the present study is to measure, compare, and evaluate various measurements of the mandibular ramus, observed in digital orthopantomographs and also to assess the usefulness of the mandibular ramus as an aid in gender determination.

Materials And Method: A retrospective study is conducted using orthopantomographs of 50 subjects (25 males and 25 females) with age range between 21–50 years, taken using CS 8100 CARESTREAM Digital Panoramic machine (73 kVp, 12 mA, 13.9 s). This study is done using archival records from HPGDC, Shimla. Mandibular ramus measurements are carried out using GIMP 2.10.30 software.

Results: In this study the data is tabulated and results are statistically analysed.

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INTRODUCTION

The identification of human skeletal remains is considered an initial step in forensic investigations and is crucial for further analysis.[1] Gender estimation of the bone is a very important part of a study in the field of anthropology and forensic sciences as further interpretations and analysis are based on it. Normally, morphological and metric analyses are used to determine the sex of the bone.[2] The reliability of sex determination depends on the completeness of the remains and the degree of sexual dimorphism inherent in the population.[1] The sex of an unknown individual can be determined based on the data from the morphology and metric features of the skull and the mandible, soft tissues, dental records as well as by DNA analysis of teeth.[2] When the entire adult skeleton is available for analysis, sex can be determined up to 100% accuracy, but in cases of mass disasters where usually fragmented bones are found, sex determination with 100% accuracy is not possible and it depends largely on the available parts of skeleton. As evident from the earlier studies, skull is the most dimorphic and easily sexed portion of skeleton after pelvis, providing accuracy up to 92%. But in cases where intact skull is not found, mandible may play a vital role in sex determination as it is the most dimorphic, largest, and strongest bone of skull. Presence of a dense layer of compact bone makes it very durable, and

hence remains well preserved than many other bones. Dimorphism in mandible is reflected in its shape and size. Male bones are generally bigger and more robust than female bones.[1]

Sex determination was considered the foremost step in identification of cases at mass disasters, explosions, air hurricanes where bodies are damaged beyond recognition and is the most challenging task for forensic experts for identification, followed by age and stature estimation as both are sex dependent.[3] It is the strongest and movable part of the skull. Its morphological features show changes with reference to age, sex, and race.[2] Moreover, occlusal status, age of the subject, and constant remodeling of the mandibular bone also results in morphological changes of the mandible.

Hence, in the present study different ramus metric measurements on digital panoramic images were considered as indicators for sex determination and we aim to determine the efficacy of mandibular ramus in sex determination.[3]

AIMS AND OBJECTIVES

The present study was designed with the following aims and objectives:

1. To measure, compare, and evaluate the various measurements of mandibular ramus as observed on digital orthopantomographs.
2. To assess the usefulness of mandibular ramus as an aid in sex determination.

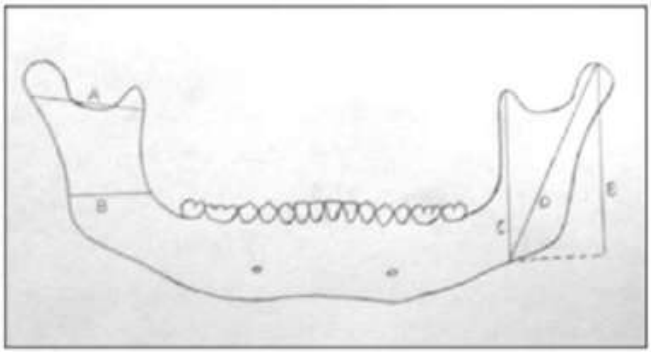
MATERIALS AND METHODS

A retrospective study was conducted using orthopantomographs of 25 males and 25 females of Himachal population in the age group between 21 and 50 years.

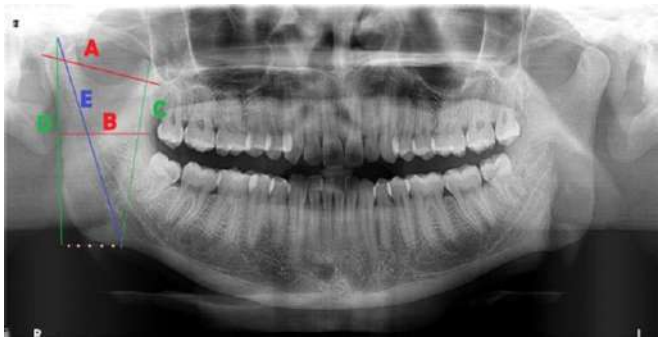
Ideal orthopantomographs of completely dentate patients were selected for the study. Pathological, fractured, developmental disturbances of the mandible, deformed and edentulous mandibles were excluded from the study. Radiographs taken by Carestream 8100C Digital Panoramic System (73 kVp, 12 mA, 13.9 s) were used for the study. Since this study was conducted on radiographs stored in the system, ethical clearance was not applicable. Mandibular ramus measurements were carried out using SPSS 24.0 version software.

The following parameters were measured using GIMP 2.10.30 software (by moving the mouse and drawing lines using chosen points on the digital panoramic radiograph) **(Figures 1 and 2)**:

Figure 1: Schematic diagram with five parameters on mandibular ramus where A: Maximum ramus breadth, B: Minimum ramus breadth, C: Coronoid Height, D: Condylar/Maximum ramus height, E: Projective ramus height.



A: Maximum ramus breadth
B: Minimum ramus breadth
C: Coronoid Height ,
D: Projective ramus height
E: Maximum ramus height



1. Maximum ramus breadth: The distance between the most anterior point on the mandibular ramus and a line connecting the most posterior point on the condyle and the angle of jaw.
2. Minimum ramus breadth: Smallest anterior–posterior diameter of the ramus.
3. Condylar height/maximum ramus height: Height of the ramus of the mandible from the most superior point on the mandibular condyle to the tubercle, or most protruding portion of the inferior border of the ramus.
4. Projective height of ramus: Projective height of ramus between the highest point of the mandibular condyle and lower margin of the bone.
5. Coronoid height: Projective distance between coronion and lower wall of the bone.

A: Maximum ramus breadth

B: Minimum ramus breadth

C: Coronoid Height ,

D: Projective ramus height

E: Maximum ramus height

STATISTICAL ANALYSIS

The data was tabulated in Microsoft excel and analysed with SPSS v.24 software. Independent t test was used for the comparison between the groups. Discriminant analysis was used for the prediction. The p value≤0.05 was considered as statistically significant (*).Discriminant function analysis was used to determine variables that discriminate between male and female and is increasingly utilized for sex diagnosis from skeletal measurements.

RESULTS

Descriptive statistics of five mandibular ramus measurements and associated univariate F ratios for both sexes are shown in **Table 1**. We noticed that each variable was a significant predictor in classifying a given sample (P value ≤0.05). The mean values showed that all dimensions were higher for males compared to females. Mean measurements between males and females are shown in **Figure 3**. The F-statistic values indicated that mandibular measurements expressing the greatest sexual dimorphism were coronoid height followed by projective height of ramus and then maximum ramus height.

The sex could be determined from calculations using the equation given below:

D= -17.226 + (0.009 x Max. ramus breadth) + (-0.012 x Min. ramus breadth)

+ (-0.013 x Max. ramus height) + (0.126 x Projective height of ramus)

+ (0.174 x Coronoid height)

With all the variables in consideration, 74% of the cases were classified correctlys. In this study, the sectioning point was found to be 0.016. Values greater than this sectioning point indicate male and values lesser than this point indicate female.

DISCUSSION

One of the important aspects of Forensic dentistry is the determination of the gender from remains of jaws and dentition.[4] Sex determination in an unidentified skeletons may be extremely complicated in cases of explosions, warfare and other mass disasters because of skeletal fragmentation. Identification of skeletal remains holds a prime importance in forensic medicine and anthropology especially in criminal investigations. Main attributes of biological identity include sex, age, stature, and ethnic background of individual which are called 'Big Four' in forensic context.[5] Panoramic radiographs have been advocated routinely as a one of the appropriate screening tool for diagnosis of oral diseases. The principal advantages of panoramic image is its broad coverage, low patient radiation dose, short time required for image acquisition and has been a very good source for retrospective studies. Several studies have been reported that panoramic radiographs are reproducible and accurate for the linear and angular measurements on mandibles. The advantages of panoramic images are its broad coverage, low patient radiation dose, and the short time required for image acquisition. The other advantages are that interference of superimposed images is not encountered, whereas the contrast, brightness enhancement, and enlargement of images provide an accurate and reproducible method of measuring the chosen points. The disadvantages are magnification and geometric distortion, the vertical dimension in contrast to the horizontal dimension is a little altered, and this technique is quite sensitive to positioning errors because of a relatively narrow image layer.[2]

In the present study, mandibular ramus measurements were subjected to discriminant function analysis.Each of the five variables measured on the mandibular ramus using orthopantomographs showed statistically

Table 1: Descriptive Statistics

Variable	Male		Female		Wilk's Λ λ	F	p value
	Mean	SD	Mean	SD			
Max. Ramus Breadth	39.00	4.67	36.07	3.75	0.889	5.947	0.018*
Min. Ramus Breadth	27.86	3.36	25.38	2.84	0.858	7.926	0.007*
Max. Ramus Height	70.15	4.82	64.68	3.91	0.713	19.362	<0.001*
Projective Height of Ramus	66.45	4.96	60.67	3.10	0.664	24.303	<0.001*
Coronoid Height	60.72	4.04	55.18	3.35	0.633	27.782	<0.001*

Table 2: Classification Function Coefficient

Variable	Male	Female
Constant	-168.855	-141.028
Max. ramus breadth	0.195	0.180
Min. ramus breadth	-0.061	-0.041
Max. ramus height	1.055	1.077
Projective height of ramus	1.530	1.326
Coronoid height	2.547	2.267

Table 3: Prediction Accuracy

True Group	Predicted Group Membership		Total	% Accuracy
	Male	Female		
Male	19	6	25	74%
Female	7	18	25	

significant sex differences between sexes, indicating that coronoid height expresses strong sexual dimorphism in terms of minimum ramus breadth, maximum ramus breadth, condylar height, projective height of ramus, and maximum ramus height. Overall, the prediction rate using all five variables was 74%. A similar study by Dayal et al6 has shown an accuracy of 75%, Ajit et al[2] their study achieved an accuracy of 83% with five mandibular parameters.

Giles measured mandibles of known sex using anthropometric measurements and reported mandibular ramus height, maximum ramus breadth, and minimum ramus breadth as highly significant, with an accuracy of 85% in American Whites and Negroes.[7]

Steyn and Iscan (1998) achieved an accuracy of 81.5% with five mandibular parameters (i.e. bigonial breadth, total mandibular length, bicondylar breadth, minimum ramus breadth, and gonion–gnathion) in South African Whites.[8]

Dayal et al. (2008) found mandibular ramus height to be the best parameter in their study, with 75.8% accuracy.[6]

Saini et al.[9] conducted a study on dry adult mandibles of northern part of India and found that ramus expresses strong sexual dimorphism in this population. The overall prediction rate using five variables was 80.2%. The best parameters were coronoid height, condylar height, and projecttive height of ramus, and breadth measurements were not very dimorphic in their sample.

In the present study, the dimensions of different parameters on mandibular ramus when correlated with sex; it was distinctly observed that the mean values were significantly higher in males when compared to females and were statistically highly significant ($P < 0.001$). This finding matched with the study of Indira et al1 and More et al.[10]

This present study, found out that the sexual differences were highest in the coronoid height> Projective height of ramus> Maximum ramus height> Minimum ramus breadth> Maximum ramus breadth.

CONCLUSION

Mandibular ramus can be considered as a valuable tool in gender determination since it possesses resistance to damage and disintegration processes. We found that mandibular ramus measurements using orthopantomographs were reliable for sex determination. Hence, we strongly suggest the use of mandibular ramus as an aid for gender determination in forensic analysis. In view of these findings, further studies on more diverse populations to assess the significance of these parameters are recommended. The limitations of this study are a failure to consistently designate sex in the subadult range and to assess the gender in edentulous cases.

Conflicts of Interests: Nil

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Table 4: Standardized and Unstandardized Coefficients in Original Samples

Variable	Discriminant Function Coefficients	Standardized Coefficients	Standardized Coefficients	Sectioning Point
Max. Ramus Breadth	0.009	0.038	0.428	0.016
Min. Ramus Breadth	-0.012	-0.037	0.493	
Max. Ramus Height	-0.013	-0.059	0.770	
Projective Height of Ramus	0.126	0.523	0.863	
Coronoid Height	0.174	0.645	0.923	
Constant	-17.226	---	---	

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Original Research Paper

Maxillary Sinus - A Tool For Gender Determination In The Himachali Population

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ABSTRACT

BACKGROUND: Sex determination is a crucial step in creating the biological profile of unknown human remains, especially that have badly decomposed, or have been distorted by any natural, or man-made disasters. Forensic science deals with skeletal examination with primary focus on identification although complete identification is hardly possible; however, anthropological identification can be done in skeletal examination. Skull is the second best for determination of sex after pelvis. Maxillary sinuses are paranasal sinuses, and possesses features of sexual dimorphism, they may remain intact even in partially charred, decomposed or fractured skulls.

AIM: To determine the reliability and accuracy of maxillary sinus dimension measurement as a method for gender through the use of reconstructed computed tomographic images.

METHODOLOGY: A retrospective study was performed on previously acquired CT scans using GE 64 slice CT Scanner, within the Government Medical College and Hospital database. The study was designed to measure volume; MSVOL, medio-lateral (width)MSW, superio-inferior (height); MSH, and anterio-posterior (length); MSL, dimensions of the maxillary sinus. A total of 120 (60 females and 60 males) CT head and neck cases were studied and measured using radiANT DICOM viewer (64-bit) and inbuilt electronic caliper.

RESULTS: Descriptive analysis and discriminant analysis were used for assessment of data obtained. In all measurements Female group had statistically significant lower values for both right and left maxillary sinus ($p < 0.05$). Comparison of the parameters between right and left sides in males and females showed no significant difference. Calculated value ''D'' is greater than reference D, it is indicative of male gender, and if ''D'' value is less than the reference values, this is indicative of female gender.

Conclusion: Maxillary sinus is good tool for sex determination in Himachali population.

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INTRODUCTION

Gender identification is a common but crucial requirement for identifying unknown human remains in the wake of natural calamities like tsunamis, earthquakes, landslides, floods and disasters created by man like terrorist attacks, mass murders, or bomb blasts. Gender identification can alone narrow the research to half of the missing person cases.[1] Sex estimation is typically the primary step in creating the biological profile, which also involves assessing age, stature, pathology, ancestry etc. For this specific purpose the usual parameters used are craniofacial height, mastoid height, bicondylar dimension, articulator dimension, pelvis, skull, long bones with assessment of epiphysis and process, the circumference of the top, mastoid bone, length of the superciliary arch the maxillary and frontal sinuses.[2] According to Sharma (2014) “main reliable bone exhibiting sexually dimorphic traits is skull because the skull has high resistance to

adverse environmental conditions over time, resulting in the greater stability of dimorphic features as compared to other skeletal bony pieces."[3]

In cases where bones are badly broken, fragmented or incinerated; the maxillae are preserved enough for the sinus evaluation[4] which can be visualized with help of Computed tomographic imaging modality.[5]

According to the outcome of the many studies conducted, the overall mean dimension for each parameter of maxillary sinus was statistically greater for males as opposed to females concluding that maxillary sinuses were bigger in dimensions in males except in the study done by Singh et al in Nepalese population which failed to provide any sexual dimorphism in relation to maxillary sinuses. This study is conducted to ascertain the reliability and accuracy of sinus parameters as a technique for gender determination using reconstructed CT images with the objectives of checking the distinction between right and left parameters of the maxillary sinus of male and females and derivation of formula for assessment of genders with the assistance of various parameters of maxillary sinus.

MATERIALS AND METHODS

It is a retrospective study done on CT scans using GE 64 slice CT Scanner, in the archived database of Government Medical College and Hospital Shimla HP from July 2021-February 2022. Reconstructed images of 120 subjects (60 male, 60 female) with age range between 25 to 85, scanned for other ailments were examined and measurements were taken which included volume, medio-lateral, superio-inferior, and anterio-posterior dimensions of the maxillary sinus, using radiANT DICOM viewer (64-bit) and inbuilt electronic caliper.

Reliability and reproducibility of the maxillary sinus measurements was ensured by carrying out intra-examiner calibrations and comparing the measurements of any 10 radiographs by the same investigator after 2 weeks from the first reading and paired t-test applied on the two measurements which showed statistical nonsignificant difference ($p > 0.05$).

Axial and coronal sections were used and measurements were taken as follows:

1. Axial section **(Figure 1)** was used for the length (MSL) (Maxillary sinus length) dimension which was the longest distance anteroposteriorly from the most anterior point to the most posterior point.
2. Axial section **(Figure 1)** was used for the width (MSW) (Maxillary sinus width) which was the longest perpendicular distance from the medial wall to lateral wall of the maxillary sinus. **(Figure 1)**
3. Coronal view **(Figure 2)** was used for the height MSH (Maxillary sinus height) which was the longest distance from the lowest point of the sinus floor to the highest point of the sinus roof in the.
4. The volume of the sinuses, MSVOL (Maxillary sinus volume): (MSW dimension × MSH dimension × MSL dimension) / 2 was calculated using a simple formula described by Sahlstrand-Johnson et al.[6]

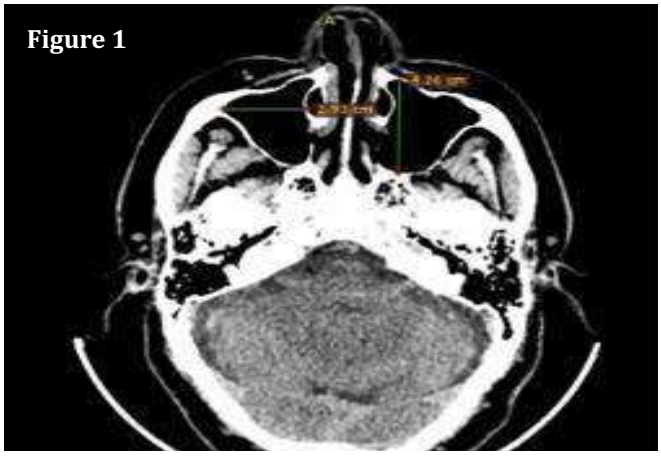


Figure 1

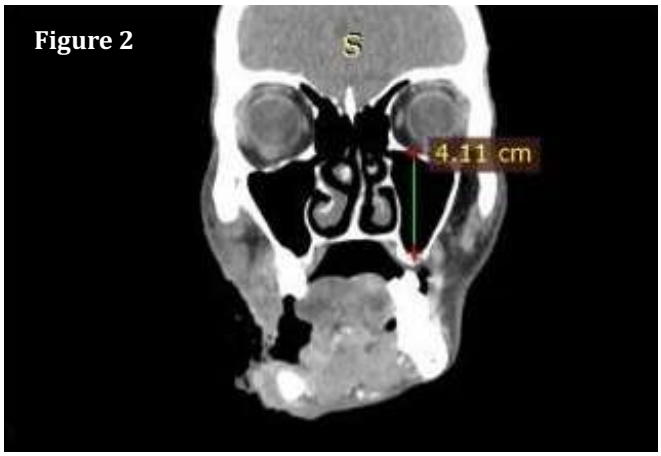


Figure 2

INCLUSION CRITERIA

- Patients admitted for CT analysis of head and neck region for diseases other than trauma to head and face.
- CT images with no artefacts.

EXCLUSION CRITERIA:

- CT pictures with facial trauma
- Previous sinus surgery
- Edentulous patients
- Any maxillary sinus pathology.

The data was tabulated in Microsoft excel and analysed with SPSS v.20 software. To compare groups Independent t test was used. Discriminant analysis was used for the prediction. Multiple logistic regression was performed to generate equations for all parameters of maxillary sinuses and a single equation combining all parameters for gender detection deriving a single value (D) that could be reliably used to calculate and classify the observations according to gender.

RESULTS

For the right-side maxillary sinus, the mean value of MSW i.e width, MSH i.e height, and MSL i.e length for males is 2.47±0.51cm, 3.46± 0.65cm, and 3.71± 0.42cm, respectively, and in case of females it was 2.2±0.44, 3.19± 0.52, and 3.5± 0.55, respectively. For the left side maxillary sinus, the mean value of MSW, MSH, and MSL for males is 2.5± 0.56, 3.42± 0.61, and 3.7± 0.4, respectively, and in case of females it was 2.27± 0.36, 3.18± 0.52, and 3.5± 0.38, respectively. In all of these measurements Female group had statistically significant lower values for both right and left sides ($p < 0.05$) (**Table 1-2**)

The mean volume of right maxillary sinus in males is 16.28± 7.29 whereas in females it is 12.92± 4.96. For left maxillary sinus, the mean value in males is 16.6± 7.05 and for females it is 13.07 ± 4.56. Female group had statistically significant lower values for both right and left sides when compared with males ($p < 0.05$; **Table 2**).

Comparison of the parameters between right and left sides in males and females showed no significant difference ($p >0.05$; **Table 3-4**).

Significant correlation ($p >0.05$) of the parameters in males and females for right and left sides is seen (**Table 5-6** respectively).

Multiple regression analysis is used; the dependent variable (gender) has been given a value 0 for women and 1 for men; the variables are the maxillary sinus parameters. The discriminate score ''D,'' is derived which aids in the predicting gender by putting the value of the specific measurement(s) in the equation. If calculated value ''D'' is greater than reference D, it indicated male gender, and if less than the reference values, it indicated female gender.

Various Discriminate analysis using different maxillary sinus measurements to discriminate between males and females are summarized in **Table 6-9**.

The right maxillary sinus height was the best discriminate variable between genders (Wilks' lambda = 0.938 with male accuracy of 70.0% and female accuracy of 56.7% and overall accuracy of 63.3%) and also all the left maxillary sinus parameters were better at predicting gender (Wilks' lambda = 0.927 and overall accuracy of 61.7%)

By combining the right sinus parameters between males and females the accuracy came out to be 60.0% for male and 55% for female (overall accuracy 57.5%) with derived formula i.e. D = -6.918 + (1.171 x RMSW) + (0.937 x RMSL) + (0.243 x RMSH)

By combining the left sinus parameters between males and females the accuracy came out to be 66.7% for male and 56.7% for female (overall accuracy 61.7%) with derived formula i.e. D = -8.481 + (0.832 x LMSW) + (1.705 x LMSL) + (0.110 x LMSH)

By combining both the right and the left sinus parameters between males and females, the accuracy came out to be 60.0% for female and 56.7% for male (overall accuracy 58.3%) with derived formula i.e. D = 3.286 + (1.815 x RMSW) + (0.592 x RMSL) + (0.340 x RMSH) + (-0.152 x RMSV) + (-3.029 x LMSW) + (-0.759 x LMSL) + (-1.944 x LMSH) + (0.528 x LMSV)

DISCUSSION

The uniqueness and interindividual variation in symmetry, shape, size and permanence of anatomical landmarks serves as basis of scientific data for forensic anthropology.[7] 100% accuracy in gender detection is possible only if complete skeleton exists. But this estimation rate is 98% in existence of pelvis and skull8,9. In cases where only skull remains are available, radiographic imaging methods can be used to assess various bones and paranasal sinuses. The present study highlights the reliability and accuracy of maxillary sinus dimension measurement, as a method for sex identification through the analysis of CT images.

According to the outcome of the current study, the overall mean dimension for each parameter was statistically greater for males as opposed to females; which is consistent with numerous published reports (Kim et al[10]., 2002; Fernandes[11] ,2004; Teke et al[2], 2007; Uthman et al[12]., 2011; Attia et al[13]., 2012; Abed-Allah and Mahdi[14], 2013; Jasim et al[15]., 2013; Kiruba et al[16]., 2014) which is probably due to sex-specific differences in body composition, nutrition, energetic intake, body compo-sition and genetics.[2]

On the other hand, a study on Nepalese population by Singh[17] (2019) concluded that the maxillary sinus cannot be subjectively analysed to determine the sex of the

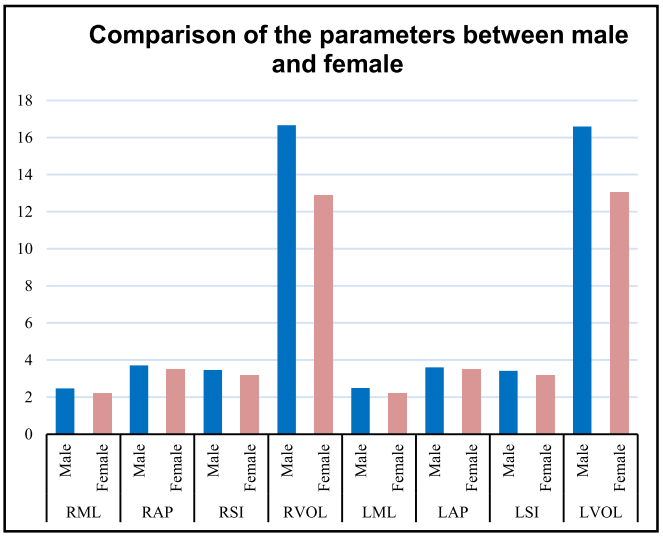
Table 1: Age Distribution of the Participants

GENDER	N	MINIMUM AGE	MAXIMUM AGE	MEAN AGE	STANDARD DEVIATION
MALE	60	22	81	52.50	14.93
FEMALE	60	23	85	52.91	16.89

Table 2. Comparison of the Parameters Between Male and Female

Parameters	Sex	N	Mean	SD	p value
RML	Male	60	2.47	0.51	0.004*
	Female	60	2.2	0.4	
RAP	Male	60	3.71	0.42	0.006*
	Female	60	3.47	0.49	
RSI	Male	60	3.46	0.65	0.015*
	Female	60	3.19	0.51	
RVOL	Male	60	16.67	7.29	0.001*
	Female	60	12.91	4.96	
LML	Male	60	2.49	0.55	0.013*
	Female	60	2.2	0.35	
LAP	Male	60	3.6	0.39	0.005*
	Female	60	3.49	0.38	
LSI	Male	60	3.42	0.61	0.023*
	Female	60	3.18	0.52	
LVOL	Male	60	16.59	7.05	0.002*
	Female	60	13.07	4.56	

(Right maxillary sinus width (RML), Right maxillary sinus length (RAP), Right maxillary sinus height (RSI), Right maxillary sinus volume RVOL, left maxillary sinus width (LML), Left maxillary sinus length (LAP), Left maxillary sinus height (LSI), left maxillary sinus volume LVOL

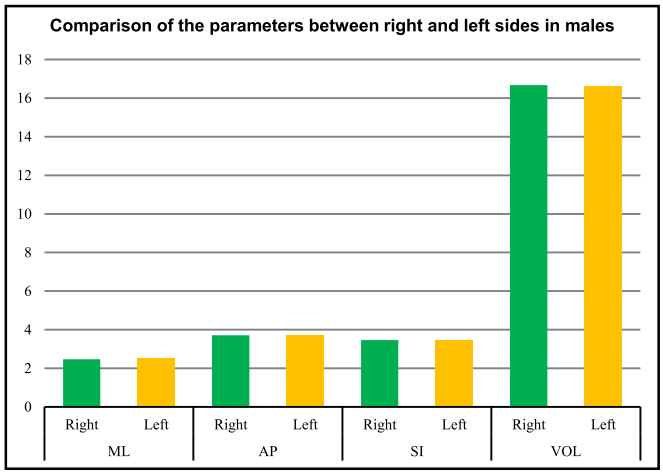


(Right maxillary sinus width (RML), Right maxillary sinus length (RAP), Right maxillary sinus height (RSI), Right maxillary sinus volume RVOL, left maxillary sinus width (LML), Left maxillary sinus length (LAP), Left maxillary sinus height (LSI), left maxillary sinus volume LVOL

Table 3. Comparison of the Parameters Between Right and Left Sides in Males

Parameters	Side	N	Mean	SD	p value
ML	Right	60	2.47	0.51	0.839
	Left	60	2.49	0.55	
AP	Right	60	3.71	0.42	0.851
	Left	60	3.69	0.39	
SI	Right	60	3.46	0.65	0.770
	Left	60	3.42	0.61	
VOL	Right	60	16.67	7.29	0.950
	Left	60	16.59	7.05	

Maxillary sinus width (ML), Maxillary sinus length (AP), Maxillary sinus height (SI), Maxillary sinus volume (VOL)

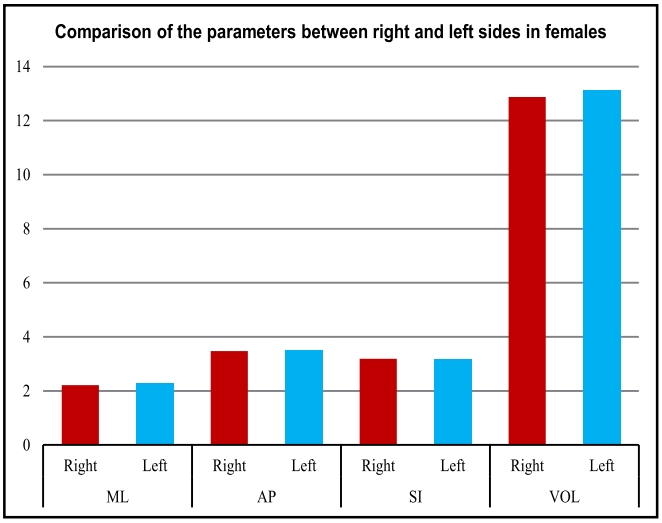


Maxillary sinus width (ML), Maxillary sinus length (AP), Maxillary sinus height (SI), Maxillary sinus volume (VOL)

Table 4. Comparison of the Parameters Between Right and Left Sides in Females

Parameters	Side	N	Mean	SD	p value
ML	Right	60	2.21	0.43	0.369
	Left	60	2.27	0.36	
AP	Right	60	3.47	0.49	0.712
	Left	60	3.50	0.38	
SI	Right	60	3.19	0.51	0.943
	Left	60	3.18	0.52	
VOL	Right	60	12.87	4.93	0.777
	Left	60	13.12	4.58	

Maxillary sinus width (ML), Maxillary sinus length (AP), Maxillary sinus height (SI), Maxillary sinus volume (VOL)



Maxillary sinus width (ML), Maxillary sinus length (AP), Maxillary sinus height (SI), Maxillary sinus volume (VOL)

Table 5: Correlation of the Parameters in Males for Right and Left Sides

Parameters	Correlation	p value
ML	0.839	<0.001*
AP	0.836	<0.001*
SI	0.877	<0.001*
VOL	0.907	<0.001*

Table 6: Correlation of the Parameters in Females for Right and Left Sides

Parameters	Correlation	p value
ML	0.726	<0.001*
AP	0.585	<0.001*
SI	0.827	<0.001*
VOL	0.849	<0.001*

*Maxillary sinus width (RMSW), Maxillary sinus length (MSL), Maxillary sinus height (MSH), Maxillary sinus volume (MSVOL) *significant)*

Table 7. Discriminate Analysis Using Right Maxillary Sinus Measurements to Discriminate Between Males and Females

Right maxillary sinus width			
D = -4.871 + 2.080 x RMSW			
Wilks' lambda = 0.931, p = 0.004*	**Male**	**Female**	**Overall**
Percent accurately predicted group membership	61.7	51.7	56.7
Functions at group centroids	0.289	-0.279	0.005
Right maxillary sinus length			
D = -7.808 + 2.172 x RMSL			
Wilks' lambda = 0.938, p = 0.006*	**Male**	**Female**	**Overall**
Percent accurately predicted group membership	70.0	56.7	63.3
Functions at group centroids	0.264	-0.255	0.004
Right maxillary sinus height			
D = -5.652 + 1.698 x RMSH			
Wilks' lambda = 0.951, p = 0.015*	**Male**	**Female**	**Overall**
Percent accurately predicted group membership	50.0	66.7	58.3
Functions at group centroids	0.248	-0.240	0.004
Right maxillary sinus volume			
D = -2.373 + 0.160 x RMSV			
Wilks' lambda = 0.915, p = 0.001*	**Male**	**Female**	**Overall**
Percent accurately predicted group membership	50.0	63.0	56.7
Functions at group centroids	0.325	-0.314	0.005

Table 8. Discriminate Analysis using Left Maxillary Sinus Measurements to Discriminate Between Males and Females

Left maxillary sinus width			
D = -5.085 + 2.133 x LMSW			
Wilks' lambda = 0.949, p =0.013*	**Male**	**Female**	**Overall**
Percent accurately predicted group membership	66.7	51.7	59.2
Functions at group centroids	0.248	-0.240	0.004
Left maxillary sinus length			
D = -9.186 + 2.555 x LMSL			
Wilks' lambda = 0.935, p = 0.005*	**Male**	**Female**	**Overall**
Percent accurately predicted group membership	68.3	48.3	58.3
Functions at group centroids	0.265	-0.256	0.004
Left maxillary sinus height			
D = -5.811 + 1.758 x LMSH			
Wilks' lambda = 0.957, p = 0.023*	**Male**	**Female**	**Overall**
Percent accurately predicted group membership	51.7	61.7	56.7
Functions at group centroids	0.228	-0.221	0.003
Left maxillary sinus volume			
D = -2.498 + 0.168 x LMSV			
Wilks' lambda = 0.918, p = 0.002*	**Male**	**Female**	**Overall**
Percent accurately predicted group membership	56.7	61.7	59.2
Functions at group centroids	0.316	-0.305	0.005

Table 9: Discriminate Analysis using Right and Left Maxillary Sinus Measurements to Discriminate Between Males and Females

	Standardized coefficient			
Right maxillary sinus width	0.563			
Right maxillary sinus length	0.432			
Right maxillary sinus height	0.143			
D = -6.918 + (1.171 x RMSW) + (0.937 x RMSL) + (0.243 x RMSH)				
Wilks' lambda = 0.919, p = 0.020*				
		Male	**Female**	**Overall**
Percent accurately predicted group membership		60.0	55.0	57.5
Functions at group centroids		0.312	-0.301	0.005
	Standardized coefficient			
Left maxillary sinus width	0.390			
Left maxillary sinus length	0.668			
Left maxillary sinus height	0.063			
D = -8.481 + (0.832 x LMSW) + (1.705 x LMSL) + (0.110 x LMSH)				
Wilks' lambda =0.927, p = 0.032*				
		Male	**Female**	**Overall**
Percent accurately predicted group membership		66.7	56.7	61.7
Functions at group centroids		0.288	-0.279	0.004

Table 10. Discriminate Analysis using Both Right and Left Maxillary Sinus Measurements to Discriminate Between Males and Females

	Standardized coefficient			
Right maxillary sinus width	-0.089			
Right maxillary sinus length	0.445			
Right maxillary sinus height	0.005			
Left maxillary sinus width	0.569			
Left maxillary sinus length	0.180			
Left maxillary sinus height	0.046			
D = -8.108 + (1.184 x RMSW) + (0.391 x RMSL) + (0.078 x RMSH) + (-0.189 x LMSW) + (1.137 x LMSL) + (0.009 x LMSH)				
Wilks' lambda = 0.912, p = 0.003*				
		Male	Female	Overall
Percent accurately predicted group membership		56.7	60.0	58.3
Functions at group centroids		0.321	-0.310	0.006
	Standardized coefficient			
Right maxillary sinus width	0.873			
Right maxillary sinus length	0.273			

individual although there was evidence of males have a larger voluminous maxillary sinus in comparison to females.

In our study among all the parameters, the right maxillary sinus height was the best discriminate variable between genders (male accuracy of 70.0% and female accuracy of 56.7% and overall accuracy of 63.3%) and also all the left maxillary sinus parameters were better at predicting gender (overall accuracy of 61.7%).

CONCLUSION

As already stated in literature that skull remains intact despite other bones get disfigured in mass disasters maxillary sinuses are mostly spared. Maxillary sinus tends to stabilize after second decade of life, so its linear dimensions measurements are valuable in studying Adult sexual dimorphism which was proven in our present study.

This study also establishes the reliability and accuracy of gender determination in Himachali population by using maxillary sinus.

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Original Research Paper

Pre and Post-Tests During Lectures: Students' Perceptions on its Effectiveness for Learning Students' Perceptions on Pre and Post-Tests

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ABSTRACT :

Introduction: Didactic lectures in which teachers talk and students listen which is just a passive mode of learning governs medical training. Few studies have suggested that if we use only didactic methods of teaching in the classroom it will limits students learning. So, many techniques have been incorporated to increase students' interest in the subject matter and enhance their learning. One such technique is using Pre and post-test techniques which has shown an increase in student learning when used. So, this study was undertaken to know the student perceptions on pre and post-test techniques which we have used during our routine medical lectures.

Materials and Method: This study was done during our routine lecture to 91 Medical students. Before starting the class, a pre-test questionnaire was given and later after finishing the class the same post-test questionnaire was given. In the end student perception based on Likert's scale was taken and analyzed.

Results: 99% of students agreed that pre and post-test technique of teaching has a positive impact on their learning. 98% of students agreed that this technique helps them to remember better the concepts taught in the class. 92% of students agreed that this technique makes them alert in class. 94% of students agreed that this technique should be used regularly. 90% of students agree that this technique of teaching is better than traditional class.

Conclusion: The students feel pre- and post-test technique when used in traditional lectures enhances student interest and learning.

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INTRODUCTION

It is observed that exclusive use of the lectures in the classroom restrains students learning and they lose their concentration after 15-20 minutes.[1-2] Some authors have observed that even extremely interested students can't keep them concentrated for a long time in didactic lectures.[3] It has been recommended that if we incorporate experiential learning activities in the classroom it will enhance students' attentiveness in the subject matter and understanding of course content can be augmented.[4]

Old-style techniques of teaching are no longer adequate. Now a days these new generation of medical students and doctors have grown up in a technological environment, the so called "millennial" It is inadequate and irrational to teach them with the approaches that have been developed years ago.[5]

Undergraduate medical education requires continuing enhancements to meet the varying demands of medical training. But the approaches of teaching medicine have transformed very little since the olden times. Teachers should learn about the modern methods and systems of medical education.[6]

It has been seen that if we give multiple choice questions to the students before the starting of class which is called as pretests it will increase students learning.[7] Pretesting is useful because it inspires more active involvement in learning, maybe by enhancing the curiosity in the topic. In addition, to this the pretest will also benefit the students to distinguish that which information in lecture is very important.[8]

It has been observed that if there is an introduction of a pre-test/post-test instrument it will support the

attainment of the learning objectives and it will lead to an improved understanding.[9]

So, this study was undertaken to know the student perceptions on pre and post-test techniques which we have used during our routine medical lectures.

MATERIAL AND METHODS

This study was done on 91 undergraduate medical students during our routine didactic lecture. Before starting the class, a pre-test questionnaire containing 5 multiple choice questions regarding the topic to be taught in class was given and they were told to fill in the answers which they know later the class was taken for 45min. After finishing the class, the same post-test questionnaire was given.

In the end a questionnaire seeking the student perception about the helpfulness of pre-test in increasing their concentration to the lecture was administered. It had 5 questions and was based on five-point Likert's scale with 5 = strongly agree, 4 = agree, 3 = neutral, 2 = disagree and 1 = strongly disagree. Later the results were analyzed.

RESULTS

99% of students agreed that pre and post-test technique of teaching has a positive impact on their learning. 98% of students agreed that this technique helps them to remember better the concepts taught in the class. 92% of students agreed that this technique makes them alert in class.

94% of students agreed that this technique should be used regularly. 90% of students agrees that this technique of teaching is better than traditional class.

Table 1 Shows the Perception of students about the Pre and Post test Method.

DISCUSSION

There are plenty of ways to increase learning but one universal principle behind all is that active participation in learning generates long-term memories. Therefore, one broad goal for such active involvement is testing.[10] This pre and post test also needs active participation of students and hence it will be helpful for enhancing the learning and knowledge of students and generates long term memories.

Muthukumar S et al studied the perceptions of 145 students. They found that 93.79% (136 students) agreed that pre-test is a useful method to be focused on the lecture and hence be more attentive and learn the important points of the lecture. [8] In our study also we found that 98% students agreed that this technique helps them to remember better the concepts taught in the class and 92% students agreed that this technique makes them alert in class.

Shivaraju PT et al did the study on 156 2nd year MBBS students. They gave the pretest before the lecture and a post-test at the end of the lecture. In their study the majority of the students (98.72%) felt that pretests helped them to improve their focus toward didactic lecture and for better performance and in our study 92% students agreed that this technique makes them alert in class. In their study 96.15% agreed that pre- and post-test are valuable technique to obtain new information, as well as key topics of the lecture.[11] In our study also we found that 98% students agreed that this technique helps them to remember better the concepts taught in the class.

Beckman WS took an unnamed written qualitative opinion from the class in which the Pre-test was given at the end of the semester about their views for the helpfulness and suitability of the pre-tests. All the responses were positive

Table 1: Shows the Perception of students about the Pre and Post test Method.

Feedback Questions	Strongly Agree	Agree	Neither Agree Nor Disagree	Disagree	Strongly Disagree
Pre and Post Test has a Positive Impact	50 (54.9%)	40 (43.9%)	1 (1.09%)		
Pre and Post Test helps to remember	50 (54.9%)	39 (42.8%)	2 (2.19%)		
Pre and Post Test Makes Alert	44 (48.3%)	40 (43.9%)	6 (6.59%)	1 (1.09%)	
Pre and Post Test should be used Regularly	43 (47.2%)	42 (46.1%)	6 (6.59%)		
Pre and Post Test used Classes are better than Traditional Didactic Classes	47 (51.6%)	35 (38.4%)	9 (9.8%)		

like, "It was helpful," or "I liked it," etc. There were no negative remarks about the pre-testing.[12] In our study there were also all positive comments about the pre and post technique.

Hill DA did a study to see that if giving a pre-test immediately before teaching it will improve the performance of student in a succeeding post-test. They found that using this type of method in class was effective as post-instructional information improved by almost half as compared with pre-test levels.[13]

Pre test when used in class make the students more focused in the lecture and post-test after the lecture evaluate that the students had learnt all the key concepts of the lecture. So, these pre- and post-tests make the student to be actively involved in education and gaining knowledge.[11] So, these should be most often used in our lectures to enhance student learning and for their active participation.

Even from our study it is clear that the students also feel that pre and post-test technique when used in traditional lectures enhances student interest and learning and hence it should be regularly used in classes.

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Original Research Paper

Histopathological Changes in Traumatized Skeletal Muscles of Neck and the Importance of Opaque Fibres in Cases of Compression to Neck

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ABSTRACT

Introduction: In some medicolegal cases it is difficult to comment about the cause and manner of death. It is specifically true in cases with faint or absent ligature mark on the neck. In such cases there might also be very minimal gross internal findings. Combining histopathological findings with gross findings can help in such difficult cases. Histopathological findings can help in deriving the cause and manner of death in such unsolved and doubtful cases.

The purpose of the study was to collect data on the various histological changes in neck muscles as an evidence of compression to neck.

Materials and Method: 100 cases of compression to neck were examined along with 10 control cases. Neck muscles underneath the compressive forces were dissected. Sample was processed and paraffin blocks were prepared. 4-5 microns thick sections were cut with a leicra microtome and stained with Haematoxylin and Eosin (H & E) stain and Modified gomoritrichome stain.

Results: A highly significant association (p value<0.0001) between presence of opaque fibre in cases of antemortem compression to neck as compared to control cases (cases with no compressive forces to neck) was found.

Conclusion: In cases of compression to neck, cervical muscular injuries produce thick and rounded fibres in transverse sections microscopically. These fibres are also seen in cross sections of muscle biopsies from patients with muscular dystrophy and also similar to wavy fibres in myocardial infarction. There is a corelation between the strength of applied force and production of opaque fibres with two different forms i.e Diffuse pattern and Focal pattern of opaque fibres.

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INTRODUCTION

In unnatural deaths, violent asphyxial deaths form one of the most important causes. Hanging, strangulations, drowning, suffocations and traumatic asphyxia are some of the important causes of violent asphyxial deaths.[1]

In deaths due to hanging there is suspension of the body by a ligature around the neck and the weight of the body acts as the constricting force.[2] Of the various methods of committing suicide hanging is one of the common method. Homicidal hanging and accidental hanging is rare. Contrary to hanging, strangulation should be assumed to be homicidal until the contrary is proved. But rarely few cases of suicidal strangulation are also reported.[3] It is important to differentiate cases of hanging and ligature strangulation as the manner of death is usually different in both.

Case history, police investigation, gross external findings and gross internal findings can help a forensic expert to derive the conclusion in cases of compression to neck. But still there remains many doubtful cases where it becomes difficult to ascertain the exact cause and manner of death. Histo-pathological examination findings can be helpful in such cases. Histo-pathological examination findings and the routine autopsy procedure findings when combined

can be helpful in such difficult cases.[4]

In cases of compression to neck, there is a high incidence of cervical muscular trauma. These traumatic changes in cervical muscles are not visible on gross examination. Under microscopic examinations, these fibers are thick and round in transverse sections. These traumatic cervical muscle fibres are known by various names such as eosinophilic hyaline change, hyaline fibres, opaque fibres, thick fibres, large-dark fibres, hyper-reactive fibres or hypercontracted fibres.[5]

MATERIALS AND METHODS

The present study was a prospective study held in the department of forensic medicine of a medical college for a period of two years. Cases of death due to asphyxia were included in the study. Prior permission cum no objection certificate to carry out the study was obtained from local ethical committee of the institute.

During the specified period as above total 100 cases of compression to neck were examined along with 10 control cases. Cases of compression to the neck included 95 cases of hanging, 3 cases of ligature strangulation and 2 cases of manual strangulation. Control cases were those where the cause of death was other than compression of neck, injuries to neck and pathologies involving skeletal muscles.

Inclusion Criteria

• All cases of medicolegal autopsy where the cause of death was compression of neck from the history given by police.

Exclusion Criteria

• Cases with no history of compression to neck.

• Decomposed body.

• Ambiguous findings.

The criteria applied for selecting the case was that the asphyxia should have occurred by compression of neck. The age, sex, and history about the case and supposed cause of death and manner of death as provided in police papers were noted. In addition, the history was taken from the relatives about the incidence.

The cases were studied with respect to different age groups and sex. Information related to the deceased such as the age, sex, circumstances of death, type of ligature material, manner and supposed cause of death were acquired from investigating police.

Whenever available, detailed examination of ligature material was carried out. Injuries over neck were examined in details. Neck structures (sternocleidomastoid muscle, strap muscles underneath the neck injury and neck structure if fractured) were dissected with utmost care at autopsy. Portion of cervical muscles, mainly sterno-cleidomastoid and/or sterno-hyoid muscles under the area of compression were dissected and removed. The specimen bore an identification number and the fixative used was 10% formalin.

The specimens were fixed in 10% formalin for 24 hours. Sample was processed and paraffin blocks were prepared. Appropriate numbers of 4-5 microns thick sections were cut with a leicra microtome and stained with Haematoxylin and Eosin (H & E) stain and special stain, Modified gomoritrichome stain. The stained sections were studied by light microscopy. Theory and practice of histological techniques by Bancroft and gamble, 2008 were followed for the procedure of processing and staining of tissue. Microscopic findings were noted. The gross and histopathological data of all cases were noted.

Descriptive statistics (percentage) was used to summarize baseline characteristic of the study subjects. Various data obtained from police inquest and histories obtained were noted. Various autopsy features were noted. Various histopathological findings were noted. Data was analysed using STATA-10 statistical software. Chi-Square test was used to analyse data. P value=0.000 was considered to be highly significant.

RESULTS

Out of 100 cases of compression to neck, 95 cases were of suicidal manner while 5 cases were of homicidal manner. There were no cases of death by accidental manner. All 95 cases of suicide were hanging cases. Of 5 homicidal cases, 3

Table 1: Distribution of Cases According to Cause and Manner of Compression

Cases	Manner of Death of Cases		
	Suicide	Homicide	Accident
Hanging	95	0	0
Ligature Strangulation	0	3	0
Manual Strangulation	0	2	0
Total	95	5	0

cases were of ligature strangulation and 2 cases were of manual strangulation (throttling). **(Table 1)**

Sex wise Distribution of Cases. (Table 2)

Out of total 05 cases of homicidal compression to neck 02 cases were females and 03 cases were males. Out of total 95 cases of suicidal compression to neck (hanging cases) 32 cases were females and 63 cases were males. Of total 100 cases of compression to neck, 34 were females and remaining 66 were males. Of total 95 suicides, 63 were males and 32 were females with male: female ratio 1:0.51.

Table 2: Sex Wise Distribution of Cases

Cases	Male	Female
Hanging	63	32
Ligature Strangulation	01	02
Manual Strangulation	02	0
Total	66	34

Histopathological (Microscopic) findings in the form of presence of opaque fibres. (Table 3)

Out of total 100 cases of compression to neck (95 cases of hanging + 05 cases of strangulation) "Opaque fibre" was observed to be present in total 92(92 %) cases and absent in 8 cases. Of 95 cases of hanging "Opaque fibre" was present in 87 (91.6 %) cases and absent in 8 (8.4%) cases. Opaque fibre" was present in all 3 cases of ligature strangulation and also in all 2 cases of manual strangulation. Out of total 10 control cases "Opaque fibre" was not observed in all 10(100 %) cases.

Presence of diffuse pattern and focal pattern of opaque fibres. (Table 4)

Out of total 95 cases of hanging "Opaque fibre" was present in diffuse pattern form in 44(46.32 %) cases and in focal pattern form in 43(45.26 %) cases. Out of total 05 cases of strangulation "Opaque fibre" was present in diffuse pattern form in all 05(100 %) cases.

Statistical association between presence of "Opaque Fibres" among cases of Compression to Neck and Control group. (Table 5)

Out of total 100 cases of compression to neck opaque fibre was present in total 92 cases and was absent in 08 cases while in control group comprising of total 10 cases opaque fibre was not observed in any of the cases. This showed statistically a highly significant association (p value<0.0001) between presence of opaque fibre in cases of antemortem compression to neck as compared to control cases (cases with no compressive forces to neck).

Table 3: Histopathological (Microscopic) Findings in the form of Presence of Opaque Fibres

Cases	Presence of Opaque fibres	Absence of Opaque fibres
Hanging (n=95)	87 (91.6%)	8 (8.4%)
Ligature Strangulation (n=3)	03 (100%)	0
Manual Strangulation (n=2)	02 (100%)	0
Total (n=100)	92 (92%)	8 (8%)
Control Cases (n=10)	0	10 (100%)

Table 4: Presence of Diffuse Pattern and Focal Pattern of Opaque Fibres

Cases	Presence of diffuse pattern Opaque fibres	Presence of Focal Pattern Opaque fibres
Hanging	44	43
Ligature Strangulation	03	0
Manual Strangulation	02	0
Total	49	43

REVIEW OF LITERATURE

Normal skeletal muscle histology:-[6]

Basically skeletal muscles are composed of long cylindrical fibres. Indeed each fibre is a syncytium with hundreds of nuclei along its length. The nuclei are elongated and lie along the periphery of the fibre beneath the sarcolemma (cell membrane). The sarcoplasm (cytoplasm) is packed with numerous longitudinal fibril called as myofibrils. Presence of transverse striations is the most important finding of skeletal muscle fibres. On staining with H& E stain transverse striations of skeletal muscle fibres are seen as alternate dark and light bands stretching across the

Table 5: Statistical Association between Presence of "Opaque Fibres" Among Cases of Antemortem Compression to Neck and Control Group.

Opaque Fibres	Cases of antemortem compression	Control Cases	Chisquaretest	P-value	Sig. at 5% level
Present	92	0	56.222**	0.000	Yes
Absent	08	10			
Total	100	10			

X2=56.222** , P=0.000 i.e Statistically highly Significant at 0.1% level i.e. P<0.001.

muscle fibre.[1]

Pathological aspects in Myocardial Infarction:-[7]

Ischemia leading to necrosis of heart muscle causes myocardial Infarction. Under microscopy it shows a typical sequential changes. The classical characteristic of coagulative necrosis become evident within 4 to 12 hours of infarction. "Wavy fibres" reflects the stretching and buckling of noncontractile dead fibers. "Wavy fibres" are regarded as "soft" findings of acute infarction. Myocyte vacuolization can also be caused by sublethal ischemia, such myocytes are poorly contractile but yet alive. Myocyte vacuolization are seen as large cleared intracellular spaces likely containing water.[2]

Muscular Dystrophies

The two most important types of muscular dystrophy are Duchenne and Becker Muscular Dystrophy. The histologic findings of DMD and BMD are identical. Their histologic findings include considerable changes in muscle fiber size which is due to concomitant myofiber hypertrophy and atrophy. DMD or BMD might also show changes in cardiac muscle such as variable levels of fibre hypertrophy and interstitial fibrosis.[8]

Nonaka[9] studied the pathology and mechanism of various neuromuscular diseases. In Duchenne muscular dystrophy (DMD) the important muscle degeneration seen is muscle fibre necrosis. He inferred that muscle fibre necrosis is likely due to some membrane instability. The at risk muscle surface membrane may cause extracellular calcium influx into the sarcoplasm which in turn leads to the production of focal myofibrillar hypercontraction (opaque fibre). This is followed by muscle fibre necrosis which permits macrophage invasion, ensued by muscle fibre regeneration.

Cullen and Fulthorpe[10] studied Duchenne muscular dystrophy and introduced a plan that divided muscle fibre breakdown process into 5 sequential stages. Stage 1 showed fine changes in the comparative volumes of the fibre components while the fibre appeared superficially normal. Stage 2 showed focal overcontraction of the myofibril which leads to excessive stretching of the sarcomeres in other parts of the fibre. This ongoing procedure causes contractile filaments to form an increasingly homogeneous mass leading to the formation of contraction clumps. In the final stage normal features of the fibre completely vanish.

Mokri and Engel[11] studied seven patients with Duchenne muscular dystrophy. Using high-resolution phase microscopy they found non-necrotic fibers with varying focal lesions. They suggested that these changes in muscle fibre of Duchenne's dystrophy may be due to an abnormality in the plasma membrane.

Witkowskiand and Dubowitz[12] also suggested that abnormality of the plasma membrane could be the cause for the pathogenesis of DMD. Their results also help to support that there could be cell surface defect in DMD.

YASIN et al[13] studied muscle biopsies of Duchenne dystrophy and Becker dystrophy patients. They also indicated an abnormality in the cell surface or cell-locomotory machinery of dystrophic cells in dystrophy patients.

Boxler and Jerusalem[14] concluded that hyperreactive fibers are not specific for Duchenne dystrophy. All 16 patients with Duchenne dystrophy biopsies showed hyperreactive fibres. While in only 34 (17%) of the 205 consecutive biopsies of other neuromuscular diseases and controls were hyperreactive fibres found.

Uchino et al[15] studied the electrophoretic patterns of myofibrillar proteins that showed three groups of opaque fibres: the first group of opaque fibres (OF a) had an almost normal electrophoretogram, the second group of opaque fibre (OF b1) showed a clear decrease of 55,000 dalton protein (desmin), and the third group of opaque fibre (OF b2) exhibited a great reduction in alpha-actinin and desmin. They suggested that opaque fibres are pathologic fibres in the degenerative process of Duchenne dystrophy.

Uchino et al[16] studied muscle biopsies of a total 21 patients of with Duchenne muscular dystrophy (DMD). The patients were 8 months to 24 years of age. They suggested that OFs may comprise two types of muscle fibres:

(1) pathological muscle fibres to be regarded as precursors of Necrotic fibres (NFs) and

(2) muscle fibres undergoing artificial changes.

Forensic Histopathology

In many cases of compression trauma to the neck discrete although occasionally marked microscopically detectable findings can be made including erythrocyte extravasation, haemorrhage & damage to the skeletal musculature of the neck. Depending on survival time cellular reaction: leucocyte and early leucocyte migration with the help of which atleast vitality at the time of trauma to the neck can be proven. In the absence of a cellular reaction segmental and discoid decay of muscle cells with loss of cross striations and newly formed pathological longitudinal

striations point to intravital trauma.[17]

Rajs and Thiblin[18] concluded that contraction bands, contraction band necrosis and 'opaque fibres' are seen in muscle fibres of neck compression.

Harman[19] also studied the early histopathological alterations accompanying the necrosis of ischemic voluntary muscle.

Nonaka et al[20] studied the pathophysiology of fibre necrosis and regeneration in diseased muscle. The sarcoplasmic reticulum (SR) was functionally disturbed. The extracellular fluid containing a high concentration of calcium (Ca) ions then permeated into the sarcoplasm through the defective membrane leading to the production of hyper-contracted myofibrils.

Tabata[5] examined the cervical muscles in cases of compression to the neck and other traumatized skeletal muscles histologically and immuno-histo-chemically. The sections were stained with haemotoxylin and eosin (H&E) and modified Gomoritrichome stain. Also immuno-histochemistry for myoglobin (Mb), fibronectin (FN) and glycophorin A(GPA) was done. In all 15 examined cases Opaque fibres were observed. Thick and round "opaque fibres" were seen in the muscles beneath the compression marks on the neck while in areas where no force was applied these fibres did not exist. Opaque fibres were seen as swollen and rounded in transverse section also they showed the loss of cross striations. Opaque fibres stained deep pink with H&E stain while on modified Gomori-trichome stain they stained blue-green and sometime red. Opaque fibres were not observed in any control cases. "Opaque fibres" are also known as eosinophilic hyaline change, hyaline, thick, large-dark, hyper-reactive or hypercontracted fibres.

Opaque fibres are produced in the area underneath the compression and at the opposite side of the area. Two types of opaque fibres 'diffuse' pattern and 'focal' pattern were described. Diffuse pattern would be formed when the force uniformly influences the muscle under the region of compression. Focal pattern is produced when the force focally affects the under compression cervical muscle.

Opaque fibres productions are attributable to damage to the plasma membrane by trauma. If force sufficient to damage the plasma membrane is applied to neck then opaque fibres are produced in cervical muscles. A close relationship between force applied and opaque changes are seen. Opaque fibres are probably produced by damage to muscles due to optimally applied force. The distribution and direction of force to the neck might be presumed by the pattern of opaque fibres in cervical muscles. High prevalence of opaque fibres underneath compression marks on the neck is a useful indication of strangulation and/ or hanging.

DISCUSSION

Distribution of cases according to cause and manner of compression. (Table 1)

Out of 100 cases of compression to neck, 95 cases were of suicidal manner while 5 cases were of homicidal manner. There were no cases of death by accidental manner. All 95 cases of suicide were hanging cases. Of 5 homicidal cases, 3 cases were of ligature strangulation and 2 cases were of manual strangulation (throttling).

Bowen[21] reported that 93.53 % of all cases of death due to compression around neck to be suicidal. In their study out of 201 cases, 188 cases were suicidal, 12 cases were accidental and 1 case was of judicial hanging. Luke[22] studied 106 cases of asphyxial deaths by hanging in New York city in the year 1967. They found two cases of accidental hanging deaths and 104 cases of suicidal deaths. Demirci et al[23] in their study found that all cases of hangings were suicidal. There was no cases of accidental hanging and homicidal hanging. All cases of strangulation were homicidal. There was no case of suicidal and accidental strangulation.

Sex Wise Distribution of Cases. (Table 2)

In our study male cases outnumbered female cases.

This finding is similar to findings in most of the studies reviewed. Bowen[21] reported that out of 188 cases of suicidal hanging 156 were males and 32 were females. Guarner and Hanzlick[24] studied 56 cases of hanging, out of which 50 victims were male (90%) and 6 were female (10%). Azmak[25] in their study found that males constituted 79.8% of all the cases. Sharma et al[26] found Male:female ratio was 2:1 in their study. Prajapati et al[27] found 73% cases were males while only 27% were females. Jayaprakash and Sreekumari[28] found 70.9% cases were male and rest were female.

But a few studies reported higher cases in females. Naik and Patil[29] reported higher number of female cases in hanging as well as in strangulation. Ahmad and Hossain[30] observed that 59% of hanging victims were females. Saisudheer and Nagaraja[31] also reported that 68% of deceased in their study were female.

Histopathological (microscopic) findings in the form of presence of opaque fibres. (Table 3)

Out of total 100 cases of compression to neck (95 cases of hanging + 05 cases of strangulation) "Opaque fibre" was observed to be present in total 92(92 %) cases and absent in 8 cases. Of 95 cases of hanging "Opaque fibre" was present in 87 (91.6 %) cases and absent in 8 (8.4%) cases. Opaque fibre" was present in all 3 cases of ligature strangulation and also in all 2 cases of manual strangulation. Out of total 10 control cases "Opaque fibre" was not observed in all 10(100 %) cases.

In the present study, finding of Opaque fibres were made on the various observations which were as follows:

1) Swollen, round and thick muscle fibres (clearly seen in transverse section).
2) Loss of cross striations of the Opaque muscle fibres.
3) Stained deeply pink by H & E stain and greenish blue in modified Gomoritrichome stain, while the normal muscle are stained lightly as compared to Opaque fibres, eosinophilia of affected muscle fibres.
4) Interstitial oedema.
5) Waviness of muscle fibres at border or Border fraying of the muscle fibres (seen more clearly in longitudinal sections).

Necrosis is the major pathway of cell death in commonly encountered injuries, such as resulting from ischemia and trauma. When damage to membranes is severe, enzymes leak out of lysosomes, enter the cytoplasm, and digest the cell, resulting in necrosis. Cellular contents also leak out through the damaged plasma membrane and elicit a host reaction (inflammation). The cell may have a more glassy homogeneous appearance than viable cells, mostly because of the loss of glycogen particles. When enzymes have digested the cytoplasmic organelles, the cytoplasm becomes vacuolated and appears motheaten. Reversible cell injury features are cell swelling, fatty change, plasma membrane blebbing and loss of micro-villi, mitochondrial swelling, dilation of the Endoplasmic Reticulum, eosinophilia (due to decreased cytoplasmic RNA). Irreversible changes which are changes of necrosis are increased eosinophilia; nuclear shrinkage, fragmentation, and dissolution; breakdown of plasma membrane and organellar membranes.[32]

Thus opaque fibres can be supposed to be the result of early ultrastructural changes of reversible cell injury due to ischemia resulting from the compressive forces applied to neck muscles. Evidence of early (reversible) ischemic injury was observed in the form of increased eosinophilia of cytoplasm, swollen, rounded, thick appearance of the opaque muscle fibers. The ultrastructural changes of reversible cell injury i.e plasma membrane alterations such as blebbing, blunting or distortion of microvilli, and loosening of intercellular attachments can be said to be the basis of the damage to the sarcolemma (cell membrane of muscle cell). The early necrotic cells show increased eosinophilia (i.e., pink staining from the eosin dye, the "E" in "H&E") this can be attributed to deeply pink staining by H & E stain and greenish blue in modified Gomoritrichome stain. For interstitial oedema it can be supposed that when compressive forces are applied, the sarcoplasm (cytoplasm) of muscle fibre is oozed to the surrounding interstitium. Nucleuses of the opaque muscle fibres were normal without showing evidence of necrosis suggestive of irreversible cell injury. In all cases the victims died before irreversible damage to muscle could happen.

Waviness of muscle fibres at border or Border fraying of the muscle fibres (seen more clearly in longitudinal sections) can be correlated with the microscopic appearance in Myocardial Infarction in cardiac muscle. In Myocardial Infarction typical features of coagulative necrosis become detectable within 4 to 12 hours of infarction. "Wavy fibres" can also be present at the edges of an infarct; these reflect the stretching and buckling of noncontractile dead fibers but are considered "soft" findings of acute infarction. Sublethal ischemia can also induce myocyte vacuolization. These are large cleared intracellular spaces, probably containing water; such myocytes are still alive but are poorly contractile. Irreversible Injury in myocardial Infarction and its light microscopic findings show at ½-4hr of ischemia usually there is none changes except variable waviness of fibers at border called "Wavy fibres".[33]

The absence of a cellular reaction segmental and discoid decay of muscle cells with loss of cross striations and newly formed pathological longitudinal striations point to intravital trauma.[34] Harman[19] said that with ischemia of 2 to 4 hours' duration the fibres are individualized, longitudinal striations disappear, and cross striations become a conspicuous cytological feature. After longer periods of ischemia abnormal anisotropic disks, Bowman's disks or conchoidal plates appear and involve the muscle fibres in increasing numbers up to 8 hours of ischemia, at which time they are nearly ubiquitous. Hence loss of cross striations of opaque muscle fibres could be attributed as a early pathological response to trauma i.e compressive

forces to neck resulting in ischemia to muscle.

Presence of diffuse pattern and focal pattern of opaque fibres. (Table 4)

Out of total 95 cases of hanging "Opaque fibre" was present in diffuse pattern form in 44(46.32 %) cases and in focal pattern form in 43(45.26 %) cases. Out of total 05 cases of strangulation "Opaque fibre" was present in diffuse pattern form in all 05(100 %) cases.

Two patterns were observed, 'diffuse' pattern and 'focal' pattern. Diffuse pattern in which opaque fibres were found distributed in most of the muscle fascicles of the muscle fibres while in the latter focal pattern opaque fibres were found distributed in one or few of the muscle fascicles of the muscle fibres.

Tabata[5] suggested that there is a close relationship between opaque changes and force. He proposed that opaque fibres are probably produced by damage to muscles due to severely applied force. Diffuse pattern would be formed when the force uniformly influences the muscle under the region of compression. While the latter focal pattern would be produced when the force focally influences the cervical muscle under compression.

Statistical association between presence of "Opaque Fibres" among cases of Compression to Neck and Control group. (Table 5)

Our study showed statistically a highly significant association (p value<0.0001) between presence of opaque fibre in cases of antemortem compression to neck as compared to control cases (cases with no compressive forces to neck).

CONCLUSIONS

Opaque fibres may help in evaluating doubtful cases where doubt regarding application of compressive forces to the neck arouse and where also doubt regarding antemortem nature of compressive forces appear to the investigator. Hence histopathology of traumatised skeletal muscle of neck can be used as a useful indicator in doubtful cases which test the mettle of the investigator.

List of abbreviations:

DMD: Duchenne Muscular Dystrophy.

BMD: Becker Muscular Dystrophy.

OF: Opaque Fibres.

NFs: Necrotic Fibres.

SR: Sarcoplasmic Reticulum.

Ca: Calcium.

H&E: Haemotoxylin and Eosin.

Mb: Myoglobin.

FN: Fibronectin.

GPA: Glycophorin A.

RNA: Ribonucleic acid.

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Figure 1: Transverse section of muscle underneath ligature mark showing waviness of borders and fraying of cell margin in H & E stain in low power (10X).

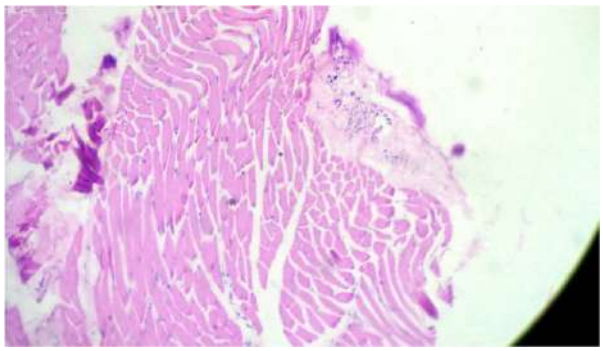

Figure 2: Transverse section of muscle underneath ligature mark showing waviness of borders and fraying of cell margin in H & E stain in high power (40X).

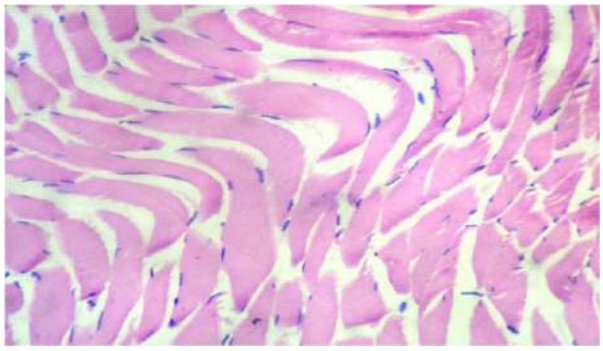

Figure 3: Transverse section of muscle underneath ligature mark showing opaque fibres with focal distribution in H & E stain in low power (10X).

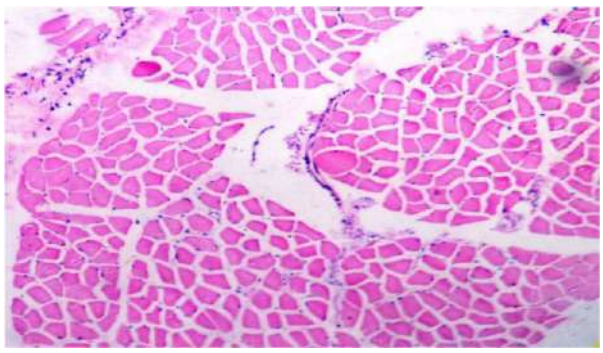

Figure 4: Transverse section of muscle underneath ligature mark showing opaque fibre in H & E stain in high power (40X).

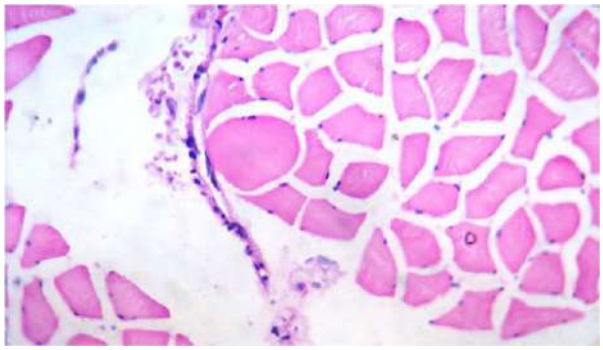

Figure 5: Transverse section of muscle underneath ligature mark showing opaque fibres with focal distribution in Gomori trichome stain in low power (10X).

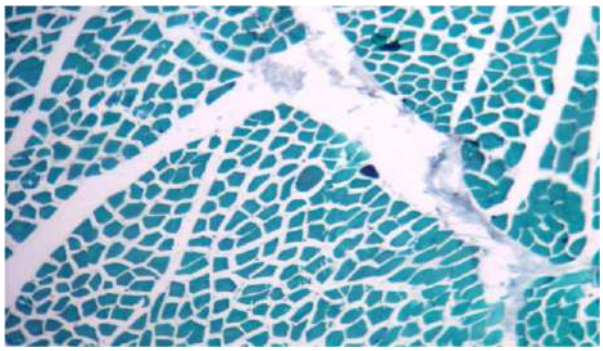

Figure 6: Transverse section of muscle underneath ligature mark showing opaque fibre in Gomori trichome stain in high power (40X).

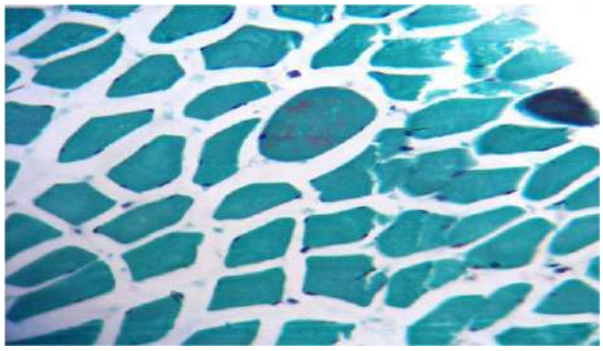

Figure 7: Transverse section of muscle underneath ligature mark showing multiple opaque fibres at different sites in H & E stain in low power (10X).

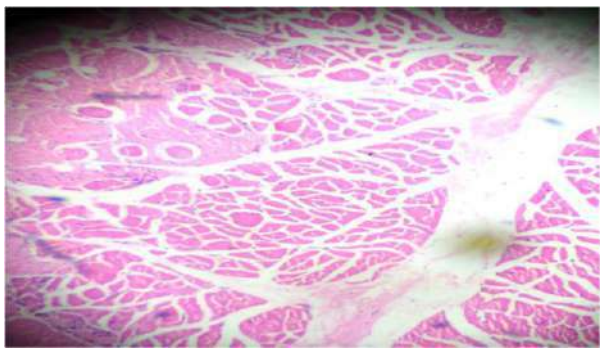

Figure 8: Transverse section of muscle underneath ligature mark showing multiple opaque fibres in H & E stain in high power (40X).

Original Research Paper

Finger-ball patterns and its comparative analyses between Bhil Females of Udaipur Rajasthan and Jaunsari Females of Dehradun Uttarakhand

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ABSTRACT

Introduction: Dermatoglyphics is a study of epidermal ridges on certain parts of body such as fingers, palms soles and toes. Dermatoglyphics is not only used in personal identification of an individual but also plays a role in population studies. The present paper aims to compare and analyze both the finger-ball patterns datasets of the Bhil and Jaunsari females.

Materials and Methods: In the present paper, a comparative Dermatoglyphic research study conducted on 100 Jaunsari females from Dehradun, Uttarakhand, and 100 Bhil females from Udaipur, Rajasthan have been discussed. The finger-ball prints were collected using the ink-pad method. In this paper the data included pattern types (W-L-A), Total finger ridge count (TFRC), Absolute finger ridge count (AFRC), and pattern type indices.

Results: Loops were found to be more prevalent than whorls in both groups (Bhils: 63.6% and Jaunsaris: 55.2%), whorls were more frequent than arches in both groups (Bhils: 28.8% and Jaunsaris: 38.9%), and arches were found to be least common in both groups (Bhils: 7.6% and Jaunsari: 5.9%, respectively).

Conclusions: In both groups, loops were found to be in higher frequencies than whorls and arches. Using TFRC and AFRC, it was determined that there was a significant difference between Jaunsari and Bhil females.

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INTRODUCTION

Every individual has distinct fingerprints that are different from one another and these complex patterns of ridges and furrows do not change over the duration of a person's lifetime. These characteristics have made fingerprints an extremely valuable tool in law enforcement. These ridge traits are helpful in understanding human diversity and have enormous significance in anthropology, medicine, and genetics, in addition to the application of fingerprints for personal identification. Dermatoglyphics is a branch of physical or biological anthropology that focuses on population studies. Population dermatoglyphics focuses on the analyses of various populations' dermatoglyphic studies.

There may be diversity in dermatoglyphic arrangements amongst populations, according to earlier research and the literature now available. Studies on Indian ethnic groups have included the Bagathas of Araku valley,[1] Sugalis of Andhra Pradesh,[2] Nagas of Nagaland,[3] Chenchu of South India,[4] Dhimals of North Bengal,[5] Khond of Vishakha patnam, Andhra Pradesh,[6] Apatani of Arunachal Pradesh,[7] Bhoi Khasis of Meghalaya,[8] Limboo of Sikkim,[9] Naikas of Gujarat.[10] These researches revealed more dermatoglyphic variation among the Indian indigenous communities.

To explore the variation in finger-ball patterns in populations, various researchers conducted most of the studies. Previous research revealed its findings among the Dumagat-Remontado of Philippines,[11] Eskimo's of Alaska,[12] Ogoni of Nigeria,[13] Elema people of Papua New Guinea,[14] Esan population of Nigeria,[15] the Jewish population of Israeli,[16] Efe Pygmies of Central Africa,[17] Australian aboriginals,[18] Kung Bushmen of Northeast Namibia[19] and Ebira population of Nigeria.[20]

In order to comprehend human variation and differentiation, the relevance of dermatoglyphics has grown among tribal and other populations from various areas of India. The purpose of the current study is to compare and examine the finger-ball dermatoglyphics of two ethnic populations-the Bhils and the Jaunsaris-who

reside in two different geographical regions of India.

The Bhils

Bhils, a dominant group in the village who were known locally as "Gameti," were found on the outskirts of the village. Both sides of the road were covered in trees and other types of vegetation in the village. The term "Mohalla" was never used by the dominant groups in the Dhinkli village; they were referred to as "Samaj." There were many landmarks in the hamlet, including Charbhuja Chowk in the main Dhinkli and Peepli Chowk/Keshav Chowk, which was the second most well-known landmark. The Bhil people's main source of economy was agriculture. They were quite simple in their way of life and very hard workers in the fields of agriculture and cultivation. Working with animals was a means of making money for certain individuals. The Bhil people who lived in the settlement of Dhinkli were very reliant on the use of alcohol and tobacco; they made spirits from the Mahua tree or the juice of the sugarcane.

The Indian state of Rajasthan is home to Udaipur, sometimes referred to as the "City of Lakes." It is situated at Rajasthan's furthest south. The majority of the district is made up of hills, rocks, and forests. It is also surrounded by the Aravali Range of mountains. Data for Bhil females were collected in the village of Dhinkli in the tehsil of Burgeon in Udaipur, Rajasthan. Bhils constituted 80% of the population of Dhinkli, with Brahmins and other tribes making up the remaining 20%. Rajasthan is known to be the native home of the Bhils, one of the largest tribal groups in India.

The Jaunsaris

The Jaunsar-Bawar region encircles the northern portion of the district Dehradun, which is located in the beautiful place of Dehradun, the capital of Uttarakhand in northern India. It can be found in the Jaunsar-Bawar valley in the Himalayas. The two distinct regions of the Jaunsar-Bawar region are Jaunsar and Bawar. The upper half of the valley is referred to as Bawar and the lower portion is known as Jaunsar. Jaunsaris or the "Jaunsari tribe" is common terms used to refer to the inhabitants of Jaunsar-Bawar. The Pandavas in the Mahabharata are where the Jaunsari people first appear. Polyandry is common among the Jaunsari (one woman marries more than one man). The Jaunsari tribe mainly consists of joint families. Since farmers constitute the majority population of Jaunsari, it is understandable that this is their primary source of income. Geographically and culturally, the Jaunsar-Bawar region is well-known for its extensive forest reserves, which are located in the high, mountainous region of the Himalayas. Five Jaunsari villages in the Chakrata and Kalsi tehsils of the Dehradun district of Uttarakhand provided the data on females in the Jaunsari community.

AIM OF THE STUDY

The aim of the current paper was to analyze and compare datasets of finger-ball patterns from Bhil females from Udaipur, Rajasthan and Jaunsari females from Dehradun, Uttarakhand.

MATERIALS AND METHODS

In the current Dermatoglyphic investigation, the finger-ball prints of 100 Bhil females from Udaipur, Rajasthan, and 100 Jaunsari females from Dehradun, Uttarakhand, were collected and analyzed. The data on the Bhil population (100 females) was taken from two schools (Rajkiya Uchh Prathmic Vidhayalaya, Dhinkli, Senior Secondary School, Main Dhinkli) and several areas of the tehsil Burgeon, Kanela Gaon, and Wada Gaon of Udaipur, Rajasthan. The data on the Jaunsari people (100 females) was collected in five villages (Sahiya, Udpalta, Nevi, Khatasa, and Bohari) in the Chakrata and Kalsi tehsils of Dehradun, Uttarakhand. Data was collected throughout the period from June 2015 to July 2015. The approval for conducting this study has been granted by Department of Anthropology, University of Delhi, India. A written consent was obtained from all participants before taking their fingerprints. We only consider healthy people who had no history of sickness or obvious malformations or injuries to their fingertips. The data were collected and analyzed by the methods used by Cummins and Midlo (1943). The finger-ball prints were taken following ink-pad method, using Gesterner's ink/Printers ink/Black ink, fingerprint paper, glass slab, cotton pad, duster, towel, soap, magnifying glass and folding lens. In order to identify someone, police enforcement organizations typically use this type of finger printing.

Before taking prints, the participants were instructed to wash their hands with soap and water to remove dust, and then dry them with a towel. On a glass slab, the ink was evenly distributed as a thin layer using a cotton pad or roller. Inking was done continuously, to produce a complete and clear print. The subjects were instructed to roll their finger from the ulnar (little finger) to the radial (thumb) side on the glass slab, and then transfer their finger prints in the same way on the fingerprint paper.

The tri-radii and other significant landmarks were included in the precautions to ensure that all prints were clear and free from smearing. For the purpose of determining the frequency, mean, standard error, and standard deviation; statistical analysis was carried out using SPSS. For both of the female groups; the frequencies of the pattern types (W-L-A), total finger ridge count (TFRC), absolute finger ridge count (AFRC), and pattern type indices were analyzed. The difference between the means of both the female groups was calculated using the student's t-test and the Chi-square test. This study calculated three different kinds of pattern indices, which are listed below.

Dankmeijer's Index= Arches×100/Whorls

Furuhata's Index= Whorls ×100/Loops

Pattern Intensity Index=2 × Whorls+ Loops/number of fingers

RESULTS

The pattern type frequencies for female Jaunsari and Bhil are shown in **Table 1**. Females in Jaunsari population have loops most frequently in both hands (55.2%), followed by whorls (38.9 %) and arches (5.9%). The loop also has the highest frequency (63.6%) in Bhil females, followed by whorls (28.8%) and arches (7.6%). Female Bhil participants (63.6%) exhibited a higher frequency of the loop than did female Jaunsari participants (55.2%) in both groups. Females from Jaunsari have a greater whorl frequency (38.9%) than females from Bhil (28.8%), whereas females from Bhil had higher arches (7.6%) than females from Jaunsari (5.8%).

According to **Table 2**'s various pattern indices, the value of Dank Meijer's index for female Jaunsari and Bhil populations was 15.12 and 26.38, respectively. Female Jaunsaris had a Furuhata's index value of 70.78, while female Bhils had a value of 45.28. Female Jaunsaris had a pattern intensity index value of 13.31, whereas female Bhils had a value of 12.12. Overall pattern type indices for Jaunsari and Bhil females revealed that Dankmeijer's index for both female groups was 19.94 and Furuhata's index was 56.98. The pattern intensity index value for both groups was 12.71.

Table 3 demonstrates that all characteristics (W-L-A) were found to significantly differ between the female hands of the Bhil and Jaunsari but the difference between the female right hand and female left hand was determined to be non-significant (p-value- .878598).

Based on the Total Finger Ridge Count (TFRC) and Absolute Finger Ridge Count (AFRC), the t-test was calculated to determine the variation among the Bhil and Jaunsari females (both hands). It was found that there was statistically significant variation between females from the Bhil and Jaunsari communities. **(Table 4-5)**

Table 6 lists the differences between the female Bhil and Jaunsari finger-ball dermatoglyphics and those of other populations. In comparison to other tribal communities, the New Zealand Samoans[21] and the Apatani[7] demonstrated the highest value of whorls (65.6 - 62.08). Among the females of Limboo,[9] Pentia[22] and Bhils (current study) displayed the highest value of loops, with values of 75.00 - 63.28 - 63.6. Females from the Bhil population and Sonowal Kachari[23] had the highest frequency of arches, with values ranging from 7.6 to 6.80.

When compared to other populations, the New Zealand Samoans[21] and the Apatani of Arunachal Pradesh[7] had greater values for the Pattern Intensity Index **(Table 6)**. Female Bhils from the current study exhibited characteristics that were similar to those of Sinhalese[24] and Bagathas.[1] Female Jaunsaris (according to the current study) also displayed significant similarities to other tribal communities **(Table 6)**. In comparison to other populations, Brahmins[25] and Bhil females (Present study) displayed the highest values of Dankmeijer's Index. Tibetans[26] showed a very low value for Furuhata's Index in comparison to other populations, whereas New Zealand Samoans,[21] Apatani,[7] and Brahmin[25] showed higher values for Furuhata's Index in comparison to other populations.

DISCUSSION

There are many finger-ball Dermatoglyphic studies that were performed on the tribal and caste population such as the present study that was conducted on the Bhil and Jaunsari females residing in two different geographical parts of India. The frequency of the finger-ball patterns was examined in 100 Bhil females from Udaipur, Rajasthan, and 100 Jaunsari females from Dehradun, Uttarakhand. The percentage of finger-ball patterns for all ten fingers in Bhil females was determined to be: loop, 63.6%; whorl, 28.8%; and arch, 7.6%. For all ten fingers on Jaunsari females, the percentage of patterns was as follows: loop, 52.2%; whorl, 38.8%; and arch, 5.9%. According to the percentage frequency for both hands in Bhil females, the ulnar loop pattern was the most common in both female groups, followed by whorl patterns (which were more

Table 1: Showing the digit-wise distribution of finger-ball pattern types among the Jaunsari and Bhil females.

Pattern Type	JAUNSARI FEMALES (n=100)						BHIL FEMALES (n=100)						Total (n=200)	
	Right Hand		Left Hand		Right + Left Hand		Right Hand		Left Hand		Right + Left Hand		Jaunsari And Bhil Females	
	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%
WHORL	198	39.6	191	38.2	389	38.9	135	27.0	153	30.6	288	28.8	677	33.85
LOOP	272	54.4	280	56	552	55.2	339	67.8	297	59.4	636	63.6	1188	59.4
ARCH	30	6	29	5.8	59	5.9	26	5.2	50	10.0	76	7.6	135	6.75
TOTAL	500	100	500	100	1000	100	500	100	500	100	1000	100	2000	100

Table 2: Showing the pattern type indices of Bhil and Jaunsari females

Pattern Type Indices	Values		Total
	Jaunsari Females (n=100)	Bhil Females (n=100)	Jaunsari+Bhil Females (n=200)
Dankmeijer's Index=Arches x 100/Whorls	15.12	26.38	19.94
Furuhata's Index=Whorls x 100/loops	70.78	45.28	56.98
Pattern Intensity Index=2 x Whorls + Loops/n	13.31	12.12	12.71

Table 3: Showing the Chi-square difference between Jaunsari and Bhil female hands.

Variables	Value of Chi-Square	p-value
JFRH vs. JFLH	0.2589	.878598
BFRH vs. BFLH	11.4775	.003219
JFRH vs. BFRH	19.5516	.000057
JFLH vs. BFLH	10.2808	.005855
JF vs. BF (both hands)	23.1481	.00001

common in Jaunsari females) and arches (which were most common in Bhil females).

It was found that Jaunsari females had a higher percentage of the whorl pattern, whereas Bhil females had a higher percentage of the loop and arch patterns. Based on Total Finger Ridge Count (TFRC) and Absolute Finger Ridge Count data (AFRC), these group differences were statistically significant (p 0.05). While the comparison between the female Jaunsari right and left hands in finger-ball patterns was found to be non-significant (p=0.88), it was noted that group differences in finger-ball patterns were also found to be statistically significant (p.05).

According to literature, there have been numerous studies on tribal populations from various Indian states' frequency distributions of finger-ball patterns. In 1964, K. C. Tripathy[27] conducted research on 48 Khonds and 62 Lanjia Saora people in the Orissa state. He discovered that the Lanjia Saora had the highest frequency of loops (55.89%), whorls (37.83%), and arches (6.27%), with the whorls coming in second place. Following whorls (37.10%) and arch (6.66%) in Khonds, loop (56.23%) was the pattern with the highest frequency. In terms of pattern intensity index, it has been discovered that these two tribal populations are similar.

In 1967, Dutta and Gupta[28] studied the fingerprints of the Asura; a traditionally iron-making tribe in Chotanagpur, Bihar, and discovered that loops were the most common pattern, occurring in 62.73% of males and 67.60% of females, whorls were next (35.92% and 28.67% of females), and arches were very uncommon (1.35% versus 3.73%). In 1967, Tiwari and Chattopadhyay[26] examined and researched the fingerprints of 150 Tibetan women and 156 Tibetan men. Tibetan males (60.24%) have more whorls than females (48.67%) do. Men were found to have more loops (38.99%) than females (49.13%), and males

Table 4: Showing t-test for variation among Jaunsari (n=100) and Bhil females (n=100) based on Total Finger Ridge Count (TFRC).

Population	Mean X	Standard Deviation S.D	Standard Error of Mean	Value of t-test	p-value
Jaunsari Females	123.47	42.34024	4.234024	3.4723	0.0006
Bhil Females	145.95	48.93818	4.893818		

Table 5: Showing t-test for variation among Jaunsari (n=100) and Bhil females (n=100) based on Absolute Finger Ridge Count (AFRC).

Population	Mean X	Standard Deviation S.D	Standard Error of Mean	Value of t-test	p-value
Jaunsari Females	154.266	75.29941	7.529941	3.9876	0.0001
Bhil Females	203.65	98.32295	9.832295		

Table 6: Showing the comparative finger-ball Dermatoglyphics of Bhil and Jaunsari females with other populations.

Female Population	No.	Pattern Frequencies			Indices			References
		W	L	A	PII	DI	FI	
Apatani (Arunachal Pradesh)	48	62.08	35.83	2.08	15.88	3.36	173.26	Jaswal et al. 1986 [7]
Bagathas (Araku valley)	235	37.57	57.58	4.85	12.84	13.75	70.06	GG Reddy, 1975 [1]
Konda (Orissa)	55	46.91	47.82	5.27	14.104	11.23	99.34	KV Satyanarayanarao, 2020 [30]
Rengma Nagas (Nagaland)	103	55.69	42.81	1.50	1.56	3.34	-	Dutta et al, 2009 [3]
Naikas (Gujarat)	100	40.8	56.3	2.90	13.79	7.10	72.46	A Mukherjee, 1988 [10]
Pentia (Koraput, Orissa)	206	34.24	63.28	2.48	13.18	7.24	54.11	Das & Kundu, 1984 [22]
Sonowal Kachari (Dibrugarh)	100	33.50	59.70	6.80	13.05	20.29	56.11	Dutta & Sengupta, 1994 [23]
Limboo (Sikkim)	150	21.33	75.00	3.66	14.08	12.60	96.06	Dorjee et al, 2015 [9]
Tibetans	150	48.67	49.13	2.20	14.65	4.50	19.06	Tiwari & Chattopadhyay, 1967 [26]
Muslims (Central India)	240	46.5	48.17	5.33	10.38	19.15	57.77	N Kapoor, 2015 [31]
Rajputs	128	39.33	55.64	5.01	13.61	12.40	129.00	Baryah & Krishan,
Brahmin		40.07	54.00	5.92	13.48	27.20	161.43	2020 [25]
New Zealand Samoans	128	65.6	33.70	0.70	16.49	1.07	194.66	C. Cho, 1998 [21]
Australian Aborigines	93	51.2	47.00	1.70	14.94	3.32	108.94	C. Cho, 2000 [32]
Sinhalese (Srilanka)	217	34.52	60.92	4.56	12.99	13.22	56.65	Buddhika et al, 2013 [24]
Jaunsari	100	38.90	55.20	5.90	13.31	15.12	70.78	Present Study
Bhil	100	28.80	63.60	7.60	12.12	26.38	45.28	

had much less arches (0.76%) than females (2.20%).

A study was done on 59 Gond and 33 Lodhi females of Western Mandla by Talwar et al. (2019).[29] The findings on the finger patterns of female Gond and Lodhi were as follows: loops were higher in Lodhi females (67.57%) than in Gond females (56.94%), followed by the whorl, which was found to be higher in Gond females (39.82%) than in Lodhi females (27.59%), and arches were similar in frequency percentage in both groups (Gond females-3.22% and Lodhi females-3.32%).

The maximum whorl frequency was observed in Tibetan females (48.67%),[26] while the lowest whorl frequency was observed in Lodhi females (27.59%),[29] according to a comparative analysis of these tribal communities. Following those Bhil females (63.6%) [Present study] were likewise discovered to have the highest frequency of loops, the highest frequency of loops was identified in Asura females (67.60%),[28] who were extremely comparable to Lodhi females (67.57%).[29] Tibetan females (49.13%)[26] had the lowest frequency of loops. Females from Bhil had higher arches (7.6%), whereas Tibetan females[26] had smaller arches (2.20%); these values were rather similar to those of Lodhi,[29] Gond,[29] and Asura[28] females (3.32%, 3.22%, and 3.73%, respectively).

CONCLUSION

Finger ball dermatoglyphics of 200 females (100 Bhil and 100 Jaunsari) had been collected and analyzed. This study aimed to determine differences in finger ball patterns between Bhil females from Udaipur, Rajasthan, and Jaunsari females from Dehradun, Uttarakhand. Whorls (Bhil females: 28.8%; Jaunsari females: 38.9%); Loops (Bhil females: 63.6%; Jaunsari females: 55.2%); and Arches (Bhil females: 7.6%; Jaunsari females: 5.9%) were the pattern categories with the highest frequency. Loops were discovered to occur at higher frequencies than whorls in both groups. Based on TFRC and AFRC, it was discovered that there was a considerable difference between the two groups. The different Pattern type indices among Bhil females revealed that the Dankmeijer's index was 26.38 compared to Jaunsari females' 15.12, the

Furuhata's index was 45.28 compared to Bhil females' 70.78, and the Pattern intensity index was 12.12 compared to Jaunsari females' 13.31.

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CONFLICT OF INTEREST

The authors declare that they have no Competing interest.

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Original Research Paper

Mandibular Canine in Sexual Dimorphism to Aid in Forensic Investigation

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ABSTRACT

Introduction: One of the important components of forensic research is sexual dimorphism. Since they are more likely to survive in all disasters, mandibular canines are ideal resources for forensic investigations to estimate sex. This study compared the mesiodistal breadth of mandibular permanent canines and intercanine distance in a group of the Delhi population in order to look at sexual dimorphism.

Material and Methods: The study incorporated 100 participants in the 18–40 years age group, of which 50 were males and 50 were females. The maximum mesiodistal widths of the right and left mandibular canines were measured on study cast models with the help of a Digital Vernier Caliper. Sexual dimorphism was calculated using statistical analysis of the collected data.

Results: The mean mesiodistal widths of the left and the right mandibular canines in males were 6.77±0.51 mm and 6.78±0.51mm which is higher as compared to 6.1± 0.38mm and 6.07± 0.46mm in females. The mean intercanine distance in males was 26.01± 2.3mm which is higher as compared to 22.71± 1.31mm in females. The p values were P<0.001 assessed which shows the comparative statistics that were highly significant. The right mandibular canine was found to show greater sexual dimorphism (11%) as compared to the left mandibular canine (10%).

Conclusions: The present study establishes a distinct, statistically significant sexual dimorphism in mandibular canines. The parameters assessed in the contemporary study are quite supportive of sex identification during forensic investigations.

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INTRODUCTION

Sex along with age and height –at death, is one of the important pieces of information required to outline the biological profile of skeletal remains. Some bones are sexually dimorphic like skulls, pelvic bones, etc.[1-2] but even if the appropriate osteological techniques are used, the disrepair state of conservation of certain archaeological remains might change or obscure the readability of dimorphic traits in an individual. Correspondingly, classification, background, and/or age may also affect the precise estimation of sex.[3,4,5] In these circumstances, DNA may be an effective substitute for sex estimation even if the analytical values and the existence of the DNA itself may powerfully limit the use of genetic markers.[6]

Sex estimation is a branch of forensic odontology that is extremely crucial, especially when information about the deceased is unavailable.[7] Sex estimation, which is a crucial component of personal identification must first be resolute. This is a frequently required step in establishing the individual's identity because the estimated age and height depend on the individual's sex.[8]

Forensic dentistry is predominantly concerned with establishing a person's identity through teeth and has played a substantial, often indispensable, role in identifying victims of mass disasters.[9]

Teeth are vital assets for anthropological, genetic, odontological, and forensic research in living and non-living populations. Teeth are the hardest (270-350 Knop Hardness Number for enamel and dentine) human body's structure.[10,11,12] They are an indispensable tool for identification due to their resilience to fire, and bacterial degradation.[13] Several odontometrical approaches can be employed to recognize sex from teeth when bodies are frequently destroyed beyond identification after major disasters.[14]

Sex estimation mostly employs visual, microscopic, and advanced techniques. Visual techniques consider male and female teeth' morphological features such as tooth size, root length, and crown diameter. The advanced way to estimate sex used PCR and enamel protein (amelogenin), whereas the microscopic method used sex chromatin.[15]

The term "sexual dimorphism" describes how males and females differ in terms of size, stature, and appearance. Since no two mouths are the same, this also applies to dental identification.[16] In addition, tooth size is influenced by a galaxy of factors due to which its morphometric study is a subject of profound interest and gives significant results. Tooth size standards are frequently used in sex estimation. The former is considered to be the more important of the two proportions - width and length.[17]

Several approaches for measuring canine teeth were investigated including Fourier analysis, Moire topography, and assessing linear measurements of teeth like mesiodistal width, buccolingual width, and incisocervical height.[18-20,15]

Sex estimation analysis is possible using either morphological or molecular methods. The morphological examination is possible on Oral and Para-oral hard tissues (Odontometric, Orthometric, and miscellaneous) or soft tissue (lip prints-Cheiloscopy, Palatal rugae pattern-rugoscopy). The odontometric method on hard tissue analysis includes mesiodistal dimensions and buccolingual dimensions of teeth, mean canine index (dental index), and distinct tooth morphology. The most simplistic and highly reliable method for analyzing sexual dimorphism is measuring teeth' mesiodistal and buccolingual dimensions.[21] An earlier study asserted that the Mesiodistal dimension was preferable to the buccolingual dimension for identifying sex because arch size influences tooth size, males with larger jaw sizes may have larger mesiodistal dimensions of teeth than females.[22]

Among all teeth, a canine is one of the toughest and most stable teeth because of its shape, structure, and root length. In addition, canines are situated in the mandible and maxilla and have a single cusp, making them less exposed to devastation or cavity formation. Therefore, canines have more forensic significance.[8]

Recent studies have manifested that the greatest sexual dimorphism is found in mandibular canines.[23-24] The mandibular canines have a mean eruption age of 10.87 years and are the last teeth to be extracted in terms of age. They are less susceptible to periodontal diseases and are more likely to survive severe trauma such as air disasters, hurricanes, or fires. These findings suggest that mandibular canines are the "key teeth" for personal identification.[25]

According to Garn et al. (1964), the measurements of the mesiodistal diameters systematically allow better sexual differentiation. The highest variations are observed on the first molar, followed by the second molar, and then by the upper and lower canine.[26]

To assist in personal identification when a person's identity is beyond recognition, the present study set out to evaluate the significance of the mandibular canine dimensions employed for sexual dimorphism As a result, the present study used the Mesio distal width of the right and left mandibular canine and intercanine as a measure for sex estimation.

MATERIALS AND METHODS

The present study was carried out in a Private Dental Clinic in Delhi, India after attaining ethical clearance from the institutional authorities. The participants were fully informed about the study undertaken and provided written informed consent. They were also promised that their refusal to participate in the study would not jeopardize their treatment.

Study Population and Study Sample

The study population entailed new patients who came to the dental clinic for dental treatment or a routine dental check-up. The study encompassed 100 participants in the 18–40 years age group, of which 50 were males and 50 were females. The study selected participants based on pre-planned inclusion and exclusion criteria.

Inclusion & Exclusion Criteria: As described in **Table 1**

Parameters used: As described in **Table 2**

Measurements Recording Convention

Table 1: Inclusion and Exclusion Criterion

Usion Criterion	Exclusion Criterion
Healthy Gingival and Periodontium State	Unhealthy Gingival and Periodontium State (Gingivitis and Periodontitis)
Healthy and Non-carious Tooth, Fully Erupted	Decayed, Attrition, Partially Erupted Restored, Orthodontic Treatment, Trauma, Deleterious Habit Like Bruxism
Normal Bite	Other Than Normal Bite (open, Deep Bite, And So On.)
Normal Alignment	Malocclusion, Crowding, Rotation, Diastema
Normal Molar and Canine Relationship	Other Than Normal Molar And Canine Relationship

Table 2: Parameters

Parameters
•Mesiodistal width of right mandibular canine
•Mesiodistal width of left mandibular canine
•Intercanine distance from right mandibular canine to left mandibular canine

Fabricating an Impression

The participants were settled on a dental chair, and mandibular impressions were taken using A- Silicone impression material (AFFINIS) on an impression tray in a well-established clinic in an aseptic environment.

Preparing The Study Cast Model

Subsequently, impressions were taken and study cast models were created with Type 4 extra hard Dental Die Stone (Elite Rock).

Instrument Used

All measurements were recorded extra orally by means of a Digital Vernier Caliper on the study cast, taking into account errors in the instrument.

Mesiodistal Width Measurement Technique

A Digital Vernier Caliper was used to measure the maximum mesiodistal width between the contact points of teeth on either side of the study model, which was accurate to 0.01 mm at that width. The divider points measured the distance between the crests of curvature on the mesial and distal surfaces of the teeth, which were defined as mesial and distal surfaces. Every reading was taken three times to reduce intra-observer error and the average of the three values was computed **(Figure 1)**.

Intercanine Distance Measurement Technique

The intercanine distance was calculated by measuring the distance between the tips of both canines. The divider points were placed on the mandibular canine tips on the study cast. The result was then recorded by holding the divider against the Vernier Caliper **(Figure 2)**.

Data Collection

All recorded measurements were computed in a tabulated manner and statistically analyzed for the purpose of establishing sexual dimorphism. Mean, standard variance, Standard error, Standard deviation, degree of freedom, T-test, and P value were calculated for each parameter.

Statistical Analyses

Means

Mean is the average of the given numbers and is calculated by dividing the sum of given numbers by the total number of numbers.

Figure 1: Mesiodistal Width Measurement of Mandibular Canine

Figure 2: Intercanine Distance Measurement

Mean = (Sum of all the observations/Total number of observations)

The formula of the mean is, Mean $\bar{x} = (\sum x)/N$

where

$\sum$ represents the summation

x represents scores

N represents the number of scores

Variance

The variance is a measure of how far a set of data are dispersed out from their mean or average value. It is denoted as 'σ2

The population variance formula is given

$\sigma^2 = (\sum(x-\bar{x})^2)/N$

Here,

σ2 = Population variance

N = Number of observations in the population

X = Xth observation in the population

$\bar{X}$ = Population mean

Standard Deviation

The spread of statistical data is measured by the standard deviation. Distribution measures the deviation of data from its mean or average position. The degree of dispersion is computed by the method of estimating the deviation of data points. It is denoted by the symbol, 'σ'.

The symbol for Standard Deviation is σ (the Greek letter sigma).

This is the formula for Standard Deviation:

$\sigma = \sqrt{((\sum(x-\bar{x})^2)/N)}$

Standard Error

The standard deviation of a sampling distribution is called the standard error. The standard error is one of the mathematical tools used in statistics to estimate variability. It is abbreviated as SE

$SE.\bar{X} = \sigma/\sqrt{N}$

where

$SE.\bar{x}$ = Standard Error of the Mean

σ = Standard Deviation of the Mean

N = Number of Observations of the Sample

T-test

A t-test is a type of inferential statistic used to determine if there is a significant difference between the means of two groups, which may be related to certain features.

$$T = \frac{\bar{X}1 - \bar{X}2}{\sqrt{(S.E.[\bar{X}1]^2 + S.E.[\bar{X}2]^2)}}$$

Where

Sample Data ($\bar{X}1$) = Mean of x1

Standard Error of Mean = ($S.E.\bar{x}1$)

Sample Data ($\bar{X}2$) = Mean of x2

Standard Error of Mean ($S.E.\bar{x}2$)

Degree of Freedom

The Degree of Freedom to be used is obtained by adding the total number of two samples and subtracting 2 from it i.e.

d.f. = (N1 + N2) - 2

Sexual Dimorphism Formula

Data acquired from various measurements were documented on a proforma and sexual dimorphism measured as per Garn and Lewis formula (45):

$((Xm)/Xf)-1 \times 100$

Where,

Xm – mean of male tooth dimension

Xf – mean of female tooth dimension.

The probability (P) Value was considered

If P > 0.05, the relation is non-significant.

If P < 0.05, the relation is significant.

RESULTS

The comparative statistics between the two sexes are shown in **Table 3, Table 4,** and **Table 5**: the left mandibular canine mesiodistal width, right mandibular canine mesiodistal width, and intercanine distance were considerably higher in males as compared to females. The mean mesiodistal widths of the left and the right mandibular canines in males were 6.77±0.51 mm and 6.78±0.51mm which is higher as compared to 6.1± 0.38mm and 6.07± 0.46mm in females. The mean intercanine distance in males was 26.01± 2.3mm which is higher as compared to 22.71± 1.31mm in females. The p values were P<0.001 assessed which shows the comparative statistics which were highly significant. The right mandibular canine was found to show greater sexual dimorphism (11%) as compared to the left mandibular canine (10%) in **Table 6**. The comparative mean bar graph of the left mandibular canine, right mandibular canine, and intercanine distance between the two sexes are shown in

Table 3: Comparative Statistics of Left Mandibular Canine Between Two Sexes

Parameters	Gender	Sum (in mm)	Mean (in mm)	Variance	Standard Deviation	Standard Error	T-Test	d.f.	p value	Significance
Left Mandibular Canine	Male	338.65	6.77	0.26	0.26	0.07	7.7	98	P<0.001	Highly Significant
Mesiodistaldata	Female	305.33	6.10	0.14	0.14	0.05				

Table 4: Comparative Statistics of Right Mandibular Canine Between Two Sexes

Parameters	Gender	Sum (in mm)	Mean (in mm)	Variance	Standard Deviation	Standard Error	T-Test	d.f.	p value	Significance
Left Mandibular Canine	Male	339.02	6.78	0.26	0.51	0.07	7.8	98	P<0.001	Highly Significant
Mesiodistaldata	Female	303.70	6.07	0.21	0.46	0.06				

Table 5: Comparative Statistics of Intercanine Distance Between Two Sexes

Parameters	Gender	Sum (in mm)	Mean (in mm)	Variance	Standard Deviation	Standard Error	T-Test	d.f.	p value	Significance
Left Mandibular Canine	Male	1300.88	26.01	5.32	2.30	0.32	11	98	P<0.001	Highly Significant
Mesiodistaldata	Female	1135.67	22.71	1.72	1.31	0.18				

Table 6: Comparative Percentage of Sexual Dimorphism in the Left and Right Mandibular Canine

Sexual Diamorphism	Percentage
Left Mandibular Canine	10%
Right Mandibular Canine	11%

Figures 3, 4, and **5**, and the individual value plot graph illustrates the difference in the range of mesiodistal width of the right and left mandibular canine and intercanine distance of male and female, which is greater in males than females in **Figures 6, 7, 8, 9, 10**, and **11** by using Minitab Software.

DISCUSSION

Teeth have long been recognized as excellent models for studying the relationship between Ontogeny and Phylogeny. Individual identification is simplified when sex is determined, and it is critical in forensics.[27] The present research aims to identify sex differences in the morphology of permanent mandibular canines. It included measuring mesiodistal width and intercanine distance. The study sample entailed patients between 18 and 40 years old who visited a dental clinic for regular dental check-ups. In the present study, the mesiodistal width of the left and right mandibular canines was assessed in a comparable cohort of male and female patients while maintaining a resemblance between groups for other factors affecting tooth size. The dimension of the mandibular canines is the foundation of the procedure considered in this study. Hence the present study was shown on mandibular canines to find out about sexual dimorphism for personal identification.

The canines are the teeth that exhibit the greatest degree of dimorphism, according to Lund et al (1999), who studied 58 dental casts of Swedish participants.[28] The mandibular canine showed the highest level of sexual dimorphism (5.7 %) among all teeth, according to Lysell and Myrberg's large examination of more than 1000 participants.[29] Accordance with Hashim and Murshid (1993), who established a study on pre-treatment Orthodontic cast of 720 Saudi males and females in the age range of 13 to 20 years and came upon that only the canines in both jaws exhibited a significant sexual difference.[30]

Any teeth measurement without considering factors like age, race, or sex should be handled with extreme caution.[31] Kaushal et al. (2003) revealed that the left mandibular canine (9.796% in casts and 8.891% intraoral) was more dimorphic than the right mandibular canine (7.96% in casts and 7.954% intraoral).[16] Nair et al. (1999) came to the conclusion that the left mandibular canine showed extreme sexual dimorphism (7.7%) and thereafter the right mandibular canine (6.2%),[32] whereas the present study found that the right mandibular canine (11%) was found to be statistically more significant than the left mandibular canine (10%) in terms of the difference in mean mesiodistal width between males and females, estimated by Garn et al. Formula (1964). This disparity can be accredited to a variety of factors, including racial, environmental, and nutritional factors.

The mesiodistal dimensions of mandibular canines were comparable to those already reported in literature taking both sexes together.[14,16,33-36] Cassidy et al. (2008) examined the size and shape of 320 adolescents' mandibular dental

Figure 3: Comparative Mean Bar Graph Representation of Left Mandibular Canine Between two Sexes

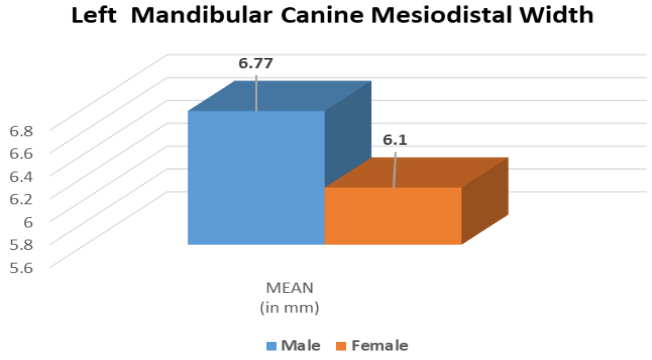


Figure 4: Comparative Mean Bar Graph Representation of Right Mandibular Canine Between two Sexes

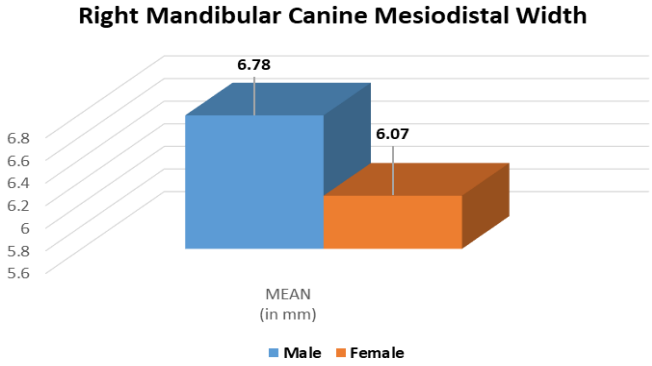


Figure 5: Comparative Mean Bar Graph Representation of the Intercanine Distance between two Sexes

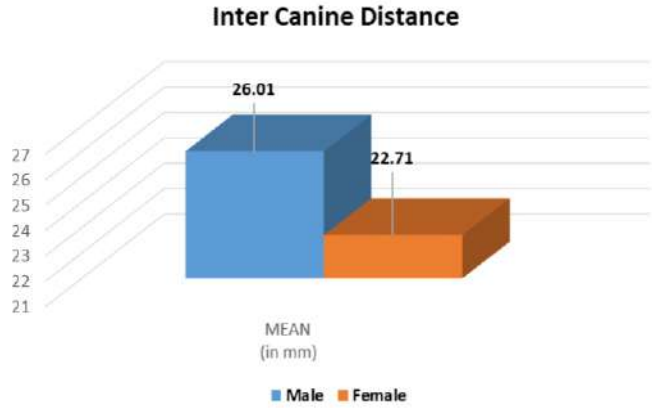


Figure 6: Individual Value Plot of Mesiodistal Width of Left Mandibular Canine Female

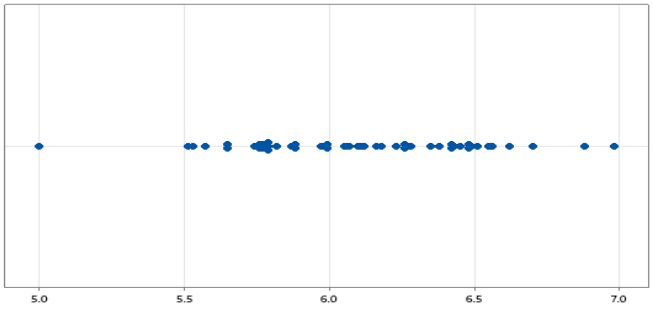


Figure 7: Individual Value Plot of Mesiodistal Width of Right Mandibular Canine Female

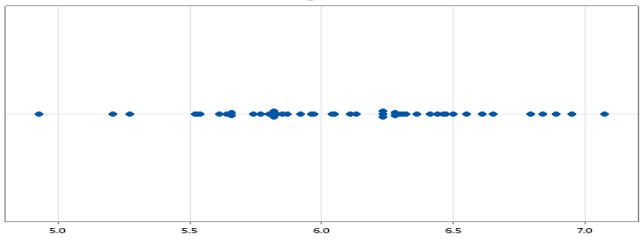


Figure 8: Individual Value Plot of Intercanine Distance of Mandibular Canine

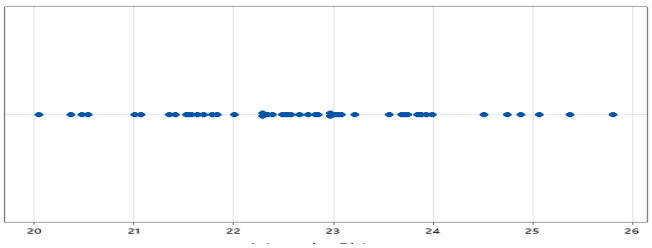


Figure 9: Individual Value Plot of Mesiodistal Width of Left Mandibular Canine

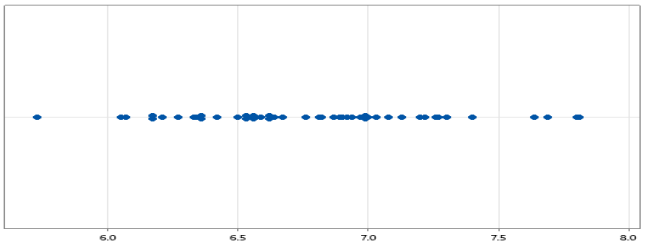


Figure 10: Individual Value Plot of mesiodistal width of Right Mandibular Canine

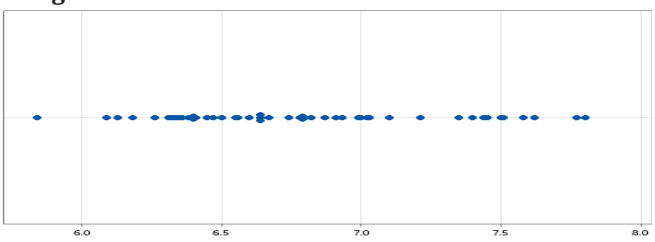
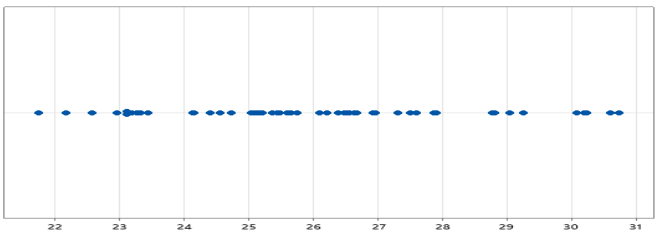


Figure 11: Individual Value Plot of Intercanine Distance of Male



arches. They found that the arch dimension is considerably larger in boys than in girls, mesiolaterally and anteroposteriorly. A well-established sex difference can be

seen before the commencement of the pubertal growth spurt.[37] Rao et al. (1989) revealed that intercanine distance and mandibular canine indices can be used to differentiate between sexes.[38] The current study found that the mean value of intercanine distance is significantly higher in males (26.01mm) than in females (22.71mm).

Genetic variables have a significant influence on the sexual dimorphism of canine size. Numerous researchers have discovered the association between both X and Y chromosomes.[39-40,18] Sexual dimorphism is governed by genetics. The "Y" chromosome, which regulates dentine thickness, has a stronger impact on tooth size than the "X" chromosome, which controls enamel thickness more strongly.[18,14] The difference between the mean mesiodistal widths of the left and right mandibular canines was found to be statistically more significant by Vishwakarma et al. in 2011.[41]

Harris et al. (2001) discovered that different tissues play a role in the sexual dimorphism of tooth size. Males have significantly larger dentine and pulp dimensions than females, while marginal enamel thickness is comparable in both sexes.[42] Moss et al. (1977) proposed that dimorphism is associated with a significantly longer period of amelogenesis in both deciduous and permanent dentitions.[43]

In the present study, we perceived that whenever the canine width is exceeding 7.2 mm, the possibility of the sex being male is higher. In the other studies, the possibility of being male occurred when the canine width was greater than 7.3 mm, 7.0 mm, 7.2, and 7.5 mm respectively.[34,20,44-45] This finding could be of immense medico-legal importance in the identification of sex in the Delhi population as it makes identification easier.

Studies on the mandibular second premolar by Acharya and Mainali in the Nepalese population and longitudinal research on the Chinese population by Yuen et al. on the mandibular incisors both revealed reverse dimorphism (where the females displayed larger teeth than the males).[46-47]

However, it should be emphasized that the method of establishing sex through canine measurement has some flaws. Only when the part of the mandible is discovered in the region where the person was born can the sex of the subject to whom it belongs be confirmed satisfactorily. The current study only evaluated the linear dimensions; greater precision might be acquired by applying Moire's topography and Fourier's analysis, which calls for advanced tools and the application of intricate mathematical calculations, correspondingly.

CONCLUSIONS

The developing science of forensic dentistry in India relies entirely on low-cost and simple means of analyzing people from broken jaws and tooth remains. A dentist with forensic scientific competence may be needed in such circumstances. It is drawn that the mesiodistal width of mandibular canine and intercanine distance are considered completely different in males and females. The present study shows that when the canine width exceeds 7.2 mm, the sex is more likely to be male. The right mandibular canine (11%) was more pleomorphic than the left mandibular canine (10%). As a result, this study confirmed a statistically significant difference in mesiodistal widths of mandibular canines between males and females, which is consistent with previous research. This research also gives insight into the use of dental casts as valuable forensic evidence for sex estimating. It can be asserted that forensic investigations can use Canines as a consistent and rapid technique for sex identification of an unidentified person because it is simple, quick, and inexpensive, requires no extensive instrument, and is appropriate for situations requiring a large number of samples to be analyzed for sex estimation. When more advanced methods for sex estimation are inaccessible, this method becomes more important.

List of Abbreviations: Not used

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Original Research Paper

Volatile Profiling of Cough Syrups For Forensic Purposes Using GC-MS

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ABSTRACT

Introduction: Cough syrup is a liquid drug used to treat coughs and colds. It comprises several components such as cough suppressing substances in conjunction with various chemically active chemicals.

Materials and Methods: A thorough qualitative examination of various cough syrups was conducted in this study, in which 20 randomly selected cough syrup samples were analysed using proper Liquid-Liquid Solvent extraction protocol, followed by volatile profiling of the samples using Gas Chromatography tandem Mass Spectrometry.

Results: The findings revealed 349 organic volatile components classified as Alcohols, Aldehydes & Ketones, Organic acids, Esters, Ethers, and other miscellaneous substances. A few analgesics (Dihydromorphine), semi-synthetic opioids (Dextromethorphan), and morphinans (Norlevorphanol) were also discovered but were not listed on the label. Because to its high efficiency and low error rates, we employed GCMS in this investigation to evaluate the organic volatile components contained in each sample.

Conclusions: GC-MS is effective, complementary techniques for the selective and sensitive quality control of cough syrups

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INTRODUCTION

A drug is any chemical (natural, semi-synthetic, or synthetic) substance that changes physiological or pathological conditions for the advantage of the recipient. The most common type of drug which is utilised in day-to-day life is for the treatment for cough and cold. Cough and cold treatments have been used to provide symptomatic relief for decades and are readily accessible as over-the-counter (OTC) medications. Despite equivocal evidence for their effectiveness, they remained the first line of therapy.[1-3] The cough syrups consist of various active ingredients which are antitussive reagents that helps in suppressing the cough in human body. Main active ingredients in well-known cough syrups are chlorphenamine, guaifenesin, acetaminophen, dextromethorphan etc. Most cough syrups are manufactured as sweet and palatable as sweet flavor have a particular effect on cough locations in the brain by promoting endogenous opioid synthesis.[4-5]

Cough-cold preparations have been frequently abused because of their simple availability, low cost, and ability to induce the desired emotion in the abusers.[6] High performance thin layer chromatography (HPTLC) and thin layer chromatography (TLC) have been used to detect and quantify active ingredients in OTC medications.[7-9] Dextromethorphan (DXM) misuse as hallucinogen peaks around the age of 19 years and swiftly declines after that.[10] Sometimes recreational users report zero physical addiction or withdrawal symptoms. Those who regularly take cough syrups containing dextromethorphan with the goal of abusing it report minor withdrawal symptoms similar to opiate withdrawal. However, there is possibility of resistance to dextromethorphan, which must be conquered in order to produce the desired "high." Dextromethorphan's exhilaration has been characterized as a PCP-kind of bliss, which has contributed to the street term "poor man's ecstasy".[11] Street names for DXM include “skittles,” “robos,” “rojos,” “velvet,” “CCC,” and “poor man's

PCP," among others. Dextromethorphan intoxication is referred to as "robo-tripping," "skittling," and "dexing" in slang. Adolescents are the most likely to abuse dextromethorphan.[12]

Though previous available studies have separated and identified components of cough syrups like organic volatile organic acids, volatile terpenes, essential oils etc[13] But No proper examination or profiling of the different types of components especially type of opioid impurities has been done by analytical methods like GC-MS as per our knowledge irrespective of their mentioning on its official label.

Therefore, the present study is done on various cough and cold syrups to separate out, identify and classify the various organic compounds like alcohols, aldehyde, esters, and organic acids and other impurities or miscellaneous compounds by employing Gas chromatography Mass Spectroscopy.

MATERIALS AND METHODS

The present study was conducted in the forensic chemistry and toxicology laboratory of Regional Forensic Science Laboratory, Dharamshala, Himachal Pradesh during the time duration of December 2021 to January 2022. Twenty (20) random cough syrups were collected from different chemist shops in Nagpur district of Maharashtra state region in plastic and glass bottles and sealed.

Sample preparation: 15ml of cough linctus sample was transferred into 250ml glass separating funnel, then 25ml of chloroform of analytical grade as solvent was added and mixed well. After that the pH of the solution was checked using litmus paper. To make the pH basic, 5ml of ammonium hydroxide was added to the solvent mixture. Then the separating funnel containing sample and solvent mixture was shaken vigorously for 10 to 15 minutes. After that the prepared solution was left untouched for around 20 minutes. Later when 2 distinct layers were visible, the organic layer (downside) was filtered and transferred into a beaker through Wattman filter paper bedded with anhydrous sodium sulphate to avoid moisture. After collecting the organic layer inside a beaker, the beaker was kept inside a fuming hood for overnight to get concentrated. Later on, getting fully dried and concentrated, a little amount of methanol solvent was added to the dried compound and shaking it properly so that all the dried components get dissolved into methanol. Followed by that, the solvent and sample layer were transferred into a glass vial through a filter paper and the sample was subjected to GC-MS analysis.

GCMS Conditions: Shimadzu GC-MS-QP2020 NX was used to analyse the specified samples. carrier gas used was helium, column -SH-Rxi-5 silicon MS (30 x 0.25mm ID, 0.25µm film thickness), oven temperature of column raised from 60°C to 250°C, and column flows of 1 ml/min in Splitless mode. The whole duration of the run was 33 minutes. For sample injection, an Auto sampler (AOC-20i) was employed, and a 10 µL of sample was injected. The temperatures of the ion source and the contact were both 230°C. The Shimadzu software (Real-time analysis) included with the equipment was used to collect spectrum, and post-run simulation tool was utilised to analyse the spectra. The chromatogram obtained was recognised by comparing the peaks to the National Institute of Standards and Technology (NIST) standard reference library 1A.

RESULTS

Compared to other analytical techniques, GCMS is believed to be the most suitable tool for the estimation and identification of organic and volatile compounds present in the cough syrup samples. The fraction of samples which were prepared by adding methanol were run and the results were matched based on the National Institute of Standards and Technology (NIST) GC-MS library. Total number of compounds found in all 20 samples analysed were 393. It has been observed that most of the alcohols and esters containing compound were found to be common in all the samples. Whereas the active ingredients remained sample specific. All the compounds found were broadly classified into 6 groups which were namely alcohols, Aldehydes & Ketones, esters, acids, ethers and additional compounds.

Alcohols

In the total of 20 samples, 86 components having alcoholic compounds were identified. Table 1 explains all the data related to the obtained alcohol components along with their retention time (Rt). Cyclohexanol, 5-methyl-2-(1-methylethyl)-, was found in abundance in most of the cough syrup samples. Other alcoholic compounds identified in most of the samples were i) Alpha –terpineol, ii) benzene methanol, iii) alpha.-phenyl, iv) ambroxol, v) 1-hexacosanol.

Acids

In all of the analyzed samples of cough syrups, fifty-seven organic compounds were found out/identified whose data is provided in Table 1. The most common type of acid to be found in maximum samples was unsaturated organic acid

i.e. Palmitic acid or N-hexadecanoic acid respectively. Along with it

i) Octadecanoic acid

ii) Oleic acid

iii) Pentatonic acid were found out to be common among all the 20 samples.

Aldehydes & Ketones

The relative amount and presence of Aldehydes and ketones were found out to be less compared to other organic components in the sampled cough syrups. A total of 37 Aldehydes and ketones in total were identified in the run samples of cough syrups. The organic compounds i.e. aldehyde and ketones found are presented in **Table 1**. Most of the aldehydes observed were

i) 2-amino-3,5-dibromobenzaldehyde

ii) 4-methoxy benzaldehyde

iii) Benzaldehyde dimethyl acetal, whereas identified ketones were

iv) 2-butanone, 1-(4-hydroxy-3-methoxyphenyl) dec-4-en-3-one

v) Alpha.-ionone in the cough syrup samples respectively.

Esters

The compounds containing esters were most abundant in all the cough syrup samples examined. Mostly, ester containing compounds are majorly responsible smell, flavouring agent and as preservatives. Most abundantly found esters in all the samples of cough syrup were Methylparaben and Propylparaben. Methylparaben is the methyl ester of p-hydrobenzoic acid. It is majorly used as a preservative.[20] Other esters like

i) Diethyl phthalate

ii) 2-phenylethyl ester

iii) n-caprylic acid isobutyl ester

iv) 1-methylethyl ester etc were also found in the samples examined using GCMS. Detailed information has been given in **Table 1**.

Ethers

In all the 20 samples, twenty-one types of ethers containing components were found in this study. Compared to other organic compounds, the constituent of ethers was very less. Guaifenesin was the only abundant type of ether containing component in all the cough syrup samples which was identified in the evaluation. Guaifenesin is categorized as an expectorant type of active ingredients helping in suppressing cough reflexes respectively. Although it is actively believed that guaifenesin brings out an action to ease productive cough to control chest congestion, it is unknown that the agent can reliably diminish coughing. **Table 1** exhibits data regarding ether containing components found in samples.

Additional compounds (Opiates and other impurities)

As a normal cough syrup is a mixture solution of various different other organic or inorganic chemical compounds, a hypothesis was marked that there may be some chances to find out some opioids along with some other trace impurities in these cough syrup samples. After analysing all 20 samples of cough linctuses using Gas Chromatography tandem Mass Spectrometry, It was observed that out of 20 samples, a total of 5 samples were found to be positive for the presence of other opioids and other impurities which were not mentioned on the label of the samples analyzed. The compounds which were found are mainly dextromethorphan, Norlevorphanol, dihydromorphine, cannabidiol, dronabinol, cannabinol etc. Some Misc. compounds which are not categorized in any functional groups were also found and mentioned in the following data of result. The detailed data is given in **Table 1**.

DISCUSSION

Cough and cold syrup medications are the liquid form preparations which are prepared in response to cure cough and cold conditions caused by external stimulus or disturbance in nasal and food passage. Presently there are more than thousands of cold and cough preparations available under various banners. Each has its specific active ingredient viz. antitussive agents like dextromethorphan, codeine, pholcodine, guaifenesin, chlorphenamine etc. and each has their own mode of action of curing the condition of cough. Faraga and Backy[13] had subjected seven samples of cough syrups to headspace solid-phase micro extraction (HS-SPME) followed by Volatile profiling using GC-MS and found organic volatile components of aromatics, aliphatic hydrocarbons, mono/sesquiterpene hydrocarbons, and oxygenated mono/sesquiterpene. The alcohols in their study were linalool, β-fenchol, pinocarveol, thymol, eugenol, etc. In comparison, our study separated and identified maximum number of alcoholic components.

Faraga and Backy[13] also identified fenchyl acetate, bornyl acetate, α-terpineol acetate, methyl salicylate, isoamyl

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Table 1: Details of volatile organic components along with their retention time classified under alcohol, acid, aldehydes & ketone, ester, ether and misc. compounds separated and identified using gc-ms.

Sample Code	Alcohols		Acids		Aldehydes & Ketones		Esters		Ethers		Miscellaneous	
	Rt	COMPOUND NAME	Rt	COMPOUND NAME	Rt	Compound name	Rt	Compound name	Rt	Compound name	Rt	Compound name
S-1	3.191 3.305	cyclohexanol 5-methyl-2-(1-methylethyl.alpha- terpineol	8.493	pentafluoropropionate	-	-	4.831 5.75	methylparaben propylparaben	6.277	trimethylsilyl (tms) derivative	7.711 8.337 8.493	chlorphenamine dextromethorphan hydromorphone
S-2	8.282	2-hexyl-1-decanol	4.173 4.954 6.040 6.110 7.290 8.305 8.120	butanoic acid pentanoic acid 1-ethylpropyl octanoate n-caprylic acid n-hexadecanoic acid n- octadecanoic acid octadecanoic acid	9.303	2-amino-3,5-dibromobenzaldehyde	4.173 4.831 4.954	3-methyl- propyl ester methylparaben butyl ester	-	-	8.351 9.303	dextromethorphan benzenemethanamine
S-3	3.187 5.083	cyclohexanol 1-phenoxypropan-2-ol	4.954 5.153 7.318	pentanoic acid 2- propenoic acid n-hexadecanoic acid	-	-	4.833 4.954 5.153	methylparaben 4-oxo-, butyl ester 3-phenyl-, 1-methylethyl ester	5.859	guaifenesin	7.718 8.347 8.495	chlorphenamine dextromethorphan hydromorphone
S-4	9.383	behenic alcohol	3.060	acetic acid	3.699 6.984	4-methoxy benzaldehyde benzaldehyde dimethyl acetal	3.060 4.827 5.575	phenylmethyl ester methylparaben diethyl phthalate	-	-	7.715 8.347 8.400 8.492 8.545 8.694	chlorphenamine dextromethorphan norlevorphanol equilin mevomethorphan8 dextrophan
S-5	3.183 8.284	cyclohexanol 2-hexyl-1-decanol	7.261 8.036 8.121 8.376 8.654 9.571	n-hexadecanoic acid oleic acid octadecanoic acid 9,12-octadecadienoic acid (z,z) glycidyl palmitate eruric acid	7.180	9z-tetradecenal	4.826 4.940 5.760	methylparaben diethyl pthalate propylparaben	6.176 7.138	guaifenesin 7,9-di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	9.931	cis-chlordane
S-6	4.229 9.384 9.775	1,2-cyclohexanediol, 1-methyl-4-(1-methylethenyl) n-tetracosanol-1 2-hexyl-1-decanol	7.316 8.060 8.59	n-hexadecanoic acid oleic acid glycidyl palmitate	5.391	4-(4-hydroxyphenyl)-2-butanone	5.576 6.899	diethyl pthalate 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester	5.576 6.899	diethyl phthalate 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester	8.341 8.397 8.493 9.043 9.138	dextromethorphan norlevorphanol equilin dihydromorphine desmomorphine

S-7	3.188 3.668 9.025	cyclohexanol, 5-methyl-2-(1-methylethyl)- 3-ethoxy-1-propanol n-tetracosanol-1	5.055 7.300	hexanoic acid, anhydride n-hexadecanoic acid	-	-	4.809 4.950 5.153 5.573 4.124 6.039 6.109 8.887	benzoic acid, methyl ester 4-oxo-pentanoic acid butyl ester 2-propenoic acid, 3-phenyl-, 1-methylethyl ester diethyl phthalate l-proline, n-(methoxycarbonyl)-, methyl ester 1-ethylpropyl octanoate n-caprylic acid isobutyl ester 1,4-benzenedicarboxylic acid, bis(2-ethylhexyl) ester	-	-	7.713 8.331 9.933	chlorphenamine dextromethorphan cis-chlordane
S-8	3.192	\ cyclohexanol, 5-methyl-2-(1-methylethyl)	7.295 8.047	n-hexadecanoic acid 9-octadecenoic acid	3.615	carvone	4.377 4.650 4.850 4.961 5.377 5.784 6.049 6.113 6.388 7.017 7.902	butanoic acid, 2-methyl-, 1-methylethyl ester l-phenylalanine, methyl ester methylparaben pentanoic acid, 4-oxo-, butyl ester isopropyl hydroxybenzoate propylparaben 1-ethylpropyl octanoate n-caprylic acid isobutyl ester l-phenylalanine, n-acetyl-, methyl ester decanoic acid, 2,3-dihydroxypropyl ester l-phenylalanine, n-acetyl-, methyl ester	3.878	ethanol, 2-(2-butoxyethoxy)	8.946 9.303 9.457	ambroxol benzenemethanamine, 2-amino-3,5-dibromo-n-cyclohexyl-n-methyl – undecylbenzene

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S-9	3.070	2-propanol, 1-[1-methyl-2-(2-propenyloxy)ethoxy]	7.306		3.898		4.853 5.322		-	-	5.120	2-oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl-, acetate
	3.193	cyclohexanol, 5-methyl-2-(1-methylethyl)			5.900	3-cyclohexene-1-acetaldehyde, α,4-dimethyl		methylparaben propylparaben			5.332	piperazine, 1-phenyl
	3.306	alpha.-terpineol										
	3.439	citronellol				2-butanone, 4-(4-hydroxy-3-methoxyphenyl)					7.718 8.364	chlorphenamine ethylphenylhydantoin
	3.591	geraniol		n-hexadecanoic acid								
	4.015	p-mentha-1,5-dien-8-ol			8.725							
	3.985	1,2-cyclohexanediol, 1-methyl-4-(1-methylethenyl)				1-(4-hydroxy-3-methoxyphenyl)dec-4-en-3-one						
	4.123	grandisol										
	4.171	1,2-cyclohexanediol, 1-methyl-4-(1-methylethenyl)										
	4.344	sobrerol 8-acetate										
	6.704	2,6,10,14-hexadecatetraen-1-ol, 3,7,11,15-tetramethyl										
	9.384	behenic alcohol										
S-10	3.187	cyclohexanol, 5-methyl-2-(1-methylethyl)	-	-	5.390	2-butanone, 4-(4-hydroxy-3-methoxyphenyl)	3.057	acetic acid, phenylmethyl ester	-	-	3.862	anethole
	3.300	alpha.-terpineol									7.722	dexchlorpheniramine
	3.429	citronellol					4.830	methylparaben			8.398	dextromethorphan
	3.586	geraniol					5.808	propylparaben			8.502	euilin
	5.499	benzenemethanol, alpha-(1-methyl-1-nitroethyl)					6.140	acetic acid, (2,4-dichlorophenoxy)-, ethyl ester			8.700	levorphanol
S-11			6.420		3.155	propane, 1,1'-[ethylidenebis(oxy)]bis	3.065		6.229			
			7.235				4.854			guaifenesin	3.432	5-hydroxymethylfurfural
			7.309				5.140				7.719	chlorphenamine
			8.054		4.545		7.147	octadecanoic acid, phenylmethyl ester			8.342	dextromethorphan
			8.132			[2-methyl]benzaldehyde,		methylparaben			8.823	9-tricosene
			8.744	tetradecanoic acid								
	3.301		8.897	palmitoleic acid	4.686	3-hydroxy-4-methoxy		diethyl phthalate				
	4.032		9.572	n-hexadecanoic acid	4.993	alpha-ionone		hexadecanoic acid, methyl ester				
		alpha.-terpineol		9-octadecenoic acid	5.025	1,3-dioxane						
		cyclohexanemethanol, 4-hydroxy-.alpha.,.alpha.,4-trimethyl		octadecanoic acid		3-buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)						
	9.384	1-hexacosanol		foctanoic acid, 3-octyl-, cis								
				eicosanoic acid								
				erusic acid								

S-12	3.190 3.305 4.086 5.895 9.384	cyclohexanol, 5-methyl-2-(1-methylethyl) alpha.-terpineol cyclohexanemethanol, 4-hydroxy-.alpha.,.alpha.,4-trimethyl benzenemethanol, .alpha.-phenyl octacosanol	5.055 6.040 7.293	hexanoic acid, anhydride 1-ethylpropyl octanoate n- hexadecanoic acid	5.390	2-butanone, 4-(4-hydroxyphenyl)	4.953 5.577 6.114	pentanoic acid, 4-oxo-, butyl ester diethyl phthalate n-caprylic acid isobutyl ester	5.985 7.135	guaifenesin 7,9-di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	8.634 7.062	benzhydryl isothiocyanate benadryl
S-13	3.191 5.505 8.950 9.383	cyclohexanol, 5-methyl-2-(1-methylethyl) benzenemethanol, .alpha.-phenyl ambroxol behenic alcohol	6.390 7.296	tetradecanoic acid n-hexadecanoic acid	4.819 5.694 8.227	cycloheptasiloxane, tetradecamethyl cyclooctasiloxane, hexadecamethyl cyclononasiloxane, octadecamethyl	5.577	diethyl phthalate	6.320	guaifenesin	9.196 9.590	cannabidiol dronabinol
S-14	3.190 3.307 6.967 7.837 8.284	alpha.-terpineol cyclohexanemethanol, 4-hydroxy-.alpha.,.alpha.,4-trimethyl 1-hexadecanol	7.297 7.558 8.130	n-hexadecanoic acid isopropyl palmitate octadecanoic acid	8.439		4.869 5.580 5.775	methylparaben diethyl phthalate propylparabe	5.997		7.516 7.447 8.823 9.199 9.384	parathion 1-nonadecene delta.9-tetrahydrocannabivarin cannabidiol 1- triacontanol
		behenic alcohol 1-decanol, 2-hexyl				p-heptylacetophenone				guaifenesin	9.589	dronabinol
S-15	3.186 5.350 8.946 9.380	cyclohexanol, 5-methyl-2-(1-methylethyl) 1,3-propanediol, 2-(hydroxymethyl)-2-nitro ambroxol 1-hexacosanol	4.005 7.296	heptanoic acid, anhydride n-hexadecanoic acid	3.062 4.549 5.397	oxiranecarboxaldehyde, 3-methyl-3-(4-methyl-3-pentenyl) benzaldehyde, 3-hydroxy-4-methoxy 2-butanone, 4-(4-hydroxyphenyl)	3.635 5.258	acetic acid, 2-phenylethyl ester diethyl phthalate	6.223	guaifenesin	3.434	5- hydroxymethylfurfural
S-16	3.186 3.190 5.363 6.906 8.963	alpha.-terpineol cyclohexanol, 5-methyl-2-(1-methylethyl) 1,3-propanediol, 2-(hydroxymethyl)-2-nitro 1-hexadecanol ambroxol	7.349 6.480 5.125	n-hexadecanoic acid tetradecanoic acid hexanoic acid, anhydride	5.924	2-butanone, 4-(4-hydroxy-3-methoxyphenyl)	4.800 5.303 6.129	methylparaben diethyl phthalate n-caprylic acid isobutyl ester	5.899	guaifenesin	3.334 7.489 7.526	5- hydroxymethylfurfural 1- nonadecene parathion

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S-17	3.190 9.232 9.379	cyclohexanol, 5-methyl-2-(1-methylethyl) ambroxol 1-hexacosanol	7.294	n-hexadecanoic acid	-	-	4.831 5.154 5.575 5.762	methylparaben 2-propenoic acid, 3-phenyl-, 1-methylethyl ester diethyl phthalate popylparaben	6.143	guaifenesin	8.340 8.575 9.120 3.071	dextromethorphan naphthalene, 1,4-dibromo phenytoin allyl isovalerate
S-18	3.190 8.257	cyclohexanol, 5-methyl-2-(1-methylethyl) 1- hexacosanol	7.312	n-hexadecanoic acid	3.020	udecanal	5.576	diethyl phthalate	-	-	7.607 8.341 8.399 8.492 9.039	cholrphenamine dextromethorphan norlevorphanol hydromorphone, pentafluoropropionate dihydromorphine
S-19	3.187 3.994	cyclohexanol, 5-methyl-2-(1-methylethyl) 2-propen-1-ol, 3-phenyl	4.715		4.545 4.687	benzaldehyde, 3-hydroxy-4-methoxy alpha.-ionone	5.576		-	-	3.432 5.103 8.632 7.450	5- hydroxymethylfurfural oxirane, 2-methyl-2-phenyl benzhydryl isothiocyanate 1- nonadecene
	4.360 5.500 5.892 7.116 8.259 9.005 9.381	benzenemethanol, 4-methoxy-, acetate benzenemethanol, .alpha.-methyl-.alpha.-(1-methyl-2-propenyl) benzenemethanol, .alpha.-phenyl benzenemethanol, .alpha.-methyl-.alpha.-(1-methyl-2-propenyl) n-tetracosanol-1 octacosanol 1-hexacosanol		oxiranecarboxylic acid, 3-methyl-3-phenyl	5.015 5.210	 3-buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen 1-penten-3-one, 1-(2,6,6-trimethyl-2-cyclohexen-1-yl)		diethyl phthalate				
S-20	3.956 5.636 3.190 8.963 6.912	2-propen-1-ol, 3-phenyl benzenemethanol, .alpha.-methyl-.alpha.-(1-methyl-2-propenyl) cyclohexanol, 5-methyl-2-(1-methylethyl) ambroxol 1-hexadecanol	4.005 4.715 6.398 7.306	heptanoic acid, anhydride oxiranecarboxylic acid, 3-methyl-3-phenyl tetradecanoic acid n-hexadecanoic acid	5.395 4.535 4.655	4-(4-hydroxyphenyl)-2-butanone benzaldehyde, 3-hydroxy-4-methoxy alpha.-ionone	4.828 5.774 4.963 5.545	methylparaben propylparaben butyl ester diethyl phthalate	5.845	guaifenesin	3.412 3.017	5- hydroxymethylfurfural octodrine

valerianate, ethyl stearate. In comparison, our study separated and identified around 57 volatile acidic components like octadecanoic acid, oleic acid etc.

Faraga and Backy[13] also found some trace number of aldehydes and ketones like p-anisaldehyde, 2′-hydroxy-5′-methoxy-acetophenone, β-damascenone, fenchone, isodihydrolavandulyl aldehyde, hexahydrofarnesyl acetone, etc. In comparison, our study found and separated around 37 compounds containing aldehydes and ketones functional groups.

Faraga and Backy[13,14] showed some esters too like methylparaben, propylparaben, benzoic acid, 4-propyloxy, propyl ester. In comparison, our study segregated greater number of esters containing components like methylparaben, propylparaben, diethyl phthalate, etc. which were abundantly found in each of the 20 samples.

Ethanol concentrations in commercial medicines syrups varied from 0.06 to 8.83% by Batista and Filho[15] Whereas in our study we have separated and identified around total of 86 alcoholic compounds from the mixture of cough syrup sample. Though we haven't carried out the quantification of the samples as it was only a qualitative analysis.

Pomponio and Hudaib[16] conducted a study for the examination of Guaifenesin-based syrups by employing micellar electrokinetic and GCMS. The main aim of the study was to develop and validate capillary electrophoric methods for the simultaneous analysis of guaifenesin and other basic active ingredient present in cough syrup preparations. The obtained data supported the suitability of GC-MS as effective, complementary techniques for the selective and sensitive quality control of cough syrups.

Dextromethorphan is a semi-synthetic opioid used as an antitussive reagents and active ingredient in many dextromethorphan-based cough syrups.[17,18] Along with it some opiates consisting analgesic properties were also identified in some samples of cough syrup examined like Norlevorphanol which is a morphinans-family opioid analgesic that was never commercialized. It is the 3-hydroxymorphinan levo-isomer. In the United States, Norlevorphanol is indeed a Schedule - I Narcotic restricted drug including an ACSCN of 9634 as well as an annual aggregate production limit of 52 grams in 2014.[19]

Another semi-synthetic opioid i.e., dihydromorphine was identified in the samples analysed. Dihydromorphine is a semi-synthetic opioid and shares structural similarities with and is generated from morphine.[20] To obtain dihydromorphine, the 7, 8-double bond in morphine is converted to a single bond. Dihydromorphine also has antitussive or cough suppressant properties and is accountable for analgesic effects in the body.[21] No other studies except ours, have shown the separation and discovery of various synthetic morphinans-family opioids and other components like THC, cannabinol, etc. from cough syrup samples which are used daily by a large number of population and which has been stated as OTC drug.

CONCLUSIONS

GC-MS is effective, complementary techniques for the selective and sensitive quality control of cough syrups

Conflict of Interest/Acknowledgements: None

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Original Research Paper

Prospective study of Correlation of waist circumference with the stature, weight and body mass index of the individuals

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ABSTRACT

Introduction: Accurate investigation of stature helps establish an individual's identity in medicolegal investigations using mutilated body remains. Instead, the body mass index (BMI) is a straightforward measure of the weight-for–height ratio that is applicable throughout human lifespan. In addition, waist circumference can be utilized for determination of identity of persons from the parts of dead bodies.

Materials and methods: The present study was conducted in the Department of Agadtantra, Ayurvidya Prasarak Mandal's Ayurved Mahavidyalaya & Seth R.V. Ayurved Hospital, Mumbai, Maharashtra, India. The duration of the study was for two years from June 2015 to June 2017. Total number of the subjects were 255 students comprising of 54 males and 201 females of age between 17 to 24 years. Measurements of stature, weight, waist circumference and BMI of all the subjects was done.

Results: The present study includes a total of 255 participants comprising of 54 (21.18%) males and 201 (78.82%) females. Single linear regressions were utilized to assess the association of waist circumference with stature, weight and body mass index for males and females separately.

Conclusion: The significance of the association was highest to determine weight followed by BMI and Stature from waist circumference. Correlation coefficient was least for ascertaining stature from waist circumference especially in females which was having very weak correlation.

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INTRODUCTION

Body mass index (BMI) is an important biometric measure commonly used across numerous disciplines to assess risk of many health conditions.[1-8] Ideally, adiposity should be defined based on body fatness, but such measurements are usually unfeasible both in clinical practice and in epidemiological studies.[9] BMI can be used as indicator of overweight and obesity.[10-11] Accurate investigation of stature helps establish an individual's identity in medicolegal investigations using mutilated body remains.[12] Instead, the body mass index (BMI) is a straightforward measure of the weight-for–height ratio that is applicable throughout human lifespan.[13] In addition, waist circumference can be utilized for determination of identity of persons from the parts of dead bodies. The objective of the study is to determine the correlation of various parameters like waist circumference with BMI, stature and weight. This study is been carried out as not many studies were being conducted for determining the correlation between waist circumference with stature, weight and BMI for ascertaining the identity of the person.

MATERIALS AND METHODS

The present study was conducted in the Department of Agadtantra, Ayurvidya Prasarak Mandal's Ayurved Mahavidyalaya & Seth R.V. Ayurved Hospital, Mumbai, Maharashtra, India. The duration of the study was for two years from June 2015 to June 2017. Students of this

institution devoid of genetic and physical deformities were involved for this study. Only those subjects who were resident of Maharashtra were involved in the study. Subjects having deformities including limb or spine, any injury including fracture, or persons underwent an operative surgery on spine or limbs were excluded from the study. Total number of the subjects were 255 students comprising of 54 males and 201 females of age between 17 to 24 years. Subjects were selected by using simple random sampling method. A proper approval was taken from the Institutional Ethics Committee and subjects were included only after taking proper consent.

Measurements

Measurements of all the subjects were taken at same time in the morning to prevent diurnal variation wile measuring the stature. All the measurements were collected in tabulated form.

Stature and weight of all the subjects were taken. The unit for stature was centimeters and for weight in kilograms. All the Measurements were done by the single author to circumvent interobserver bias.

Stature measurement was done using a stadiometer having movable headboard. Measurement was taken from vertex to floor with person standing straight in anatomical position and with the head in Frankfurt horizontal plane (i.e., the line joining the inferior margin of the orbit and upper margin of external auditory meatus). Subjects were standing on even surface in erect posture with feet closely approximated and both the hands on each side.

Weight: The subjects were asked to stand in erect posture in anatomical position with feet closely approximated on digital weighing machine.

Waist circumference: The subjects were made to stand in erect posture in anatomical position with feet closely approximated and waist circumference were measured encircling the waist above level of iliac crest using measuring tape.

Body mass index was calculated for each subject using weight and height in the formula.

Statistical Analysis: Analysis of the data obtained was done statistically using Statistical Package for Social Sciences version 16.0 computer software (SPSS, Inc., Chicago, IL). The mean, standard deviation (SD) and 95% confidence interval of all measurement variables were calculated. Pearson's correlation coefficient was considered to determine the degree of correlation of waist circumference with body mass index, weight and stature along with standard error and coefficient of determination (R2). Single linear regressions were utilized to assess the association of waist circumference with stature, weight and body mass index.

RESULTS

The present study includes a total of 255 participants comprising of 54 (21.18%) males and 201 (78.82%) females. Range of stature of the subjects involved in the study was from 145.0 to 179.0 cm with mean 161.172 (SD 6.775). Weight of the all the subjects was ranging from 36 kgs to 85 kgs with the mean 53.24 (7.72). Similarly, Body mass index of the all the subjects was ranging from 14.19 to 35.53 with the mean 20.48 (2.61). Waist circumference of the all the subjects was ranging from 60 cm to 121 cm with the mean 77.69 (12.92).

Stature of the participants ranged from 152.0 to 179.0 cm in males and 145.0 to 175.0 cm in females. Mean stature in males (167.8 cm) was larger than that in females (159.4 cm). Similarly weights of the participants ranged from 41 to 85 kgs in males and 36 to 85 kgs in females. Mean weights in males (57.4 kgs) was larger than that in females (53.24 kgs). Waist circumference of the participants ranged from 66 to 102 cm in males and 60 to 120 cm in females. Mean waist circumference in males (84.08 cm) was larger than that in females (77.69 cm). Body Mass Index (BMI) of the participants ranged from 14.19 to 27.44 in males and 14.19 to 35.53 in females. Mean BMI in males (20.4) was almost equal to that in females (20.48).

Scatter diagram and regression line were plotted showing relation between Waist circumference with stature, weight and Body mass index in males and females. Linear regression equations were obtained from the scatter diagrams by obtaining stature, weight and body mass index from waist circumference. Waist circumference was used as dependant variable to obtain stature, weight and body mass index of the individual.

DISCUSSION

The present study was carried out to determine the relationship between parameters like Waist circumference, stature, weight and BMI. This can be particularly useful when only mutilated remains of hip regions are recovered by the police. Waist circumference thus known can be helpful for determining identity parameters like weight, height and BMI. Height and weight of the individual are very important parameters in

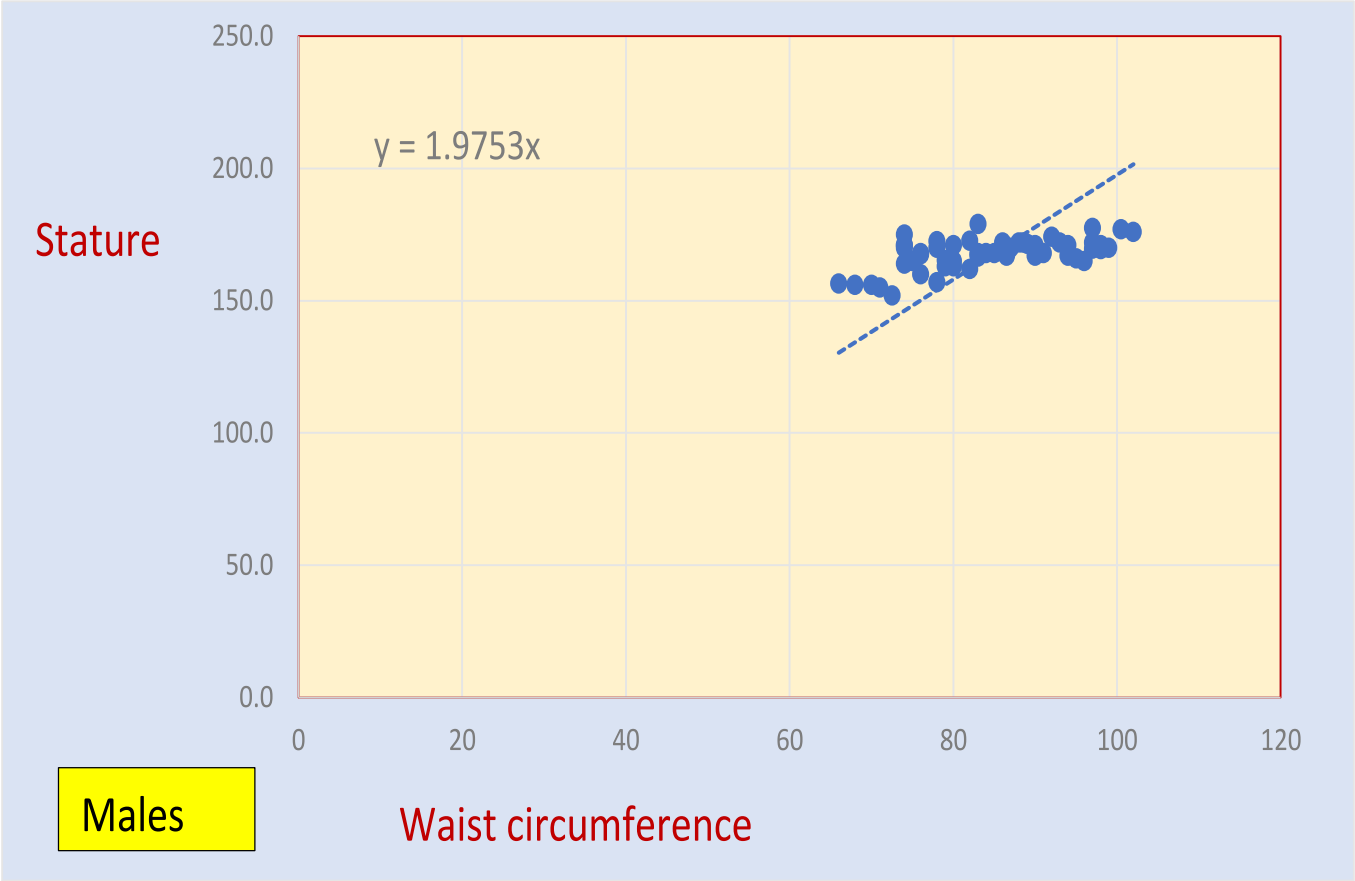
Table 1: Regression Statistics for the Waist Circumference With Height (stature) in Males was as Follows.

Regression Statistics	
Multiple R	0.59
R Square	0.34
Adjusted R Square	0.33
Standard Error	4.81
Observations	54

ANOVA					
	df	**SS**	**MS**	**F**	**Significance F**
Regression	1	629.8709	629.8709	27.17341074	3.25712E-06
Residual	52	1205.343	23.17968		
Total	52	1835.214			

	Coefficients	**Standard Error**	**t stat**	**p-value**	**Lower 95%**	**Upper 95%**	**Lower 95.0%**	**Upper 95.0%**
Intercept	136.9425	5.946871	23.02766	6.08379E-29	125.009223	148.8757612	125.009223	148.8757612
X Variable 1	0.366437	0.070295	5.212812	3.25712E-06	0.225378611	0.507494609	0.225378611	0.507494609

Graph 1: Scatter diagram and regression line showing relationship between stature and Waist circumference in males (all dimensions in centimetres)



As the R value is 0.59, it indicates strong positive correlation between waist circumference and stature in males. Similarly, the p value is less than 0.05, it can be interpreted that the correlation is statistically significant (Refer to Table 1). The equation derived from the scatter diagram (graph 1) is y = 1.9753x.

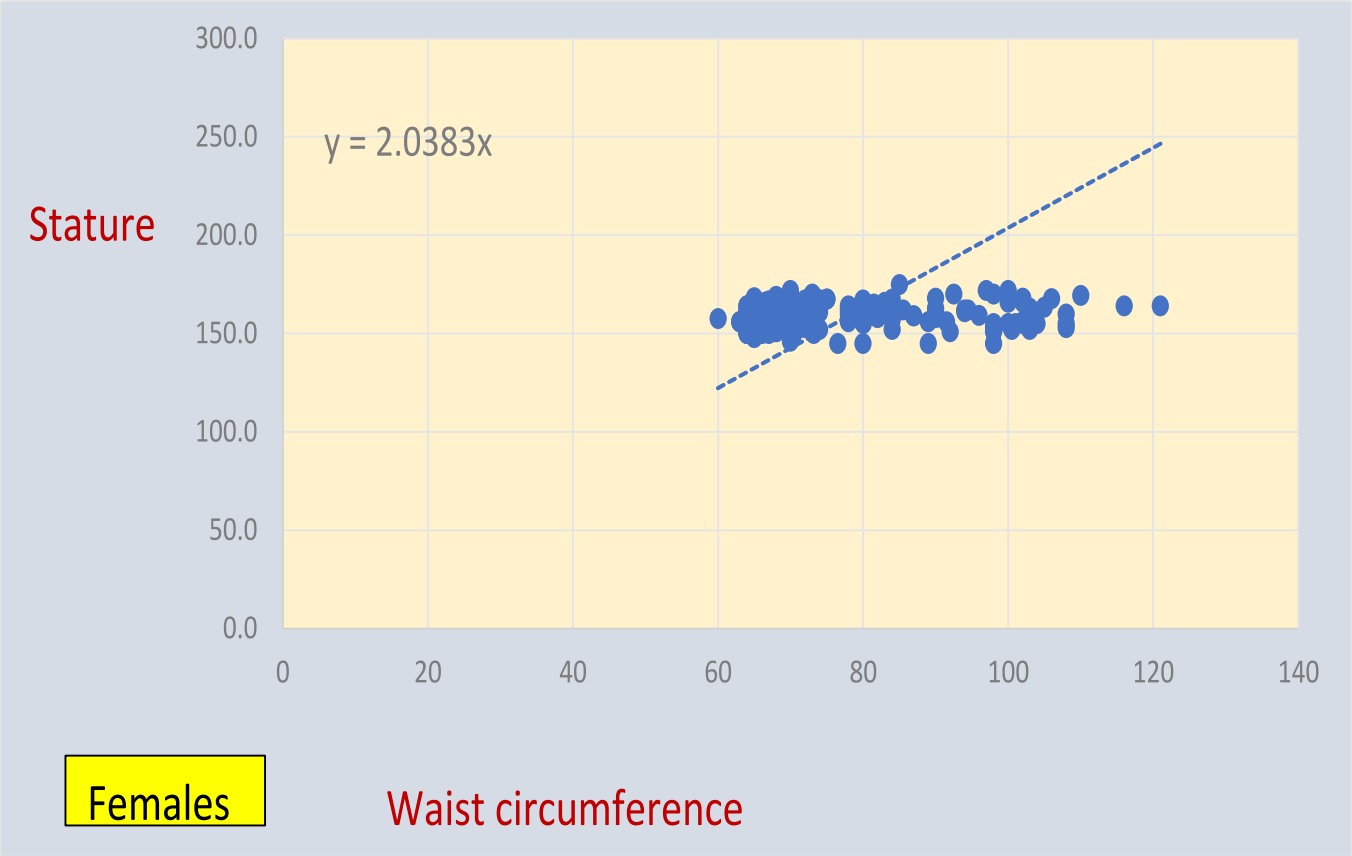
Table 2: Regression Statistics for the Waist Circumference with Height (Stature) in Females Was as Follows:

Regression Statistics	
Multiple R	0.11
R Square	0.01
Adjusted R Square	0.01
Standard Error	5.84
Observations	201

ANOVA					
	df	SS	MS	F	Significance F
Regression	1	79.44771	79.44771	2.332526	0.128283991
Residual	199	6778.1	34.0608		
Total	200	6857.548			

	Coefficients	Standard Error	t stat	p-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	155.7807503	2.4075	64.70644	1.3E-135	151.033265	160.5282	151.0333	160.5282
X Variable 1	0.047687353	0.031224	1.527261	0.128284	-0.013885222	0.10926	-0.01389	0.10926

Graph 2: Scatter diagram and regression line showing relationship between stature and Waist circumference in females (all dimensions in centimetres)



As the R value is 0.11, it indicates weak positive correlation between waist circumference and stature in males. Similarly, the p value is less than 0.05, it can be interpreted that the correlation is statistically significant (Refer to Table 2). The equation derived from the scatter diagram (graph 2) is y = 1.9753x.

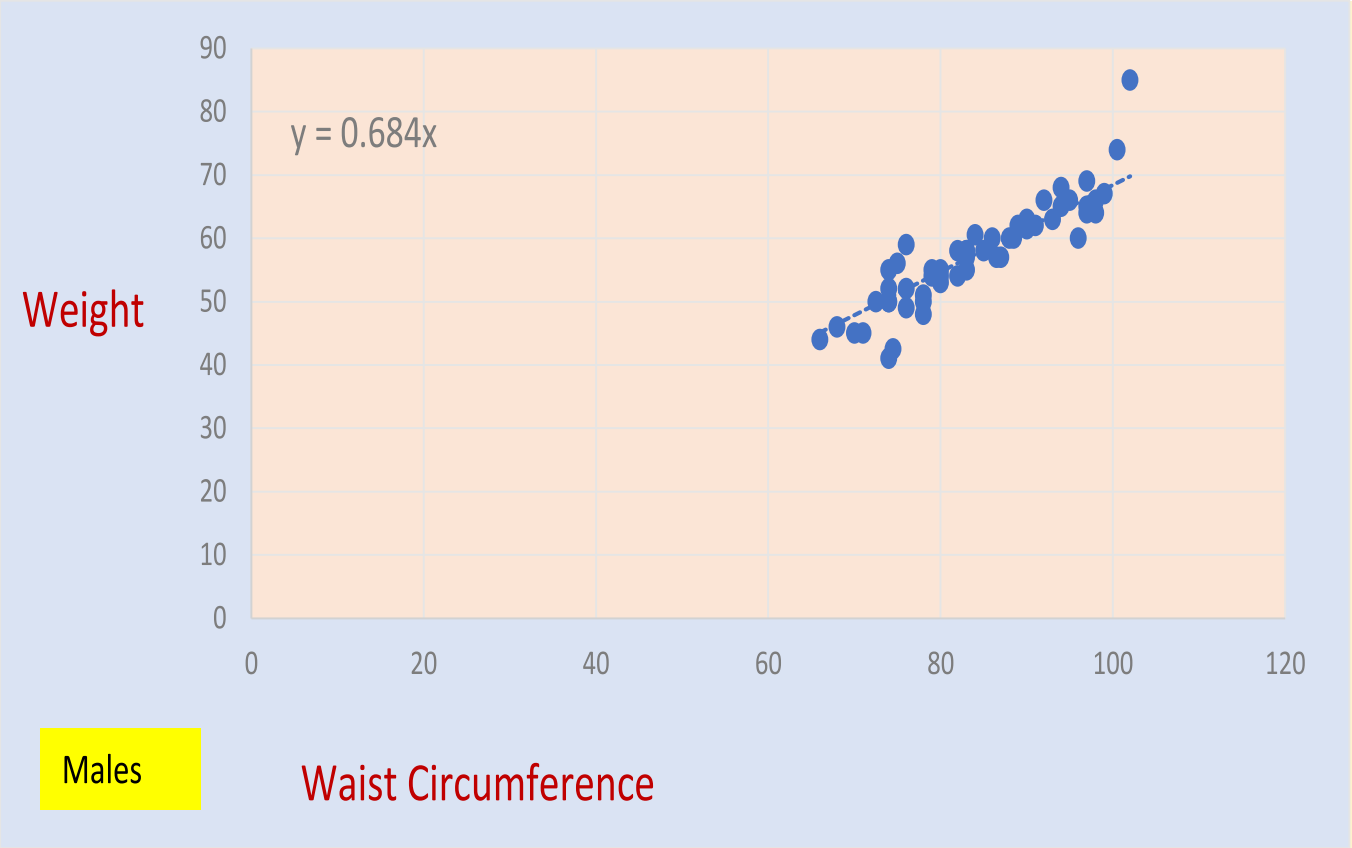
Table 3: Regression statistics for the Waist circumference with weight in males was as follows

Regression Statistics	
Multiple R	0.90
R Square	0.82
Adjusted R Square	0.81
Standard Error	3.57
Observations	54

ANOVA					
	df	SS	MS	F	Significance F
Regression	1	2966.679	2966.679	233.2044	7.3419E-21
Residual	52	661.511	12.72137		
Total	53	3628.19			

	Coefficients	Standard Error	t stat	p-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-9.46984	4.405565	-2.14952	0.036267	-18.31025331	-0.62943	-18.3103	-0.62943
X Variable 1	0.795259	0.052076	15.27103	7.34E-21	0.690759827	0.899757	0.69076	0.899757

Graph 3: Scatter diagram and regression line showing relationship between Weight (kgs) and Waist circumference (cm) in males.



As the R value is 0.9, it indicates very strong positive correlation between waist circumference and weight in males. Similarly, the p value is less than 0.05, it can be interpreted that the correlation is statistically significant (Refer to table no 3). The equation derived from the scatter diagram (graph 3) is y = 0.684x.

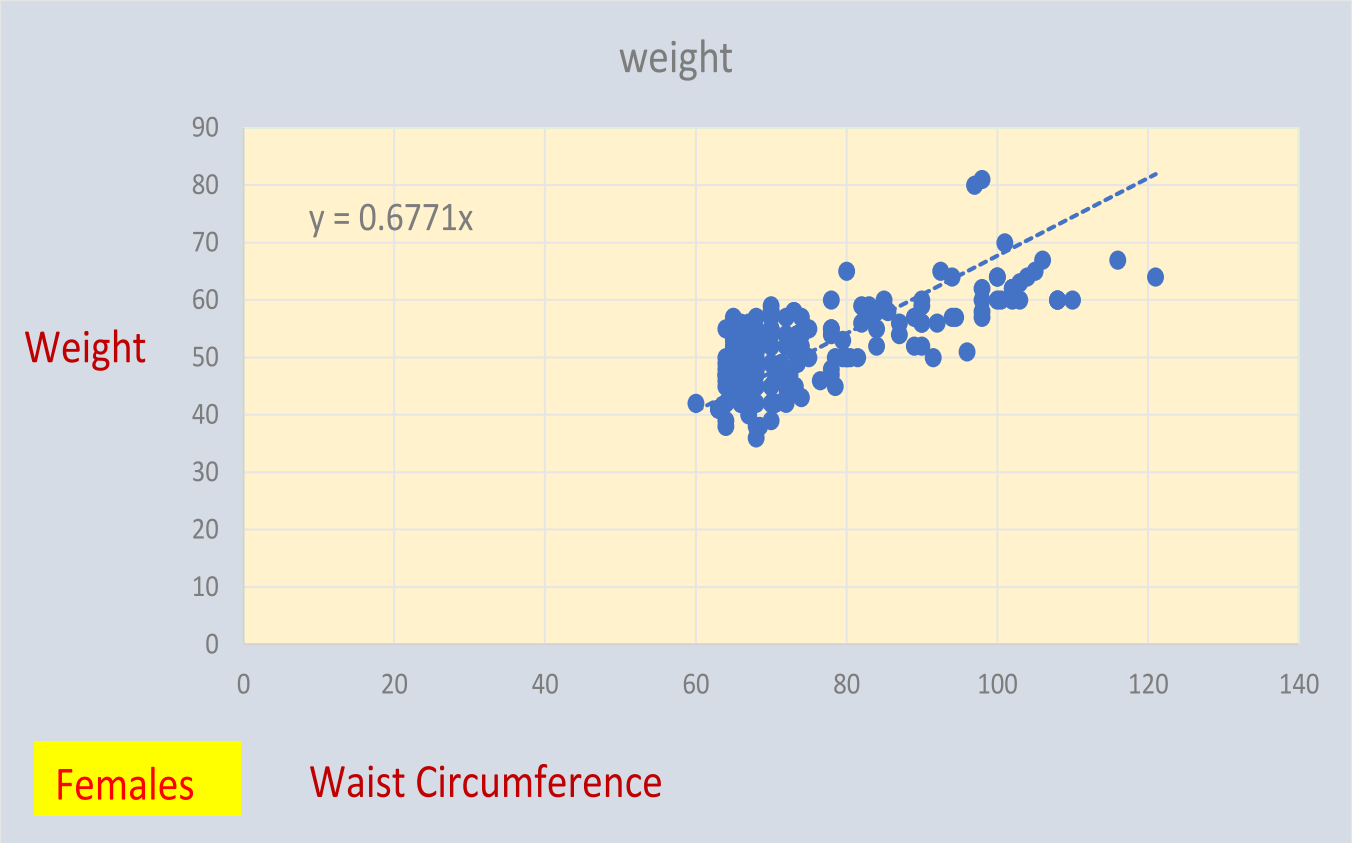
Table 4: Regression statistics for the Waist circumference with weight in females was as follows

Regression Statistics	
Multiple R	0.70
R Square	0.49
Adjusted R Square	0.49
Standard Error	5.14
Observations	201

ANOVA					
	df	SS	MS	F	Significance F
Regression	1	5069.516	5069.516	191.7575	5.56E-31
Residual	199	5260.987	26.43712		
Total	200	10330.5			

	Coefficients	Standard Error	t stat	p-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	23.17823	2.121026	10.92784	4.46E-22	18.99566	27.3608	18.99566	27.3608
X Variable 1	0.380931	0.027509	13.84765	5.56E-31	0.326685	0.435176	0.326685	0.435176

Graph 4: Scatter diagram and regression line showing relationship between Weight (kgs) and Waist circumference (cm) in females.



As the R value is 0.7, it indicates strong positive correlation between waist circumference and weight in females. Similarly, the p value is less than 0.05, it can be interpreted that the correlation is statistically significant (Refer to table no 4). The equation derived from the scatter diagram (graph 4) is y = 0.6771x.

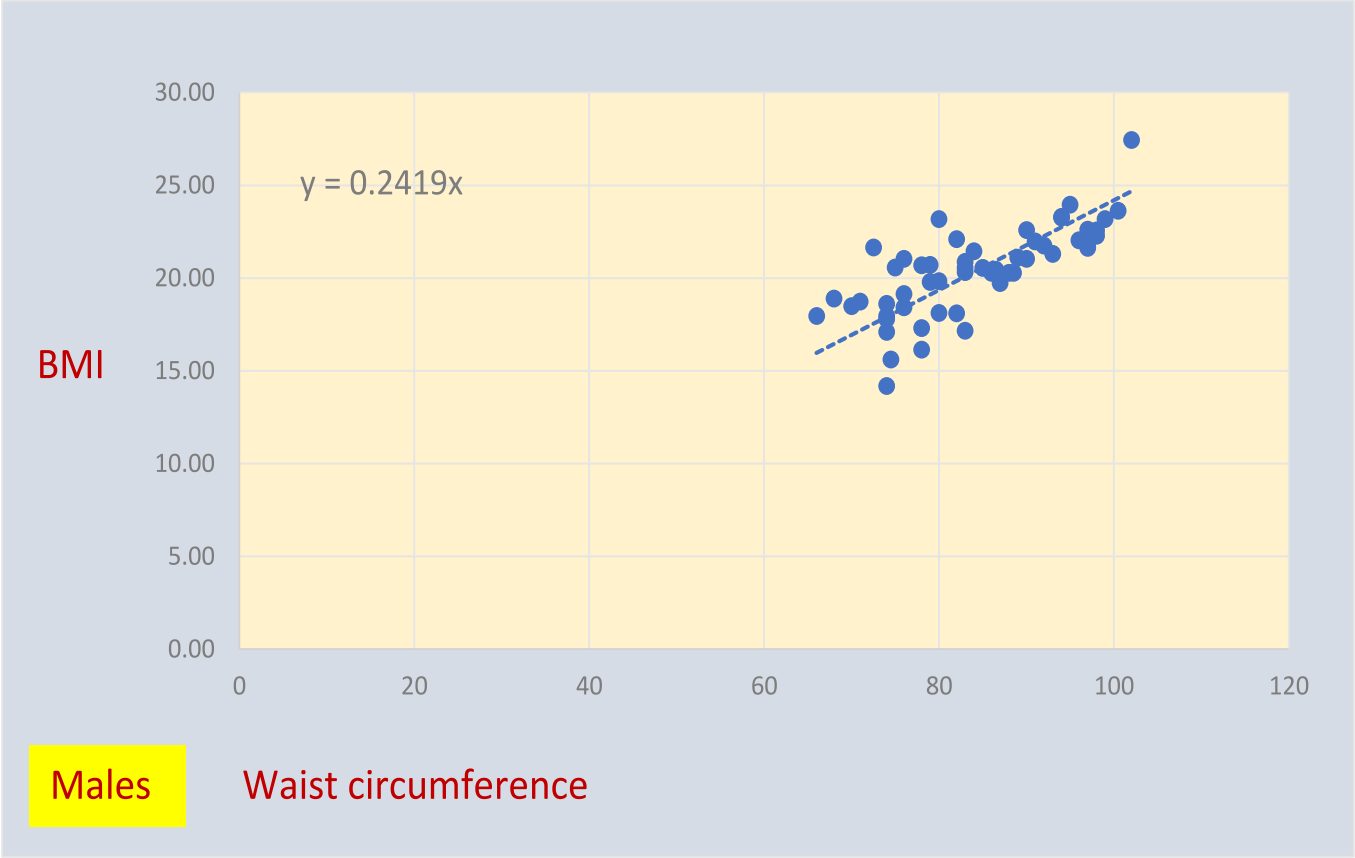
Table 5: Regression statistics for the Waist circumference with BMI in males was as follows

Regression Statistics	
Multiple R	0.75
R Square	0.56
Adjusted R Square	0.55
Standard Error	1.60
Observations	54

ANOVA					
	df	SS	MS	F	Significance F
Regression	1	166.7764527	166.7764527	65.219	9.66277E-11
Residual	52	132.9731546	2.557176051		
Total	53	299.7496074			

	Coefficients	Standard Error	t stat	p-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	4.543854302	1.975218298	2.300431455	0.02547	0.580288816	8.507419789	0.580288816	8.507419789
X Variable 1	0.188556035	0.023348199	8.075827837	9.7E-11	0.141704446	0.235407623	0.141704446	0.235407623

Graph 5: Scatter diagram and regression line showing relationship between BMI and Waist circumference (cm) in males.



As the R value is 0.75, it indicates very strong positive correlation between waist circumference and BMI in males. Similarly, the p value is less than 0.05, it can be interpreted that the correlation is statistically significant (Refer to table no 05). The equation derived from the scatter diagram (graph 5) is y = 0.2419x.

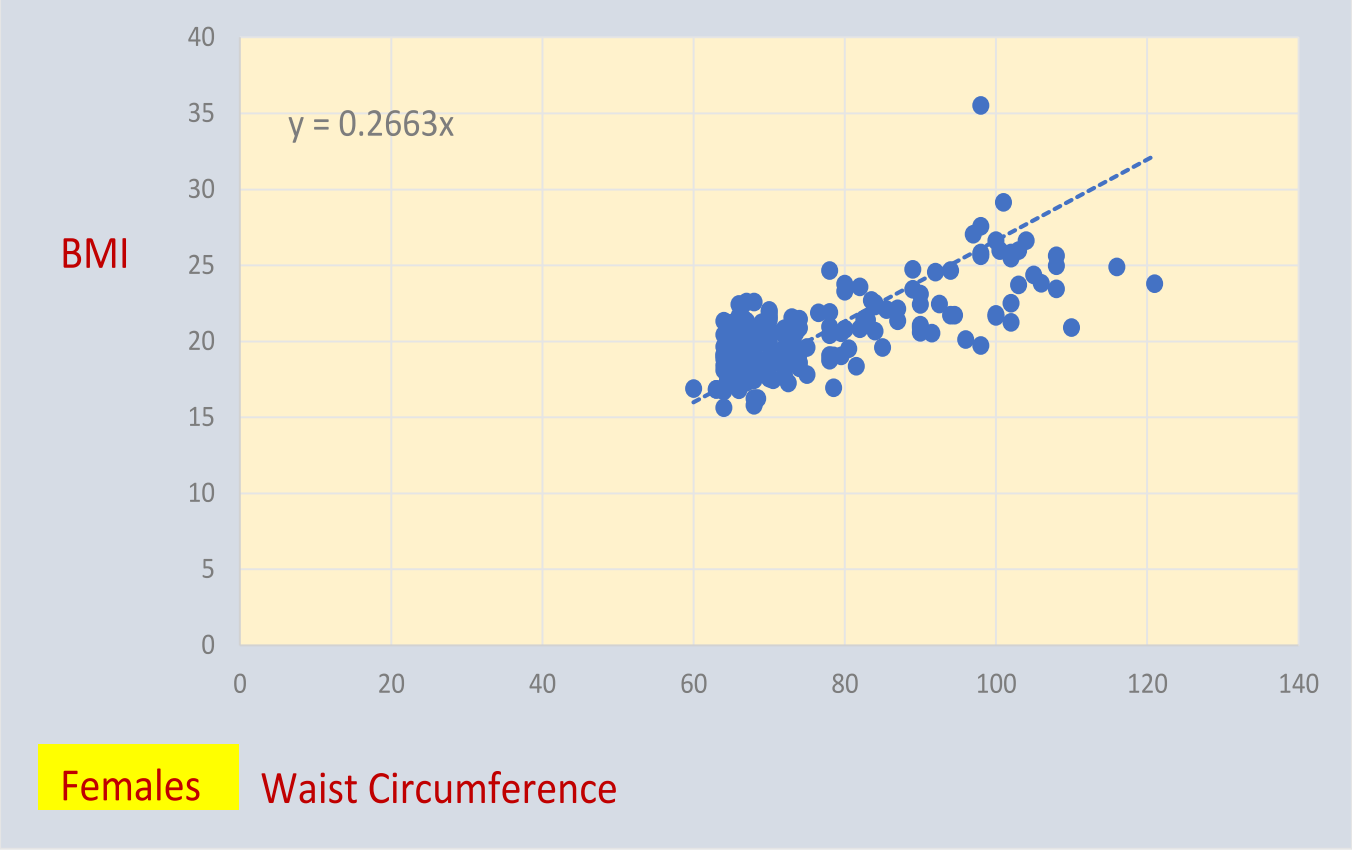
Table 6: Regression statistics for the Waist circumference with BMI in females was as follows

Regression Statistics	
Multiple R	0.70
R Square	0.49
Adjusted R Square	0.48
Standard Error	1.92
Observations	201

ANOVA					
	df	SS	MS	F	Significance F
Regression	1	691.4982868	691.4983	187.9707245	1.47371E-30
Residual	199	732.0722917	3.678755		
Total	200	1423.570578			

	Coefficients	Standard Error	t stat	p-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	9.819204	0.791205493	12.41043	1.44317E-26	8.258981296	11.37943	8.258981	11.37943
X Variable 1	0.140688	0.01026155	13.71024	1.47371E-30	0.120453002	0.160924	0.120453	0.160924

Graph 6: Scatter diagram and regression line showing relationship between BMI and Waist circumference (cm) in females.



As the R value is 0.7, it indicates strong positive correlation between waist circumference and BMI in females. Similarly, the p value is less than 0.05, it can be interpreted that the correlation is statistically significant (Refer to table no 06). The equation derived from the scatter diagram (graph 6) is y = 0.2663x.

knowing the identity of the individual. In addition, parameter like BMI can be helpful in predict the obesity of the individual which can be help in far better prediction of individuality of the person. Once the identity of the person is determined, it provides the link for recognition of the culprit. The present study was conducted in 255 participants comprising 54 males and 201 females of age ranging from 17 years to 24 years. This age group is included as the maximum height is achieved in the human by this age.

The stature obtained from the findings of present study was considered to be on higher side in males compared to females which is consistent with that of the findings in the Indonesian study by Fatmah M.14 and Singh JP et al 15. There can be multiple factors including the changes in puberty where testosterone causes massive increase in height as compared to estrogen. This may also be contributed due to other factors like genetic, lifestyle factors, etc.

Significant correlation (R=0.78) between Waist circumference and BMI was found in study by Marcin Gierach M 16. It also found more distinct correlation among females (r=0.8) compared to males (r=0.76) [15]. These findings are consistent with the present study with correlation coefficient of 0.725. But correlation is slightly more pronounced in males (0.75) than in females (0.7). The slight variation between the findings can be due to environmental, genetic or sample size differences.

Study by Chinedu SN et al 17 shows that in females, correlation of BMI with Waist circumference ®= 0.75) is more pronounced than in males (r=0.55). In general, correlation of BMI with weight (r=0.76) is more than with waist circumference (r=0.63). In present study, Correlation coefficient of Waist circumference with BMI is in consistency for females but for males the It is on higher side which is opposite to those findings in study by Chinedu SN et al.

Ashtary-Larky et al 18 found that there is moderate correlation between waist circumference with BMI (0.43) and weight (0.46). These findings do not match with that of present study which were having strong correlation of waist Circumference with BMI while very strong correlation for Weight.

Most of the studies conducted were with objectives of correlating BMI with waist circumference, weight and height for determining the relation with medical conditions like coronary artery disease, dyslipedimia, obesity, metabolic syndromes etc.16,19 Till date use of correlation of these parameters for forensic purposes are very limited. BMI and Waist circumference were predominantly utilised for assessing intensity of the abdominal obesity or overall obesity.20

CONCLUSION

Determination of Identity of the person forms the mainstay for finding the link to trap the culprit. Recognition of the individual can be done even from the mutilated remains comprising of hip regions. Findings of the study can be concluded that waist circumference can be utilized for ascertaining the weight, height and BMI. The significance of the association was highest to determine weight followed by BMI and Stature from waist circumference. Correlation coefficient was least for ascertaining stature from waist circumference especially in females which was very weak correlation. Therefore, waist circumference can be utilized for reveling the identity from cases having only mutilated remains.

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Original Research Paper

Correlation of Deaths Due to Suicide with Menstrual Phase

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ABSTRACT

Introduction: The menstrual cycle is under complex hormonal control and endometrial morphology closely reflects the endocrine status and the interplay between the ovarian hormones. The menstrual cycle continues to be the focus of major medical problems.

Material and Method: Present study was conducted in the Mortuary of the Forensic Medicine & Toxicology Department of Government Medical College in the Rajindra Hospital Patiala over a period of one year from 1st July 2021 to 30st June 2022 on female dead bodies brought to the mortuary.

Observation and Results : A total of 116 cases of female came for postmortem during period of study. Out of these maximum 56(48.2%) were of suicide. Out of suicide case maximum 30(53.6%) were of poisoning. Maximum 49.9% cases of suicide were with late secretory phase. Findings of our study matches with some studies done earlier and contradicts with some also.

Conclusion: These findings will help in support routine suicidal risk assessments for women who suffer from moderate-to-severe menstrual disturbance. Furthermore, psychosocial treatments for women at risk should be developed to improve well-being.

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INTRODUCTION

Menstruation is defined as a periodic and cyclical shedding of progestational endometrium which is accompanied by loss of blood during the reproductive age that is time interval between menarche (onset of menstruation) and menopause (cessation of menstruation).[1] The normal menstrual cycle takes place at an approximate interval of 28 days but one cycle may extend up to 35 days in different women. The menstrual flow generally lasts for 4±2 days, and the average blood loss is 40±20 ml. The menstrual cycle is under complex hormonal control and endometrial morphology closely reflects the endocrine status and the interplay between the ovarian hormones.[2]

Menopause, on the other hand, is experienced by most women at around 51 years of age. It is simply defined as the cessation of menstruation. At this time, the ovaries stop releasing eggs and the levels of oestrogen and progesterone also fall. This phase of woman's life is also known to present with gross changes in the uterine parameters. The changes occurring in the uterus during pregnancy and parturition are also significantly affected by age of the woman and her hormonal balance.[3]

The menstrual cycle continues to be the focus of major medical problems. Millions of menstruating girls around the world are coping with uncontrollable mood swings, struggling with pain and survival. Behavioral changes, such as hangovers, can lead to unusual behaviors, involvement in minor or serious crimes, and life endangering behaviors such as suicide attempts.[4] The psychological and physical signs and symptoms that appear in girls four days before menstruation, are called premenstrual syndrome, disappear some time after

menstruation. When the signs and symptoms approaching length are mild, it is called "morimina". Premenstrual syndrome (PMS) isn't necessarily small or a weird idea, Studies show that PMS is common menstrual disorder among.(5)Aim of current study is primarily based on whether there is an association between suicide and end and menstrual cycle levels in all suicidal women undergoing post-mortem examination at Government Medical College, Patiala.

MATERIAL AND METHODS

Present study was conducted in the Mortuary of the Forensic Medicine & Toxicology Department of Government Medical College in the Rajindra Hospital Patiala over a period of one year from 1st July 2021 to 30st June 2022. All the female dead bodies brought to the mortuary irrespective of cause of death and where consent was available were included in the study.

During post-mortem, the uterus along with fallopian tubes and ovaries were examined after removal of rectum and vagina. Using scissors, the vagina was opened with a midline posterior cut, which extended to cervix. Findings of uterus in reference to menstrual phase are then correlated with cause of death.

RESULTS

A total of 116 cases of female came for postmortem during period of study. Out of these maximum 56 (48.2%) were of suicide. **(Table1)** Out of suicide case maximum 30(53.6%) were of poisoning . Maximum 17 cases(30.3%) of suicide were with late secretory phase. Out of 116 cases, 16 cases (13.8%) belonged to early proliferative phase, 17 cases (14.7%) belonged to late proliferative phase, 20 cases (17.2%) belonged to early secretory phase, 24 cases (20.7%) belonged to late secretory phase, 16 cases (13.8%) had menses The maximum incidence of suicide was seen in 15-25 age group with 23 cases (41%), followed by 26-35 age group with 15 cases (26.8%).

Table 1: Distribution of Manner of Death

Manner of Death	Frequency	Percentage
Natural	20	17.2%
Accidental	33	28.4%
Suicide	56	48.2%
Homicide	7	6.0%

Table 2: Distribution of Method of Suicidal Deaths

Method	Frequency	Percentage
Hanging	9	16.1%
Poisoning	30	53.6%
Burns	2	3.5%
Drowning	15	26.8%

Table 3: Age wise Distribution of Cases

Age Group	Frequency	Percentage
15-25	23	41%
26-35	15	26.8%
36-45	8	14.3%
46-55	6	10.7%
56-65	4	7%

DISCUSSION

Our results matched with study conducted in neighbouring Delhi in 2019 which showed that in suicidal cases of female, 49% cases were in secretory phase.[6] Our results differed from study conducted in 2015 in Kerala, which showed 40% of cases were in the proliferative phase and 20% were in the menstrual phase and 20% each were in early and late secretory phases.[7]

Our results differed from study done in 2015 in Turkey, 64 cases (62.7%) were during mensturation.[8] Our results also differed from study conducted in neighbouring Delhi in 2007 in which majority of females who did suicide were having menses 54.4%.[9] In 1998 Spanish researchers conducted a study on 113 Spanish Women who attempted suicide. They found that the incidence of suicide attempts in the menstrual phase (36%) was significantly higher than that in the remaining phases.[12]

Researchers conducted a study in Gdansk, Poland in 2005, of 151 women of childbearing potential admitted to the Clinic of Internal Medicine and Acute Poisoning at Gdansk Medical University for suicide attempts. They found that woman who tried to do suicide, were more common among women who were undergoing menstruation.[13]

A study to find correlation between suicidal behavior and the menstrual cycle was conducted in Oxford, United Kingdom in 2006 which suggested that a positive relationship exists between aspects of the menstrual cycle and non-fatal suicidal behaviour. They found that such behaviour appears to be more common in those phases of the menstrual cycle when oestrogen levels are lowest that is during the late luteal and follicular phases), and in those suffering from PMS.[14] In 1959, a postmortem study was conducted by researchers entitled “Lethal hazards of the luteal phase of the menstrual cycle” in London. They found that out of 102 postmortems conducted 89 deaths occurred during the luteal phase of the cycle. The death was significantly more frequent after than before

Table 4: Correlation of Manner of Deaths with Menstrual Phase

Manner of Death	Early Pro-liferative	Late Pro-liferative	Early Secretory	Late Secretory	Menses	NA	Total	p value (x^2)
Natural	5	3	2	2	1	7	20	0.068 (3.33) S
Accidental	5	5	5	3	4	11	33	0.011 (6.55) S
Suicide	6	8	11	17	10	4	56	0.001 (16.10) HS
Homicide	0	1	2	2	1	1	7	0.053 (3.73) S
p value (X2)	0.037 (4.36)	0.027 (4.90)	0.012 (6.28)	0.015 (5.87)	0.005 (8.04)	0.104 (2.65)		
	S	S	S	S	HS	NS		

Table 5: Correlation of Endometrial Phase in Suicide Cases

Endometrial Phase	Frequency	Percentage
Early Proliferative	6	10.7%
Late Proliferative	8	14.2%
Early Secretory	11	19.6%
Late Secretory	**17**	**30.3%**
Menses	10	17.8%
Not Applicable	4	7.1%

ovulation, and significantly more frequent in midluteum than in the remainder of the luteum.[11] From the results obtained from the present study, we can see that there is a significant corelation between late secretory phase of the menstrual cycle and suicide. So we could arrive at a conclusion that premenstrual syndrome could be one of the precipitating factor in the present study

CONCLUSION

There should be extensive research to correlate suicidal tendencies with menstrual phases. The high risk patients with menstrual syndrome should be identified and observed routinely. Also, new studies which include hormonal analysis should be done in future for more accurate relationship of menstrual phase and hormone levels with suicide. These findings will help in support routine suicidal risk assessments for women who suffer from moderate-to-severe menstrual disturbance. Furthermore, psychosocial treatments for women at risk should be developed to improve well-being.

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Table 6: Comparison with other Studies in Reference to Distribution Based on Endometrial Phase in Suicide Cases

Study Location	Year	Endometrial Phase with Highest Incidence	Percentage
Patiala (Current Study)	2023	Secretory Phase	49.9%
Delhi (6)	2019	Secretory Phase	49.0%
Kerala (7)	2015	Proliferative Phase	40.0%
Turkey (8)	2015	Secretory Phase	26.4%
Delhi (9)	2007	Menses	54.4%
Gdańsk,Poland (10)	1998	Menstrual Phase	36.0%
London (11)	1959	Luteal Phase	87.3%

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Original Research Paper

Determination Of The Sex In The Population Of Salem District Using Various Cephalometric Measurements - A Cross Sectional Study

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ABSTRACT

Introduction: During medicolegal situation, mass disasters or any conditions requiring forensic identification, the skull bones can be exposed to radiation to obtain lateral cephalometric radiographs. . The landmarks present in the radiographs can be used to determine the sex of the individual after measurement.

Aim: The aim of our study is to develop a formula to identify sex of the population of Salem district.

Materials and methods: A cross-sectional study was conducted using 100 digital lateral cephalometric radiographs of 6 to 30 years old population of Salem district. All the lateral cephalometric radiographs will be traced and analyzed digitally by using a standardized Carestream CS8100 SC software.

Result: The mean values of males higher for variables such as AO (A to Occlusal Plane), BO (B to Occlusal Plane), Chin thickness (Pog' to N-B) than females. This study showed a overall accuracy of 92.6% for determination of sex using lateral cephalometric radiographs.

Conclusion: This study was able to develop formula for gender identification in Salem population.

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INTRODUCTION

Forensic science is the branch of science which deals with the investigation of human remnants, followed by generation of scientific evidence and producing it to the court of law.[1] Forensic odontology and forensic anthropology are the two main displines of forensic science which deals with the structures of craniofacial region. The teeth and bones present in the maxillofacial region are the most resistant structures among other structures of the body.[2-3] Especially the bony structures of the skull display a variety of landmarks which can be taken as reference points for investigation of human remnants.[4] The identification of human remnants is utmost important in in case of medicolegal situations, mass disasters, terrorist attacks and war situations.[4-6]

The identification of sex of a human remnant is the basic step in the hierarchy of recognizing an individual.[5,7] Even though pelvis bone seems to be more accurate for determination of sex of an individual yet skull bone can be used for identification of gender when the remnants of pelvis bone are not available.[4,8,9] The accuracy of determination of sex of an individual is 100% when complete skeleton is present, 98% when evaluated with skull and pelvis bone and 92% when only skull bone is available. The skull seems to the second bony region of the body for determination of sex of an individual after pelvis bone.[4,9,10] Thus, sex determination using skull bones can be used as alternative method in the forensic identification procedure.[4]

The two methods in determination of sex of individual are

morphological and morphometric. Morphological methods can lead to inter-investigator variations.[4,9,11] This variation can result inappropriate identification of sex of an individual. The morphometric method is generalized, can be applied to every member of a particular population and helpful in precise identification. The morphometric method uses various landmarks for measurement.

The radiographs such as orthopantomogram and cephalograms can be used to visualize the landmarks of the skull. The cephalometric radiograph reveals wide range of soft tissue and hard tissue landmarks for measurement.[6,11] The cephalometric radiographs are also available easily for the study purposes because, the lateral cephalograms are the routinely prescribed radiographs for the diagnosis of various skeletal deformities. So, the evaluation of various linear and angular measurements using lateral cephalometric radiograph for this study can be performed without the exposure of human participants into radiation.

During medicolegal situation, mass disasters or any conditions requiring forensic identification, the skull bones can be exposed to radiation to obtain lateral cephalometric radiographs. The landmarks present in the radiographs can be used to determine the sex of the individual after measurement. This method is the most convenient, accurate and easier approach of identifying sex of an individual.[11] Even though, there are many previous studies which derived formula for determination of sex, the measurements vary for different population. Hence, reference values for each population are essential. These reference values can be used to derive formula for every population which helps in accurate determination of sex of individual. So, the aim of the study is to develop a formula to identify sex of the population of Salem district.

MATERIALS AND METHOD

A total of 100 samples were included in the cross-sectional study. Among them --- males and females. The age group of the study population was between 6 to 30 years. The digital lateral cephalometric radiograph of the study population was collected from the Salem district which was taken for diagnostic purposes before orthodontic treatment, orthognathic surgeries, developmental deformities and other skeletal deformities.

The inclusion criteria of the study were lateral cephalometric radiograph with good clarity and absence of artifacts and patients belonging to the age group of 6-30 years. The exclusion criteria of the study were lateral cephalometric radiograph which showed missing teeth, syndromes, cleft palate, cleft lip, congenital anomalies, developmental disorders of teeth or jaw, patient had an history of orthodontic treatment and orthognathic surgery, cases with history of head and neck injury, patients undergone surgery in the head and neck region and radiographs showing craniofacial pathologies.

All the lateral cephalometric radiographs were traced and analyzed digitally using a standardized Carestream CS8100 SC software.

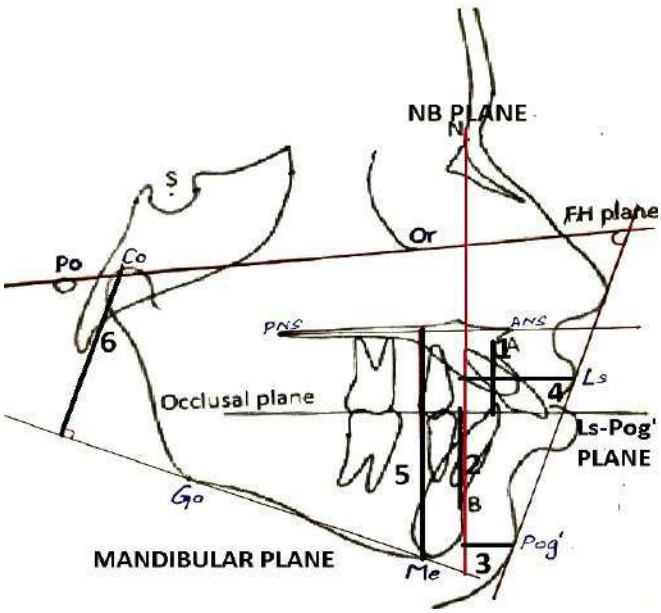
A total of 10 cephalometric points such as Point A (A), Nasion (N), Point B (B), Condylion (Co), Menton (Me), Gonion (Go), Anterior nasal spine (ANS), Posterior nasal spine (PNS), Soft tissue pogonion (Pog'), Labialis superior (Ls) and planes including N-B plane (formed between point N and point B), Mandibular plane (formed between Me and Go), Palatal plane ((formed between ANS and PNS), Occlusal plane were used to construct following linear measurements:

1. AO (A to Occlusal Plane)
2. BO (B to Occlusal Plane)
3. Chin thickness (Pog' to N-B)
4. Upper lip thickness (Ls to N-B)
5. Anterior facial height (Me to Palatal Plane)
6. Posterior facial height (Co to Mandibular Plane)

STATISTICAL ANALYSIS

Statistical analysis was performed using the Statistical Package for the Social Science (SPSS) version 17.0 software. Descriptive statistics (mean and standard

Figure 1: Showing the parameters used



deviation) was computed for each variable and Student's t-test was used to determine if statistically significant differences existed between the sexes. The level of significance was kept at $p \leq 0.05$.

All the predictor variables were subjected to stepwise discriminant function analysis, which has the potential to optimally separate the sexes; further the statistical significance was assessed using Wilks' lambda. The variables having the higher discriminant function coefficient were included in the discriminant function for developing the formula.

Di = K + di1z1 + di2z2+ …….+ dipzp

where Di is the discriminant function score, di is the discriminant function coefficient, z is the score of the predictor variable and K the discriminant function constant.

RESULTS

The intra-observer reliability calculated during the second examination after 2 months revealed the ICC value to be .965. Hence the measurements made at two different points showed negligible difference. Therefore, the initial measurements were taken into consideration for calculation.

Table 1: Depicts the students T-test for the comparison of variables (Upper lip thickness, AFH, PFH, AO, BO, chin thickness) between male and female. The mean values of males were significantly higher for variables such as AO (A to Occlusal Plane) (p = 0.089), BO (B to Occlusal Plane) (p = 0.082), Chin thickness (Pog' to N-B) (p = 0.032)than females.

Table 2: Shows the accuracy of the discriminant function coefficient for all the predictor variables which were included in this study, from which the highest accurate values were included for the generation of the discriminant function.

The discriminant analysis produced the best discriminant functions and the predictor variables included in the functions were AO (A to Occlusal Plane), BO (B to Occlusal Plane), Chin thickness (Pog' to N-B), Upper lip thickness (Ls to N-B), Anterior facial height (Me to Palatal Plane), Posterior facial height (Co to Mandibular Plane), based on the greatest univariate discriminant coefficient. Before the formula was calculated with the greatest univariate discriminant coefficient AO (0.56), the predictor variables were subjected to a test of significance using Wilks' lambda. It was found that the entire assigned predictor variables showed statistical significance at $p < 0.05$ **(Table 2)**.

DISCUSSION

Human identification from remnants has been researched since long time to develop specific methods to establish identity of a person.[4,12] The human remnants can be tooth, bone, mucous secretions such as saliva and blood etc. among these remnants the teeth and bone are the most resistant and available in many situations.[2,3] The identification of gender and chronological age is the basic identity of an individual. This study focused on the determination of sex by lateral cephalometric radiograph.

Although pelvic bones are most reliable for determination of sex, skull bones also display many features for determination of sex.[4,8,9] The determination of sex using skull bone can be employed as alternative method when pelvic bones are not available. The development of alternative method can act as another source of obtaining information during forensic evaluation. The evaluation of

Table 1: Students T-test for the comparison of variables (Upper lip thickness, AFH, PFH, AO, BO, chin thickness) between male and female.

Variable	Male (1) μ ± SD	Female(2) μ ± SD	T-test	Significance
AO	19.228± 1.9982	18.037± 1.466	3.346	0.039*
BO	17.293 ±3.231	16.863 ±2.412	0.742	0.082
Chin thickness	11.494± 3.108	11.370 ±1.861	0.238	0.032*
Upper lip thickness	22.291± 3.050	21.007 ±3.199	2.052	0.795
Anterior facial height	56.672± 4.682	53.989 ±3.762	3.119	0.191
Posterior facial height	36. 748± 3.037	35.128 ±3.022	2.664	0.872

**- Statistically Significant*

Table 2: Wilks Lambda to Test the Significance of Predictor Variables

Test of Function(s)	Wilks Lambda's	Chi-square	Df	Sig.
1	0.898	10.417	1	0.001***

Table 3: Shows the Canonical Discriminant and Function Coefficient

	Function
	1
AO (A to Occlusal Plane)	0.561
(Constant)	-10.479

Table 4: Shows the Group Centroids

Sex	Function 1
Male	0.305
Female	-0.366

Table 5: Depicts the Classification Accuracy Check Using Cross Validation

		Sex	Predicted Group Membership		Total
			Male	Female	
Original	Count	Male	52	2	54
		Female	2	40	45
	Percentage	Male	96.3	3.7	100.0
		Female	11.1	88.9	100.0
Cross-Validated	Count	Male	52	2	54
		Female	5	40	45
	Percentage	Male	96.3	3.7	100.0
		Female	11.1	88.9	100.0

a) 92.6% of original grouped cases correctly classified.
b) Cross validation is done only for those cases in the analysis. In case validation, each case is classified by the function derived from all cases other than that case.
c) 92.6% of cross-validation grouped cases correctly classified.

skull using lateral cephalometric radiograph for determination of sex is the simpler and precise method as compared to other methods.

The accuracy of determining sex using morphological or anthropometric methods ranges from 77% to 92.5%. Although there are several studies based on determination of sex using lateral cephalometric radiographs, the formula generated cannot be used for all the population because of diversity in food, cultural practices, climatic conditions among different populations.[4] This study was aimed to develop formula for identification of sex in Salem population.

Previous study done by Darshan Devang Divakar et al.[4] showed the mean linear measurements of males were greater than females which was similar to the results of our study and the overall reliability of the same study was 100% which was greater than our study. The results of Rawad Qaq et al.[12] overall accuracy was 82.4% for both females and males which was lower than our study with 92.6% accuracy. Also, there were some limitations in the study, such as the sample size was less, the formula generated cannot be used for all the population, this method cannot be employed when the skull bones are damaged. The advantages of the study were this study was able to develop formula for gender identification and formulated an alternative method for gender determination in the specific population.

CONCLUSION

This study showed a overall accuracy of 92.6% for determination of sex using parameters such as AO (A to Occlusal Plane), BO (B to Occlusal Plane), Chin thickness (Pog' to N-B), Upper lip thickness (Ls to N-B), Anterior facial height (Me to Palatal Plane), Posterior facial height (Co to Mandibular Plane) constructed in lateral cephalometric radiograph. This study can be helpful in forensic identification procedure in case of flood, medicolegal situations, earthquakes, terrorist attacks and war situations.

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Original Research Paper

Forensic Medical Examination of Severity at Closed Injuries of the Chest According to the Materials of the Specialized Clinic

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ABSTRACT

Introduction: A closed chest injury is a frequent type of damage to the organs of the external respiratory system. Victims with such an injury may become the object of a forensic medical examination. The aim of the work was to determine the severity of closed blunt trauma of the chest based on the materials of a specialized clinic in order to determine additional diagnostic criteria for the severity of such an injury.

Materials and Methods: 123 medical cards of inpatients department, patients with chest injuries, who were treated at the Kharkiv Institute of General and Emergency Surgery named after V.T. Zaitsev. Forensic evaluation of closed injuries of the chest organs was carried out according to the degree of severity of physical injuries based on the materials of clinical observations.

Results: Severe injuries were found in 22 (17.9%) cases of injuries with the occurrence of a life-threatening phenomenon, namely, acute respiratory failure. Injuries of medium severity were found in 71 (57.7%) cases of chest injuries, mainly with rib fractures, due to the absence of danger to life. Mild injuries were found in 30 (24.4%) cases of chest injuries without rib fractures with intrapleural injuries and complications or mild chest injuries without any complications.

Conclusions: It was determined that additional diagnostic criteria for closed injuries of the chest, which must be taken place when determining the severity of physical injuries, should be considered: dynamics and duration of restoration of post-traumatic morpho-functional changes of injured organs, loss of part or all of the respiratory organ (lungs), occurrence of life-threatening phenomenon.

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INTRODUCTION

The uninterrupted functioning of the external respiratory apparatus, which includes the airways, lungs, chest with muscles, ensures the normal vital activity of the human body.[1-2] At the same time, a frequent type of damage of organs of the external respiratory system is a closed chest injury, which is one of the leading causes of life-threatening consequences, both in peace time and in war time.[3-6] Victims with non-fatal closed blunt trauma of the chest (CBTC) become the object of a forensic medical examination to determine the severity of physical injuries.[7-10]

During the forensic medical evaluation of the specified injuries, when determining the severity of bodily injuries, the approaches of forensic doctors differ when applying certain qualifying signs of the severity of bodily injuries. This is evidenced by the study of special literary sources.[11-15] Forensic medical evaluation of CBTC when classifying them as life-threatening is controversial. Some experts suggest that serious bodily injuries should be classified as

life-threatening in all cases of CBTC with the occurrence of intrapleural injuries, in cases of hemopneumothorax, regardless of its nature and expressiveness and the presence or absence of life-threatening phenomena.[11-12] At the same time, in the opinion of other scientists and in accordance with the current normative documents of Ukraine, in direct, according to the "Rules of forensic medical determination of the severity of bodily injuries" (confirmed by the order of the Ministry of Health of Ukraine № 6 of 17.01.1995), severe bodily injuries it must be established only in the presence of life-threatening phenomena listed in paragraph 2.1.3 "o".[13-15]

The purpose of the work: forensic-medical determination of the severity of closed blunt trauma of the chest based on the materials of a specialized clinic to determine additional diagnostic criteria for the severity of such an injury.

MATERIAL AND METHODS

Present Retrospective study has been carried out from medical records of admitted patients of closed blunt trauma to chest during last ten years (2009-2018) at Kharkiv Institute of General and Emergency Surgery named after V.T. Zaitsev. The Institute receive on an average 18 patients of blunt trauma chest every year. But the data of only 123 patients (108 males and 15 females) could be archived and included in the present study.

The study cases were divided into 4 groups, depending on the dynamics of morpho-functional post-traumatic changes in the chest organs responsible for respiratory function, final results and the presence of life threatening phenomena in patients.

The First group (subgroups a and b) included patients with full restoration of the functioning of the chest organs and disappearance of post-traumatic morphological changes up to 6 days (subgroup "a") and in the period from 7 to 21 days (subgroup "b").

The second group (subgroups a to g) included patients with positive dynamics, but incomplete restoration of the function of the chest organs and the disappearance of posttraumatic morphological changes: in the period up to 6 days (subgroup "a"), in the period from 7 to 21 days (subgroup "b"), in the period of more than 21 days to 31 days (subgroup "c"), in the period of more than 1 month up to 2 months (subgroup "d"), in a period of more than 2 months up to 3 months (subgroup "e"), in a period of more than 3 months, up to 1 year (subgroup "f"), in the period of more than 1 year (subgroup "g").

The Third group included patients who lost part or their entire respiratory organ (lung) as a result of the injury.

The Fourth group included patients who developed an acute life-threatening condition, namely, acute respiratory failure, as complication of CBTC.

RESULTS

The Institute receives on an average 18 patients with CBTC for treatment annually, which is 0.3% of the total number of patients undergoing treatment in the clinic. Out of 123 study patients 108 were males (87.8% of cases) and 59 were of age 20-50 years (47.9%). The impact of blunt hard objects on the thorax was the commonest mechanism of injury.

Table 1:

Second Group: 71 (57.7%) patient's i.e. more than half had rib fractures and 55 (44.7%) patients with rib fractures had intra-pleural injuries and complications. First Group: While the patients in the first group had no damage to the bones of the skeleton.

We systematized the identified lesions in the patients in the observation groups and highlighted the morpho-clinical variants of CBTC **(Table 1)**. It can be seen from the table that more than half of the patients with the investigated injury - 71 (57.7%) observations, mainly in the patients of the second group had rib fractures. At the same time, 55 (44.7%) patients with rib fractures had intrapleural injuries and complications. It is worth noting that the patients of the first group had no damage to the bones of the skeleton. In 22 (17.9%) cases of CBTC in the absence of rib fractures mainly in patients of the second group, in 1 (0.8%) case in patients of the first group, in 3 (2.4%) cases in patients of the third group, in 5 (4.1%) in cases of patients of the fourth group, intrapleural injuries and complications occurred. In 16 (12.7%) cases of CBTC in patients of the first and second groups, it was noted that the chest was contused, without any complications. Rib fractures were present in some patients of the fourth group - 13 (10.6%) of observations.

Post-traumatic morphological changes were detected mainly by X-ray examination of chest organs, which was carried out in all patients. This study revealed rib fractures, air, blood, fluid in the pleural cavity, and changes in the parenchyma of lung tissue. In 17 (13.5%) observations, a computed tomographic study was carried out in order to clarify the results of the radiological examination, or in those cases when the radiological examination was not sufficiently informative.

Table 1: Morpho-Clinical Variants of Closed Blunt Chest Injuries

The Details of the Injuries	Groups of Observations											In Total	(%)
	I		II							III	IV		
	a	b	a	b	c	d	e	f	g				
CBTC, rib fractures			4	12								16	13.0
CBTC, rib fractures, presence of intrapleural injuries and complications:													
- hemothorax				7	1			1			1	10	8.2
- clotting hemothorax					2	3				1	2	8	6.5
- pneumothorax						1					3	11	9.0
- hemopneumothorax			2	5		2	2	1			6	21	17.1
- encapsulated pleurisy, hydrothorax			3	7		1	1					2	1.6
- post-traumatic rib chondroma								1			1	1	0.8
- bronchopleurothoracic fistula												1	0.8
- pneumonia, sternum fracture						1						1	0.8
CBTC, without fractures of the ribs, the presence of interpleural injuries and complications:													
- hemothorax			2	3	1	1						7	5.7
- clotting hemothorax				1		1						2	1.6
- pneumothorax	1		2	1		1					1	6	4.9
- pneumothorax, lung rupture												1	0.8
- hemopneumothorax			1	1	1					1	2	5	4.1
- post-traumatic pleural empyema					1							1	0.8
- relaxation of the dome of the diaphragm											1	1	0.8
- post-traumatic lung abscess						1						1	0.8
- diaphragmatic hernia								1	1			2	1.6
- encapsulated pleurisy						1		1				2	1.6
-sternum fracture											1	1	0.8
CBTC, contusion without complications CBTC, contusion & presence of intrapleural injuries:	2	3	4	6	1							16	13.0
-pulmonary contusion, pulmonary hemorrhage										1		1	0.8
- post-traumatic pneumonia				4	1							5	4.1
- contusion, lung gangrene, hemothorax										1		1	0.8
Total	**3**	**3**	**18**	**47**	**8**	**13**	**3**	**5**	**1**	**4**	**18**	**123**	**100**

Among the medical care provided to patients in the observation groups, conservative therapy was provided in 53 (43%) cases. Among the surgical treatment, the largest number of manipulations performed regarding drainage of the pleural cavity according to Bülau – 57 (46.3%) observations. Drainage of the pleural cavity according to Seldinghir was performed in 6 (4.9%) cases. More difficult surgical interventions, such as lobectomy, diaphragm plastic surgery, unilateral pulmonectomy, decortication, lobe resection, lung apices, and others, were performed in 9 (7.3%) cases.

Based on the results of the forensic medical evaluation of the clinical observations of CBTC, we established the following degree of severity of bodily injuries. Severe bodily injuries according to the "danger to life" criterion of paragraph 2.1.3. "o" "Rules..." were established in 18 (14.6%) patients with CBTC, who had signs of acute respiratory failure. At the same time, 13 (10.6%) patients had rib fractures, intrapleural complications and injuries were present: in 1 (0.8%) case hemothorax, in 2 (1.6%) cases clotting hemothorax, in 3 (2.4%) cases of pneumothorax, in 6 (4.8%) cases of hemopneumothorax, in 1 (0.8%) case of bronchople-urothoracic fistula. In 5 (4.1%) patients there were no rib fractures, but there were intrapleural injuries and complications: in 1 (0.8%) case pneumothorax, in 2 (1.6%) cases hemopneumothorax, in 1 (0.8 %) relaxation of the dome of the diaphragm, in 1 (0.8%) case, sternum fracture. 1 (0.8%) patient had

pleurisy, and 1 (0.8%) patient had pneumothorax.

Also, we estimates severe injuries in 4 (3.2%) cases in patients of the third clinical group with CBTC according to the criterion "health disorder associated with permanent loss of working capacity by more than one third" paragraph 2.1.6 "The rules...". At the same time, 1 (0.8%) patient had a pulmonary contusion, pulmonary hemorrhage, and underwent surgical intervention - "Left sided lobectomy". 1 (0.8%) patient had contusion, lung gangrene, collapsed hemothorax, empyema of the pleura, surgical intervention - "Unilateral pulmonectomy" was performed. In 1 (0.8%) patient, there was a rupture of the apex of the lung, a total right-sided pneumothorax, surgical intervention was performed - "Resection of the apex of the lung". 1 (0.8%) patient had multiple rib fractures, hematoma, lung contusion, pulmonary hemorrhage, and the operation was performed - "Resection of the lower lobe of the lung".

As injuries of moderate severity, which caused a long-term health disorder for a period of more than 3 weeks (more than 21 days), paragraph 2.2.1 "b" of the "Rules...", we assessed: 16 (13.0%) cases of CBTC with rib fractures, without complications; 41 (33.3%) cases of CBTC with rib fractures, the presence of intrapleural complications and combined injuries, of which 9 (7.3%) cases of hemothorax, 5 (4.1%) cases of clotting hemothorax, 8 (6.5%)) cases of pneumothorax, 15 (12.1%) cases of hemopneumothorax, 2 (1.6%) cases of encapsulated pleurisy, 1 (0.8%) case of rib chondroma, 1 (0.8%) case of sternum fracture, pneumonia.

Also, according to the same criterion, 13 (10.6%) cases of CBTC, without rib fractures, with the presence of intrapleural injuries and complications from them were classified as moderate injuries: 2 (1.6%) cases of diaphragmatic hernia, 1 (0.8 %) case of clotting hemothorax, 2 (1.6%) cases of hemothorax, 1 (0.8%) case of hemopneumothorax, 3 (2.4%) cases of pneumothorax; 1 (0.8%) case of pleural empyema, 1 (0.8%) case of lung abscess, 2 (1.6%) cases of encapsulated pleurisy. In addition, 1 (0.8%) case of chest injury with pneumonia was assigned to the same degree of severity.

9 (7.3%) cases of CBTC were assessed as mild bodily injuries that caused a short-term health disorder lasting more than 6 days, but less than 3 weeks (21 days) in paragraph 2.3.2 "a" "Rules..." without rib fractures, the presence of intrapleural injuries and complications, of which: 5 (4.1%) cases of hemothorax, 1 (0.8%) case of clotting hemothorax, 1 (0.8%) case of pneumothorax; 2 (1.6%) cases of hemopneumothorax. Also, 14 (11.4%) cases of chest contusion without complications, 4 (3.2%) cases of chest contusion with intrapleural complications, of which 2 (1.6%) cases of pneumonia, 2 (1.6%) cases of pneumonia, pleurisy.

2 (1.6%) cases of chest contusion without complications, 1 (0.8%) case of CBTC, without rib fractures, with the presence of pneumothorax were assessed as mild bodily injuries under paragraph 2.3.2 "b" of the "Rules...".

DISCUSSION

The conducted scientific work and modern literature show that the search for objective forensic medical diagnostic criteria for determining the severity of injuries in this type of injury remains one of the most relevant and promising areas of research in forensic medicine [2, 3, 5, 9]. Our study provides insight into the nature of chest injuries in living persons, whereas the forensic literature [7, 8, 9] tends to focus on characterizing injuries in cases of fatal injuries. There are few publications on the topic of forensic medical evaluation of closed chest injuries in living persons.

There are few scientific works in which the data of the forensic medical assessment of the clinical current of chest injuries, possible consequences, final results and determination of their severity are considered [5, 6, 9]. In separate publications [9, 13] there was a description of cases in which the course of some chest injuries was discussed, but there was no forensic medical assessment. In our study, the results of the forensic medical examination and evaluation of such injuries are preferred. Our observations indicated the need to establish links with clinicians to allow them to provide a more detailed description of the clinical manifestations of life-threatening events in the medical documentations of chest trauma victims. The importance and necessity of conducting objective research methods, including computer tomography, is shown. At the same time, it was also noted by other authors.[2,6,12,14]

CONCLUSION

1) Patients with injuries of the organs of the external respiratory system, namely, with CBTC, on average, make up 0.3% of their total annual number in a specialized surgical hospital.
2) According to their morphology, injuries with fractures of the rib cage of the chest (56.7%), including the

presence of intrapleural injuries and complications (44.7%) prevail in patients with CBTC undergoing inpatient department treatment. Injuries with signs of danger to life make up 14.6%.

3) In the structure of the forensic medical evaluation of CBTC, based on clinical observations, severe injuries taken place for 17.9% of cases and are established mainly in the event of acute respiratory failure. 57.7% of cases of CBTC, mainly with rib fractures, qualify as medium-severity injuries due to the lack of danger to life. 24.3% of cases of CBTC without rib fractures with the presence of intrapleural injuries and complications or light injuries without any complications qualify as mild bodily injuries.

4) The available scientific and methodical literature does not contain strong diagnostic morpho-clinical signs for qualitative forensic medical assessment and prediction of the final results of CBTC.

5) The following additional diagnostic criteria for the evaluation of CBTC, which must be taken place when determining the severity of physical injuries, should be considered: the dynamics and duration of restoration of post-traumatic morpho-functional changes of injured organs, loss of part or all of the respiratory organ (lungs), occurrence of life-threatening phenomena.

The perspective of further research consists in conducting scientific work on the study of all possible morpho-clinical manifestations of the studied injury with the subsequent development of a strong algorithm for conducting forensic medical expert research in these cases.

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Review Article

Significance of Forensic Botany in Criminal Investigation: A Review of Case Studies

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ABSTRACT

With a focus on taxonomy, field botany, anatomy, and ecology, forensic botany is a broad arena that covers many zones of plant sciences. Expanding the use of forensic botany in criminal investigations, particularly in cases of war crimes, genocide, homicide, sexual assault, major physical assault, illegal trade in endangered species, and wildlife crime, presents a tremendous opportunity on a global scale. The use of plant anatomy as a forensic technique in criminal investigations can be significant. The presentation of four instances in which anatomical information about plants or plant parts was used in research understanding of how to prepare plant parts. Forensic botany must include the study of these fragments as well as the interpretation of the data. This study aims to illustrate the value of forensic botany at crime scenes. The following collection of unusual case studies, which aims to demonstrate the value of forensic botany, will look at the determination of post-mortem or the post-depositional interval, evidence for a victim's post-mortem transfer, evidence for the identification of a primary crime scene, and evidence for the identification of a victim's dismemberment site. The review was executed by manual search strategy on google scholar, and PubMed, and data are summarised in tabular form to capture relevant information to establish the purpose. The published article exhibited great potential in criminal investigation, especially the victim's location, the period of its deposition, and the possible sequence of events that occurred at a specific scene.

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INTRODUCTION

Forensic science is a vital field of proficiency and skills that can be extremely beneficial in criminal investigations. Technical skills are used in forensic science to detect, identify, and prosecute criminals.[1]

The Latin word forum is the source of the adjective forensic. Forums were utilized by the early Romans for a diversity of reasons, including markets, public debate, and commerce and commercial transactions. In terms of applicability, forensic science is a huge area. It goes by numerous names, including Forensics, Forensic/medical jurisprudence, Forensic Anthropology, Forensic Botany, Forensic Archaeology, Forensic Psychology, Criminalistics, and Forensic Hypnosis, among others. Pathologists, biologists, physicists, chemists, and medical officials work together in forensic science to solve crimes. Through the provision of scientific evidence, forensic science can efficiently help find missing persons, establish their actual identities, and relate and prosecute who victimized whom.[1]

Forensic botany is a broad field that includes taxonomy, field botany, anatomy, and ecology, among other aspects of plant science. There is a significant opportunity to expand the use of forensic botany in criminal investigations around the world, particularly in cases of war crimes, genocide, homicide, sexual violence, major physical assault, illegal trade in endangered species, and wildlife crime.[2]

Forensic Botany is an integration of Botany and Forensics. Forensic botany utilizes the plant sciences in matters related to law, i.e., using plants or plant products as evidence to help solve crimes such as murder, kidnapping, etc. and help determine the victim's cause of death.[3,18]

Forensic botany is a branch of environmental forensics that

emphasizes the use of plants and plant components in criminal investigations for the persistence of legal cases. Botanical pieces of evidence are plant components that can constrict the inquiry channel and aid in the acquittal of the righteous. Environmental forensics is the use of scientific methods to answer concerns about the source and effectiveness of contaminants released into the environment.[4]

The various fields of forensic botany are helpful in determining the uniqueness of criminals. These branches assist in this by directing either plant configuration or plant connotation with the environment in which they are found.

According to the techniques of forensic and criminalistic investigations, forensic botany is the branch of botany that provides anatomical, histological, dendrochronological, ecological, molecular, palynological, and limnological information that is used as evidence.[5]

A leaf is any typical flattened green extension from the stem of a vascular plant and they are the key sites for photosynthesis. They originated in the apical bud laterally with the tissues of the stem itself. Botanical evidence is a shred of valuable evidence because it can be easily unheeded by the culprit. It can lead to a concrete crime scene and is easy to scrutinize. Forensic botany can deliver adequate auxiliary data as evidence throughout criminal investigations. Furthermore, due to a lack of comprehension and botanical expertise among the examiners, this subject of study remains underused. It is quite easy to trace any component of the plant, especially the leaf if it is present as evidence at the murder scene. Once the plant is acknowledged it can aid in shaping a sample's topographical origin and it can also offer the link between the crime scene and the suspect.[8-15]

Because leaves are so easily visible, they are frequently used to identify plants. They normally have two parts: the blade, which is the wider and more visible part of the leaf, and the "stalk," or petiole, which connects the blade to the stem. At the place where the petiole joins the stem, there is a bud. To begin, keep in mind that not all of the leaves on a plant are the same size or appearance. They come in a variety of sizes, colors, and shapes, and those that get a lot of sun may look different than those that get a lot of shade.

Leaves can be broad and narrow. Broad leaves have a broad blade with a visible vein network. Narrow leaves are narrow and lack a broad blade; they are typically described as "needle" or "scale-like." The pattern of leaf attachment to a stem or twig is another significant feature in plant identification.

There are two major types of patterns: alternate and opposite, as well as a third, less prevalent pattern called whorled. Only one leaf is linked to a stem at a time (a node), and the leaves generally alternate from one side to the other as one proceeds along the stem, or they may be arranged in a spiral pattern around the stem. Two leaves attached at the same point (a node) on a stem, but on opposite sides of the stem, are referred to as opposite leaves. A whorled arrangement occurs when more than two leaves emerge from the same position (node) on a twig. The leaves may radiate from the twig-like spokes on a wheel. Simple leaves are those that contain only one leaf blade, with or without a stalk or petiole. Compound leaves feature several blades and a complicated leaf stalk structure. Leaves have either smooth edges, termed as entire, or small notches or “teeth” along the margin.[16]

A leaf is a flattened green protrusion from the stem of a vascular plant that serves as the major site for photosynthesis. They begin in the apical bud, together with the stem's tissues. Botanical evidence is significant because it is easy to overlook by the perpetrator, it might lead to the actual crime scene, and it is simple to investigate. If any component of a plant, particularly a leaf, is found as evidence at a crime scene, it is relatively straightforward to identify the plant and the soil type in which it thrives. Once the plant has been identified, it can be used to govern the geographical origin of a sample as well as to create a link between the crime scene and the suspect. This evidence is frequently overlooked by investigators, but civil and criminal courts recognize them as valid scientific evidence.[8-15]

Investigators amass evidence that could contain fingerprints after a crime is committed. Plants can be found at both indoor and outdoor crime scenes. Fingerprints can be found on a varied range of surfaces, including plants. Every person carries a unique form of identification such as fingerprints. Therefore, it can help aid in criminal investigations by precisely identifying the perpetrators involved, searching for the suspect, and aiding in finding a missing person. Therefore, when searching for and gathering evidence, it's crucial to look at both indoor and outdoor plants.[17] This study aims to investigate the important role of plants, particularly leaves, in narrowing investigations by creating a link between the crime scene and the suspect, which is often neglected by investigators.

Objectives

The purpose of this review paper is to; (1) show how crucial forensic botany is at crime scenes, (2) Use plants or plant-based products as evidence to help solve crimes like murder, kidnapping, etc., (3) to help determine the cause of death of the deceased, is known as forensic botany. Fragments of plant material are frequently discovered at crime scenes (on both living and dead bodies, or on incriminating objects), and they can offer ad hoc evidence for a variety of forensic investigations, including locating crime scenes, establishing death dates, or proving the possession of prohibited species.

MATERIALS AND METHODS

Search strategy

Online electronic databases such as Scopus, PubMed, and Google Scholar were used to conduct a literature search method. In the title, abstract, and keywords, the keywords "Forensic Botany," "Plant Leaves", "Botanical evidence", "Case studies on Forensic Botany" and "Forensic Science" were used to filter the results. The titles and abstracts of the search results were initially sifted according to the inclusion criteria detailed. **(Figure 1)**

Eligibility Criteria

Articles were screened by title and abstract and then possible inclusion of some articles was selected. The authors' eligibility criteria for inclusion and exclusion were supported by the study's final inclusion. The inclusion criteria include case studies based on various botanical evidence such as leaves, shrub species, chestnut, marijuana leaf, the stack of loose hay, no restriction for sample size, studies within the English language, and articles in the past 20 years. The exclusion criteria are based on abstract and title.

RESULTS

Literature search and screening

The review paper for analysis included database sources n=100, 32 articles excluded after screening titles and abstracts, 68 articles needed full evaluation, 37 articles excluded that defied inclusion criteria, and 31 articles included for the review.

Data Extraction

The results are summarised of various case reports in a tabular manner to capture relevant information, vital for the review paper. An outline of these case studies incorporating Country, Author Year, Issue Type, Botanical Evidence, Case Report, and Outcome of the Study. **(Table2)**

Findings

Table 2 illustrates the eleven case studies in summarised form that contain virtually, fully or at least partially skeletonized human remains, and the botanical sampling procedures were applied using various ad hoc protocols. Aquila et al., 2010, Southern Italy solved Accidental issue type after examining botanical evidence (Xanthium orientalis subsp. Italicum) found on the clothing of the victim. The botanical components of each path were identified and analyzed.[27] Caccianiga et al., 2011, Pre-Alpine Valley in Northern Italy solved the Homicidal issue type after examining botanical evidence (shrub) on women's skeletal remains revealed along the river's edge that the offender sited the victim's body in neighbouring bush and shielded it with the evidence. Caccianiga et al., 2010, Rugged woodland area in Canton Ticino (Switzerland) solved Homicidal issue type after examining the botanical evidence (Castanea sativa). Several wood splinters that were discovered embedded in a fracture line along the frontal region of the skull were examined under a microscope, revealing the splinters' compatibility with Castanea sativa (chestnut). Caccianiga et al., 2011, Northern Italy solved the Homicidal issue type after examining botanical evidence (Solidago gigantia leaf) decided that the body had been dumped on top of the leaf from the late fall because it was located immediately beneath the victim's skull. Caccianiga et al.,2017, Northern Italian Province solved the Homicidal issue type after examining the botanical evidence (Solanum lycopersicium) a considerable uniformity of specimens was observed between the samples that were taken from the luggage and the samples that were collected from the region during the on-site inspection.[28] David L Dilcher.,

Table 1: Inclusion And Exclusion Criteria

Characteristic	Inclusion Criteria	Exclusion Criteria
Population	Any Country	Based on the abstract and title
Intervention	Complete Studies, Within The English Language	Incomplete studies, other languages
Result	Highly Significant Botanical Evidence Perceived	That defies inclusion criteria
Methodological Study	Case Study, Qualitative Data	Quantitative

Table 2: Tabular summarization of various case studies

Country	Author Year	Issue Type	Botanical Evidence	Case Report	Outcome of Study
Southern Italy	Aquila et al., 2010	Accidental	Xanthium orientalis subsp.italicum 	A woman in her 80s was discovered underneath the train station's bridge. She had dementia and was experiencing spatial and temporal disorientation. Excoriations and ecchymosis on the hands and knees, as well as hand scratches, are signs of injuries.	The dementia-affected woman fell accidentally from a distance.
Pre-Alpine Valley in Northern Italy	Caccianiga et al., 2011	Homicidal	Prunus laurocerasus (a shrub species)	After the victim's skeletal remains were found lying along the overgrown banks of a river, forensic archaeology procedures were put in place to aid in the victim's recovery. The area's spontaneous vegetation was examined. Human skeletal remains were discovered. The year before, a girl vanished after meeting a young male subject. Using an episcopic microscope, the samples were examined.	The perpetrator placed the woman's body in a neighboring bush and covered it with Prunus laurocerasus plant trimmings retrieved from a nearby fly-tip to masquerade it.

Country	Author Year	Issue Type	Botanical Evidence	Case Report	Outcome of Study
Northern Italy	Caccianiga et al.,2011	Homicidal	Sorghum halepense (prevalent species), Epilobium hirsutum and Rubus ap. Solidago gigantea leaf	In a field in a sparsely populated industrial area, a girl's largely skeletonized corpse was discovered. Violence and wrongdoing are clearly visible. The vegetation species in the immediate vicinity, the adjoining area, and the area underneath the victim's deposition were acknowledged through an on-site assessment. 	In the late Autumn, the corpse had been left on top of the leaves. The victim was discovered at the primary crime scene and underwent no post-mortem transfer.
Northern Italian Province	Caccianiga et al.,2017	Homicidal	Solanum lycopersicum, a tomato, persimmon (Diospyros kaki) 	Human remains that were just slightly skeletonized were found inside a partially zipped suitcase. A suitcase with botanical samples from the surroundings was collected. The interior of a suitcase included laminar species that resembled cuticles and had a vegetal look and leathery firmness. The retrieved items were	Finally, botanists determined that the fruit fragments belonged to Solanum lycopersicum.

Country	Author Year	Issue Type	Botanical Evidence	Case Report	Outcome of Study
Gainesville (Florida)	David L Dilcher.,2001	Accidental	Leaf fragments of Marijuana	The little leather pouch with some plant bits that the police had been given was examined. It had many trichomes and was readily crushed. The small leather pouch belonged to an itinerant, whom police stopped for interrogation. 	A cuticular examination led to the conclusion that it wasn't marijuana. A person who was in police custody was liberated.
Gainesville (Florida)	David L Dilcher.,2001	Suicidal	Taxus leaves 	A young college student slashed his wrist and then killed himself by consuming some plant matter. When police were asked to identify the plant matter, they found a little, twisted, partially digested, flattened Conifer needle.	The plant material was determined to be Taxus leaves, which are known to contain the alkaloid taxine. Escalating the depression, it contributed to his passing.
Gainesville (Florida)	David L Dilcher.,2001	Accidental	Stack of loose Hay 	Before he passed away, a small boy was having fun in a barn filled with hay. Police requested that the boy's tracheal material be examined.	Just before he passed away, the youngster inhaled some ground corn that was inside a bag. These were remarkably reminiscent of the starch granules in ground corn used as animal feed.

Country	Author Year	Issue Type	Botanical Evidence	Case Report	Outcome of Study
Gainesville (Florida)	David L Dilcher.,2001	Homicidal	Shrub woody vegetation 	A man was charged with killing his wife, taking her body to the bank of the river, and setting it ablaze in a 55-gallon oil drum. Judge – from the 55-gallon oil tank being lit on fire to the woody plant stems being damaged by fire. Determine– When the fire occurred in the year.	A cross-section of small-diameter woody plants created distinct yearly growth rings that could be used to identify the seasons, such as early Spring, Summer, or Early Autumn. But no wood samples ever did come.
West of Denver, Colorado	Norris et al.,2016	Homicidal	The root of the plant Cross section of a young woody eudicot root showing growth rings. 	Cher Elder Case Thomas Luther murdered Elder, a 20-year-old, in 1993. Cher's ex-boyfriend provided information in 1995 that her grave was located precisely west of Denver, Colorado. A forensic botanist looked at the plant roots near the grave.	She was able to identify roots using Herbochronology, demonstrating Luther was alive and well at that time. She was probably around 2 years old. On the grounds of second-degree murder, he was found guilty in 1996.
Taipei, Taiwan	Coyle et al.,2005	Suicidal	Solanum nigrum L 	The body of a young woman was found lying in a gutter in an urban section of Taiwan. She was visible on a surveillance system tape, but	The site of the body's discovery contained a portion of a broken stem. The edge of a railing above the gutter was where the investigators noticed some potted plants.

Country	Author Year	Issue Type	Botanical Evidence	Case Report	Outcome of Study
				after a truck passed by her, she was no longer visible on the surveillance tape. It was assumed that she was struck by the vehicle and her body moved to the gutter to hide the accident. Some plant material (a tiny berry and stem) was found in the victim's hair that was unusual flora for the area, possibly from the genus Solanaceae.	The botanical evidence from the victim's hair was used to identify those plants as Solanum nigrum L., and there was a physical match between one plant's stem and the stem fragment discovered in the gutter. Investigators assumed that a heavy object, such as a person, had struck the plant hard. Additionally, the plants were out of reach for those walking on the street due to the 3.5-meter height of the railing where they were discovered. The female dropped from the top of the building, making contact with the plants as she did so, and part of the plant material was transported to the gutter and the victim's hair. Her impact injuries were determined to be the cause of death several days later by autopsy, and her relatives later informed the police that the young woman had attempted suicide previously and suffered from despair.

2001, Gainesville (Florida) solved the Accidental issue type after examining the botanical evidence (leaf fragments of Marijuana) which was forbidden to possess; thus, the police took the offender into custody. David L Dilcher., 2001, Gainesville (Florida) solved the Suicidal issue type after examining the botanical evidence (Taxus leaves) According to the authorities, the leaf material was found in the stomach of a young college student who had killed himself. After consuming some of this plant matter, he slashed his wrists. David L Dilcher., 2001, Gainesville (Florida) solved the Accidental issue type after examining the botanical evidence (Stack of loose Hay). Just before he passed away, the boy consumed the contents of a bag containing ground corn. David L Dilcher., 2001, Gainesville (Florida) solved the Homicidal issue type after examining the botanical evidence (Shrub woody vegetation). It would be feasible to tell the season of the year the damage occurred by judging the point in the yearly ring's growth when the damage to the wood occurred.[29] Norris et al., 2016, West of Denver, Colorado solved the Homicidal issue type after examining the botanical evidence (the root of the plant) around the grave. Herbochronology allowed researchers to conclude that the grave was around two years old and that Luther was alive and well at that time [30]. Coyle et al., 2005, Taipei, Taiwan solved the Suicidal issue type after examining the botanical evidence (Solanum nigrum L). The spot where the body had been discovered had a broken stem. The edge of a railing above the gutter was where the investigators noticed some potted plants and there was a physical match between the stem on one plant and the stem piece discovered in the gutter.[31] Regardless of the strategies used, all of the cases produced incredibly distinct and helpful outcomes.

DISCUSSION

The main goal of forensic botany is to establish links between criminal activity and the evidence. In order to link a suspect to a victim or scene, various plants or plant material, such as pollen at the crime scene or a rare plant kind located nearby, can aid in the identification of the suspect. These evidences are frequently neglected by investigators, but civil and criminal courts acknowledge them as appropriate scientific evidence. Morphological and anatomical features validate the source only when present as nondegraded and unfragmented material; however, if present in degraded stages, DNA sequencing, and other biomolecular techniques can be used to establish the link between the plant part and the source, making them useful in forensics. The main issue for molecular biology analysis is the reproducibility of evidence when it is deteriorated and fragmented.[19-25]

This was only made possible by the meticulous care with which the on-site experts-who weren't necessarily from a botanical background-performed the inspections, recoveries, and sample processes. Due to a general lack of personnel specifically trained and capable of identifying and gathering botanical evidence and a general lack of culture regarding the discipline and potential of forensic botany, this field is regrettably undervalued far too frequently in both national and international contexts, this can be its limitations. In addition to concentrating on the human remains themselves, sampling procedures such as the collection of local plant samples will reveal a recovery site's entire historical context. At the very least, the area's dominant plant species should be mentioned in addition to the tree species that make up the majority of an ecosystem's unique ecology. Their identification will give basic ecological information about a specific location and match to samples that are most likely to be found beside a victim's remains.[26]

However, it must be stressed that all contexts with any physical or stratigraphic connection to a victim, whether direct or indirect, shall always be recovered, bagged, and stored for subsequent research. Once the victim has been found and is available for additional in-depth study, instances that call for a later botanical inspection can be handled. Nevertheless, it should be taken into account that botanical components and environs are vulnerable to quick changes and are at a high risk of environmental dispersion (due to flora, fauna, and meteorological events) that may change complete distribution patterns.

CONCLUSION

Forensic botany only becomes relevant when plants are implicated in criminal activity. Using plants or plant-based products as evidence to help solve crimes like murder, kidnapping, etc., as well as to help determine the cause of death of the deceased, is known as forensic botany. Bringing together botany and forensics is forensic botany. While the forensic aspect deals with identifying pertinent evidence at the crime scene, gathering and packaging evidence, maintaining the chain of custody, conducting scientific tests on the samples collected, and the admissibility of the evidence in court, the botanical aspect focuses primarily on anatomy, growth, development, taxonomy, and classification of plants that aid in the

identification of the specific species of plant. When assessing the cause of death-whether it was an accident, suicide, homicide, or other cause-or the season in which the burial may have occurred, plant evidence might be helpful. Plant evidence can also be utilized to find bodies that have gone missing and to assess whether a crime scene is a primary or secondary site. It is concluded from the above case studies, that forensic botany can offer great potential in the identification of a victim's location, the period of its deposition, and the possible sequence of events that occurred at a specific scene in the case of skeletal or partially skeletonized human remains in which some other means of identifications are simply ineffective.

It is true that, up to this point, there has been much discussion over botanical evidence in court cases. The forensic science community's representatives must therefore become more aware of the potential that this field may offer in a wide range of situations.

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Review Article

Reliability of Fingerprint Ridge Density as an Effective Determinant of Sexual Dimorphism

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ABSTRACT

Introduction: Human identification is critical, although it might be difficult given that each person has a particular trait. Fingerprint is the best identification of both living and deceased individual. There are patterns like ridge density, macro characteristic and micro characteristic in fingerprints. Fingerprint of ridge density plays an important role in differentiation male and female gender.

Materials and Method: An extensive search of all materials related to the topic was carried out in the PubMed and Google Scholar search engines. Relevant research articles focusing on "Reliability of fingerprint ridge density as an effective determinant of sexual dimorphism" published in the period 2001–2021 was included in the review. A total of about 300 studies which were similar to the current study objectives were included and analysed whereas other have been excluded. From the list of 300 articles finally 26 numbers of articles published by previous authors have been chosen and cited based on the relevance and compatibility with the objectives of this review.

Results: The ridge density of fingerprint is useful to determining the possible gender of an individual in routine lifestyle or in crime investigation.

Conclusion: The Fingerprint of ridge density are more useful in identify the sexual dimorphism among individuals of ethnic Tamil descent.

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INTRODUCTION

The Identification of an individual is a unique parameter for living and deceased person. In day to day life there are many techniques such as poroscopy, anthropometry, fingerprint, and age and sex determination based on height, dental examination, blood grouping and DNA profiling are used for identification of every individual. Identification is the process of determining a person's uniqueness. It can be total (absolute) or partial (partial).[1]

The term used for denoting the study of fingerprint is scientifically called dermatoglyphics where the terms derma means skin and glyphe means curve and this was first coined by Cummins and Midlo in the year 1926.[1] In forensic and medico legal investigations, gender identification is critical. Fingerprint identification is one of the most extensively used biometric technologies and in this the word dactyloscopy is used to denote the study of fingerprints.[3] Since from the ancient times dating back to 7000- 6000 B.C there are evidence that Assyrians and Chinese have used fingerprints for human identification.

There is proven evidence that the fingerprint ridge pattern is unchangeable and unique in every human and these patterns do not ever change in one's lifetime. There are no two fingers with identical prints, and there is a high mathematical probability that no two will ever match. The chances of two people having identical finger impressions are believed to be one in sixty-four thousand million people on the planet. Surprisingly, even identical twin's fingerprints are not identical.[4]

The number of ridges that occur in a defined space is known as ridge density. Ridge density has two important components are breadth and distance between two lines.

Fingerprints of ridge densities nowadays useful in identity of gender discrimination. Further these fingerprints are classified into macro and micro characteristics. Hence, this study was aimed at the application of finger ridge density in determining the gender of an individual.

In 1926 the term dermatoglyphics was coined by Harold Cummins. In Intrauterine period the fingerprints and palm prints are begin from 6-7th week and completed after 10-20 weeks of gestation. At 14th week of gestation, the lower part of the dominant ridges of the fingers appears. Later, unique patterns of ridge formation appear on the surface of the skin of the fingers and this organization of the ridge systems thus formed is permanent and irreversible for one's life period.[5]

HISTORY OF FINGERPRINT

In 700 A.D., the science of fingerprints was known in ancient Assyria and was utilized for identification.[6] In 3000 BC, the Chinese employed fingerprints as official records and used fingerprints as sign legal documents. In a study published in 1788, J.C.A Mayer of Germany was the first to suggest the hypothesis that the arrangement of friction ridges is never replicated in two persons. Sir William Herschel, an English Civil Servant stationed in India, obtained the indigenous' finger patterns to sign their contracts. In 1880 Henry Fauld, a Scottish physician, published his thoughts on the relevance of fingerprints in personal identification and human ridge patterns could be used in criminal investigations.

In 1892, Sir Francis Galton put forward a classification system for fingerprints in which he described the macro characters of fingerprints as Loop pattern, Whorl's pattern and, and Arches pattern of fingerprint. Edward Henry in the 1900s further worked on the Galton's classification and sub divided the system and introduced newer patterns such as arch, tented arch, right loop, left loop, and whorl. He further introduced anatomical landmarks like core and delta which form an intersection of multiple ridges of fingerprint known as Triradii. In United States, FBI utilizes and rely fingerprints in most of crime investigation in day-to-day practice.

FINGERPRINT COLLECTION AND ANALYSIS

The fingers get contact with any liquid content such as blood, greasy oil, ink, sweat and sebum secretion from sweat present on the friction ridges forms an Impression on any surface area. There will be basis appearance of ridge patterns impression on any objects.

Appearance of the latent prints of friction ridges are dependent on factors such as the type of material on which it is deposited and how much is that material rough in texture and the quality of the deposited material are thus the latent fingerprint may manifest differently than other fingerprints. Each occurrence of deposition of the friction ridge patterns has its own set of circumstances that are never repeated. Fingerprint examiners are obliged to complete thorough training for this reasons.[7,8]

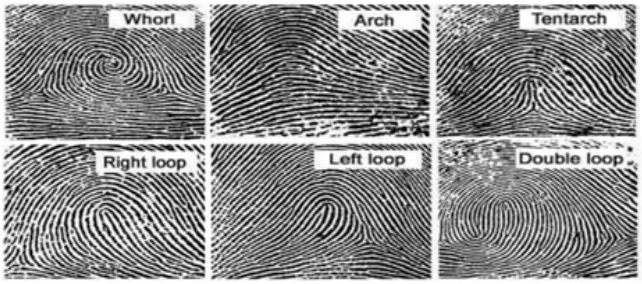
There are some types of prints by which we can collect and analyse the fingerprints. Latent prints, visible prints and plastic prints are most commonly used for detecting the fingerprints in the any crime investigation.

FINGERPRINT PATTERNS

There are various categories of fingerprint patterns like Macro characteristic, Micro characteristic and ridge density with ridge thickness

Macro characteristic are Loops which appears on both sides of fingerprint and at same direction it re curves till termination. One delta and one core are present in each loop pattern. Whorls are circular type line in nature with two deltas. Arch has ridges contain no line, delta, or core which extend from one ending to another ending with no reverse turn as shown in **Figure 1**.

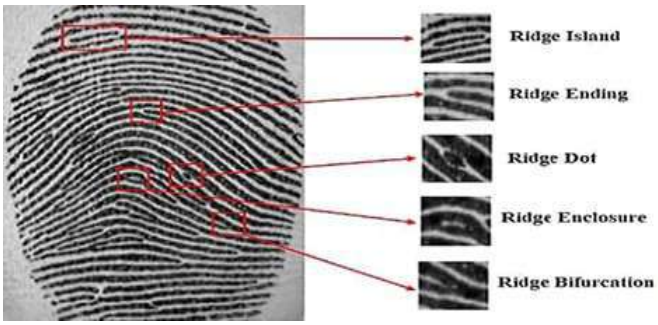
Figure 1: Types of Macro characteristic of fingerprints: Whorl, Arch, and Loop



The Ridge Density (RD) is the multiple ridges occur in particular areas of a finger. RD has two components like width and varies between the lines for calculating the ridge densities. The diameter of the epidermal ridges can differ in every individual and even in both sexes as shown in **Figure 2**. RD is more useful in investigating sexual dimorphism in every person.

Micro characteristic are the ridge lines end or fork are known as minutiae. About 40–100 minutiae are found in a good grade fingerprint. These minutiae points are the local ridge discontinuities, which can come in a variety of shapes and sizes like islands, short ridges, enclosure, termination, bifurcation, Ridge ending, dots, bridges, spur, lakes and so on as shown in **Figure 2**.

Figure 2: Ridge density and Micro characteristic of Fingerprints



MATERIALS AND METHODS

An extensive search of all materials related to the topic was carried out in the PubMed and Google Scholar search engines. Relevant research articles focusing on "Reliability of fingerprint ridge density as an effective determinant of sexual dimorphism" published in the period 2001–2021 was included in the review. A total of about 300 studies which were similar to the current study objectives were included and analysed whereas other have been excluded. From the list of 300 articles finally 26 numbers of articles published by previous authors have been chosen and cited based on the relevance and compatibility with the objectives of this review.

RESULTS

Gungadin S carried out a study on 500 people of age under 18 to 60 years in which the ridge of upper part of each radial border was tailed and the mean value was calculated with the goal of establishing a link between sex and fingerprint ridge density. According to this study, female ridge density was 14 ridges/25 mm2 and this shows that the ridge density more in females as compared to male where the ridge density was 13 ridges/25 mm2. It has been proved statistically that women show significant high ridge density compared with men.[9]

By evaluating 100 males and females from the FPRD, Nayak et al. were able to determine the sex differences in the Indian population aged between 18 and 25 years. Male and female fingerprints had statistically significant differences in FPRD, according to the study. The ranges of ridge density in female are from 12 to 15.9 ridges/25mm2 and males from 9.6 to 12.5 ridges/25mm2 which showed mean value of female 14.198 ridges/25mm2 and male 11.049 ridges/25mm2 where mean between male and female is 3.149 ridges/25mm2. As a result, females had a substantially higher ridge density than men. The ridge density of an unknown print left at the crime scene can be utilized as a presumptive indicator of sex. However, this study has a significant flaw in that it requires fingerprints from all ten fingers.[10]

Nithin MD et al in a study tried to determine the gender by counting the finger ridges in a well-defined area, in a south Indian population of 550 participants (275 males and 275 females) all between the ages of 18 and 65. From all the participants rolled out fingerprints were obtained. They applied Baye's theorem and noted that compared to males the females have higher ridge count. From the observation they came to the conclusion that a finding of ridge density which is more than 14 ridges/25 mm2 is female and ridge density of 13 ridges/25mm 2 is male. These findings are useful as a tool for fingerprint experts since the degree of ridge density can be utilized as a presumptive indicator of gender.[11]

Gutiérrez-Redomero et al. performed a study on Mataco-Mataguayo population and in that study the significant findings were in the age group above 12 years the female participants of the study have greater ridge density compared to the male participants. In this study focus was on the radial region of two ethnic groups of the namely the Mataguayo population and Spanish ethnic groups. The findings showed that in the radial area the ridge density is equal in both the population. In this study 99 males and 110 females under the age group of 6-25 years were included and the ridge count of distal radial and ulnar and on proximal regions of each finger were analyzed. According to above study specific findings were described in which the mean female ridge density is 13.94 ridges/25mm2 and mean male ridge density are 12.32 ridges/25mm2 which shows that females have higher ridge density and thinner ridges when compared with men and more over Females had a higher frequency of ridge endings (50.24 index fingers, 50.62 middle fingers) and enclosures (2.87 % in index and 3.08 % in middle finger) than male. In terms of broad pattern type, there was also a substantial gender difference in minutiae characteristics. Males have more minutiae than females when it comes to loop and whorl pattern types. In the case of the Arch pattern, females have a higher number of minutiae than males.[12]

Krishnan K et al in a study attempted to distinguish sex from fingerprint ridge density in the radial, ulnar, and lower portions of a fingerprint. A total of 194 people (97 men and 97 women) between the ages of 18 and 25 were included in the study, and fingerprints were taken from each participant's finger. So far, a total of 1940 fingerprints have been collected, with epidermal ridges in the radial,

ulnar, and lower portions of each fingerprint being counted. On a fingerprint, the radial and ulnar areas are the 5mm x 5mm areas on the radial and ulnar sides of the central core respectively, while the lower area is the 5 mm x 5 mm area adjacent to the flexion crease of the terminal phalanx. The t-test was used to compare the density of fingerprint ridges in the radial, ulnar, and lower areas, as well as between sexes. Females have a much higher ridge density than males in all three locations, according to the findings. The ulnar and radial areas of the fingerprints have much higher ridge density than the bottom area. According to the findings, fingerprint ridge density can be a meaningful and useful morphological characteristic in determining the sex of a latent fingerprint of unknown origin from the crime scene. When a severed hand is brought in for a medico-legal investigation, the findings may be useful in identifying mangled remains.[13]

In a study conducted by Ray AK et al. a study on Anthropometric investigations of the digits, palms, and feet demonstrates the relative distribution of dermal ridges among persons in various geographic zones. Plain arches were the most prevalent digital pattern in females (31%) compared to males (10%), according to a cross-sectional study conducted at Kalinga Institute of Medical Sciences (KIMS) in Odisha State, India. Loops were the most prevalent digital pattern in males (38%) compared to females (28.5%). There are considerable gender variations between these patterns. There was significant sexual dimorphism, with males 11/25mm2 having a lower finger ridge count than females 12/25mm2.[14]

In their study Neeti Kapoor and Ashish Badiye found that female ridge density > 28 ridges/mm2 have higher thumbprint ridge density when compared with male ridge density < 23 ridges /mm2. The study was held in a population of 100 males and 100 females of ethnic Marathi origin in the age group of 18 – 30 years.[15]

Hilal M et al did a study to determine sex differences based on fingerprint ridge density and whether it might be used in forensic medicine to determine sex. The study included 300 students (150 males and 150 girls) from Sohag University's Faculty of Medicine. Plates were consistently covered with a small layer of black printer ink to gather fingerprints. Subjects were asked to spread their fingers on the smeared plate and then transfer their fingerprints to a card that had been produced. Men and women's epidermal ridges were counted within a 5mm × 5mm square made on a transparent film mounted to a lens. The average number of fingerprint ridges in females right and left hands was 17.73 1.69 and 17.74 1.73 respectively, according to the findings. Both hands fingerprint ridges had a mean value of 17.74 1.63. Males fingerprint ridges had mean values of 14.82 1.52 and 15.22 1.32 in the right and left hands, respectively. Both hands fingerprint ridges had a mean value of 15.02 1.33. The mean of right finger ridges of 15.9 is the most accurate cut point to differentiate females from males, according to Receiver Operating Curve results. Meanwhile, for the left fingers the mean of 16.3 ridges is the most precise cut spot. Females had a statistically significant higher ridge density than males according to the current study. The mean ridge densities of an unknown print left at the crime scene can be used as a presumptive indicator of sex.[16]

In a study Rivalderia et al. used a sample of 335 people from two Argentinean populations to assess topological, digital, bilateral, sexual, and population variations in FPRD 172 people were from Buenos Aires and 163 people were from Chubut. Fingerprints were taken with the use of an adhesive and graphite. It was discovered in this study that females have a higher ridge density than males in all locations and on all fingers.[17]

In an extensive study done by Soanboon P et al. on northeastern Thai teenagers on fingerprint ridge density and variability to determine sex differences in the Thai population. This study included local northeastern Thais aged 14 to 24 who are descended from northeastern Thai heritage. In both counting categories, significant inequalities between genders and age groups were discovered. Females have a higher thinner ridge density than males. There was also a decline in Ridge Density values as people got older. The Bayes' theorem-based RD threshold for sex discrimination was achieved in all groups and counting areas, allowing it to be used in forensic investigations.[18]

Abdullah SF et al conducted a study on people born in Malaysia between the ages of 18 and 60 years which resulted that female have ridge density more than 14 ridges/25mm2 and males have lesser than 12 ridges/25mm2. As a result of the findings, we can deduce that women in Malaysia have a higher ridge density than men. It demonstrates similar sex difference tendencies to previous research conducted on other races in different nations. The ridge density of fingerprint is one of the most reliable criteria for identification factor in gender differentiation. Many studies of ridge density of fingerprint were analyzed by many scientists which has approved that ridge density is a definite criterion for gender

differentiation.[19]

Kumar et al. did a study on 200 South Indians participants (100 males and 100 females) ranging in age from 18 to 65. In males, the ridge density in the radial region was determined to be 13.56 ridges/25 mm2, while females had 16.92 ridges/25 mm2. Males have coarser ridges and females have finer ridges, female have 16 ridges/25 mm2 higher density than male 15 ridges /25mm2, according to this study.[20]

In their study M.K. Thakar and colleagues looked at the ridge characteristics and ridge density of fingerprints to see if there were any gender disparities among Punjabi people. In the study rather than using the traditional method of ridge counting, comparison was done between the general pattern type and ridge density, and minutiae or ridge features which noted that that higher number of minutiae and ridge density found in females than males.[21]

Sucharitha TM et al in a study observed that fingerprint ridge density differed in genders among medical students in an Andhra Pradesh medical college. They took the fingerprints of a total of 120 medical students in which the mean ridge density of Males is 128.5 (range 124-134) and females are 148 in all ten fingers with 148.67(range 145 - 153). Average ridge density of male and female in 0.25sq.cm are 12.85:14.86. Females have a higher ridge density than males with more than 14.86 ridges / mm2 of a finger in a 0.25 sq. when compared with males12.85 ridges / mm2.22 In below table the ridge density of fingerprints is compared with other authors, which is a useful data for determining the gender.

DISCUSSION

In the various previous studies, it has been observed that fingerprint ridge density has been effectively used to determine stature. In the previous studies of other authors it has been found that in some studies higher ridge density have been found among males whereas in few others higher ridge density was noted in the female subjects of the studies.

In studies done by Amira et al[23] it was noted that ridge density was higher in females than compared to males, and similarly Jasmine Kaur Dhall and Anup Kumar Kapoor also got concurrent results in their study[24]

Nagurka ML et al., out of 115 study participants of Taiwan origin, containing 57 males and 58 females between the age group of 18 - 35 years, it was found that the ridge density feature alone wasn't an effective factor for gender determination and the other two parameters like ridge count and finger size of the left little finger showed a positive correlation of 75 % in gender identification.[25] Natarajan Moorthy T et al they investigated the fingerprint collected from Malaysian population for determination of gender through ridge density of 400 individual found that females have greater ridge density compared to males with values of 12.39 to 14.14 and 11.06.11.87 respectively.[26] In disagreement with most studies there was one study by Khadri S.Y et al[27] where the males exhibited higher ridge density than females. The findings based on the observation of several authors have been shown in the **Table 1**.

Table 1: Showing Observation of the study of various authors with mean ridge density in males and females

Sr. No.	Author	Year	Male (Ridge/ $25mm^2$)	Female (Ridge/ $25mm^2$)
1	Gungadin.S	2007	14	13
2	Nayak et al	2010	11.04	14.19
3	Nithin et al	2011	14	13
4	Gutierrez Redomero	2011	12.32	13.94
5	Khadri S.Y et al	2013	12.4	12.0
6	Ray AK et al	2015	11	12
7	Neeti Kapoor	2015	<23	>28
8	Abdullah SF	2016	<12	>14
9	Natarajan Moorthy T et al	2016	16.87	15.63
10	Kumar et al	2017	13.56	16.92
11	Amira et al	2017	11.0	> 11.87
12	Sucharitha TM et al	2021	12.85	14.86

CONCLUSION

Fingerprint plays an important role in data of identification and the unique patterns of ridges and their density are highly useful for determining the possible gender. The ridge density patterns are useful to determine the differences in both males and females. As compared with many authors, their studies showed that with the help of fingerprint ridge density the scope of ascertaining gender from fingerprints is possible and useful in day to day lifestyle and in crime investigation. However, there is need to more studies in this regard involving multiple ethnic groups and also utilize other details in human fingerprint such as micro characters or minutiae to differentiate between male and female fingerprint effectively. The combination of fingerprint ridge density and fingerprint micro characteristics can prove to be very useful and reliable in determining sexual dimorphism in various medico legal scenarios.

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Original Research Paper

"Detection of Forensically Relevant Body Fluids using Specific Biomarkers Through Flow Cytometry

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ABSTRACT :

Introduction: Detection and identification of body fluids have been at the forefront of forensic professionals and researchers for the past two decades, due to the decisive role played by genetic and non-genetic information imparted by the body fluids.

Techniques such as spectroscopic methods, DNA methylation, messenger RNA profiling, etc. are being explored extensively for the detection and identification of body fluids. Although these techniques are promising, their specificity and the need for intricate bioinformatic procedures for their data interpretation are still under investigation.

On the other hand, Flow cytometry is an emerging single-cell analysis technique that has already established itself in various domains of basic and applied sciences. The main attribute of this technique to analyze multiple parameters of a single cell in a complex cellular population shows its potential for incorporation in the forensic analysis of body fluids.

Mixed body fluid identification has always been a challenge to a forensic serologist as due to the low quantity of samples, it gets difficult to analyze them through traditional techniques. However, this study focuses on the detection and identification of mixed body fluids based on specific protein biomarkers.

Aim: Detection of body fluids (blood, saliva, and urine) based on specific protein biomarkers using cytometric bead array flow cytometry.

Materials and Methods: Samples were collected from 10 healthy donors, selected through stratified sampling from Prayagraj after written consent. For this study, the samples were subjected to cytometric bead array flow cytometry for the detection of specific body fluid biomarkers. Body fluids were analyzed by calculating median fluorescence intensities for selected biomarkers in body fluid combinations.

Results: MFI values of specific biomarkers in pure body fluid samples ranges between 90 –150 and in binary mixtures of body fluids (1:1 ratio), ranges between 80 – 130. In case of other proportions (ratios of 1:3, 1:5, 3:1, and 5:1) MFI values of biomarkers range between 40 – 80 and 100 – 240 for positive detections. However, MFI values below 20 were considered as negative detection for that biomarker.

Conclusion: The study concludes that flow cytometry successfully identifies the body fluids based on their biomarkers even in trace quantity or in mixed states. However, in mixed state conditions, the MFI for the body fluid in larger quantity was considerably large in contrast to the one which was present in traced amount.

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INTRODUCTION

(What is known about body fluid identification and the limitations of the existing techniques.) Body fluids are rarely encountered in pure or pristine conditions at a crime scene. Apart from the genetic information imparted by the body fluids that help in the identification of the donor, the non-genetic information also plays a decisive role by directing the course for further investigations, as well as in contributing to the evidence in a case,[1] therefore, the detection and examination of body fluids recovered at a crime scene have a pivotal role in any forensic investigation.

In the present scenario, the analysis of body fluids in forensic laboratories is based on traditional techniques which include chemical tests and microscopic examinations. However, due to their limitations in terms of specificity, sensitivity, and sample destruction, and the scientific and technological advancements, since the last decade, body fluid identification has witnessed various novel approaches which have shown great potential[2-3] especially which were based on biomarkers for the identification of body fluids. Some of them are DNA methylation approaches, mRNA profiling, micro-RNA profiling, and spectroscopic techniques based on proteomics.[4-7] Despite these techniques being promising, their specificity and the need for intricate bioinformatic procedures for data interpretation open new avenues for much simpler and specific techniques for the identification of body fluids in mixed state. (Application of flow cytometry in body fluid analysis.)

Few studies based on the analysis of single-cell present in the heterogeneous cellular population of body fluids have been reported in the forensic domain, among which Flow cytometry is an emerging technique based on the examination of the optical attributes of individual cells with lasers at predetermined wavelengths.[8] Flow cytometry has established itself in the clinical and biomedical domains and has become the go-to technique for single-cell analysis in other fields of basic and applied science.

In the Forensic domain, Flow cytometry has been applied for the segregation of sperm and vaginal cells,[9-10] blood and saliva mixtures[11] and also in the separation of sperm from buccal epithelial cells[12] Flow cytometry has also been applied to detect the number of contributors of the body fluid samples, along with the generation of the genetic profiles of the individual donors.[13-15]

(Limitations of existing studies and scope for further research) However, the implementation of the flow cytometric technique for the analysis of body fluids has explored a small variety of samples and is mostly focused on addressing the issue of mixed genetic profiles.[11,13] Nevertheless, these studies have demonstrated the potential of utilizing protein biomarkers for the detection and identification of body fluids. This in turn has resulted in a new avenue for creating assays based on proteins for forensic applications, especially in body fluid analysis. One of the main benefits of the protein-biomarker method is the wide range of potential targets and the stability of proteins even under conditions when other bio-molecules break down.

(What is done in this study)

In this study, the detection of body fluids and their binary mixtures based on detection of specific protein biomarkers, through the flow cytometric technique has been explored. Biomarkers are the biological molecules present in the body fluids which are under normal range in healthy individuals but dysregulated under abnormal conditions.

The biomarkers utilized in this study include GMCSF (Granulocyte-Macrophages colonystimulating factor), EpCAM (Epithelial Cell Adhesion Molecule), and Uromodulin for the detection of blood, saliva, and urine in the body fluid samples, respectively. GMCSF, EpCAM, and Uromodulin all are glycoproteins present in the blood, saliva, and urine respectively. They have been utilized extensively in biomedical science for various diagnostic and therapeutic purposes.[16-21]

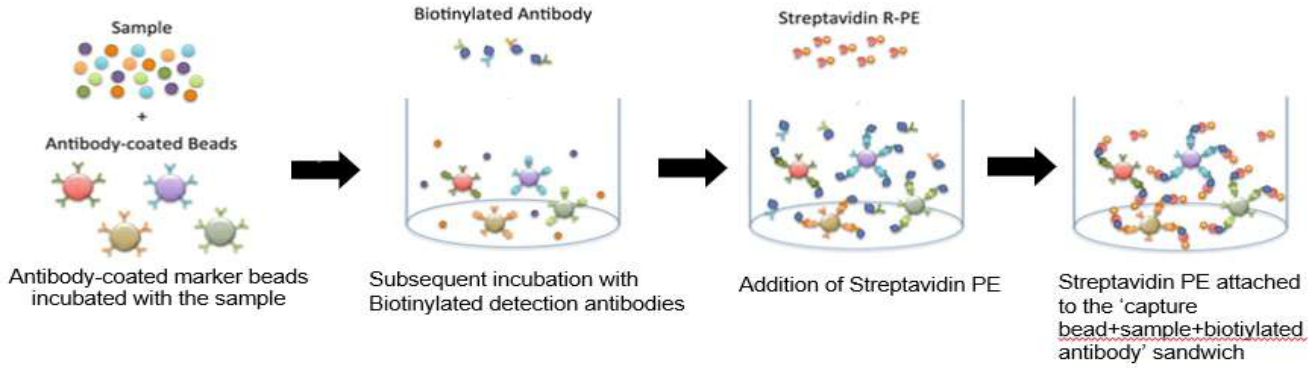
The cytometric bead-array (CBA) flow cytometry technique has been utilized in this study, where an array of beads coated with antibodies, having a known amount of fluorescence, targeting selected proteins were used,[22] followed by incubation of capture beads with the sample, and subsequent addition of biotinylated detection antibodies resulting in a sandwich of capture beads, analyte, and detection antibodies **(Figure 1)**. The addition of fluorescent dyes (such as Streptavidin phycoerythrin) results in the detection of analytes present in the sample through fluorescence (Median Fluorescence Intensity/ MFI).

MATERIALS AND METHODS

Sample collection

Blood,saliva, and urine samples were collected from 10 of age group of 20-30 years, selected through stratified

Figure 1: Principle of the Cytometric Bead Array (CBA)



sampling from Prayagraj, after getting their written consent. Blood samples were collected by venipuncture method in EDTA-coated tubes, whereas saliva and urine samples were collected in sterile plastic containers, by spitting in the container and direct deposition of mid-stream urine, respectively. The collected samples were stored at 4^0C for further research use.

Preparing samples of varying proportionsThe samples were diluted with phosphate buffer saline (PBS, Sigma Aldrich) in a ratio of 1:3 and the experiments were carried out in two phases. In the first phase, pure body fluid samples that is, blood, saliva, and urine samples diluted with PBS were analyzed for the presence of specific biomarkers.

In the next phase, different mixing ratios of diluted body fluid samples in a binary fashion were analyzed. The combinations of the mixtures are given in **Table 1**.

Flow cytometric analysis –

Pure body fluids and different body fluid mixtures were analyzed for the presence of specific biomarkers; GMCSF from blood origin, Uromodulin from the urinary origin, and EpCAM from the epithelial origin using multicolour flowcytometry-based bead array protocol (LEGENDplex™, Biolegend). 25 µl of body fluid samples (pure and mixtures) were incubated with the microbeads at 4^0 C for 30 minutes, and detection antibodies were subsequently introduced. The samples were then evaluated using a 3-laser flow cytometer MACS Quant analyzer 10 (Miltenyi) **(Figure 2)**. The microbeads identify the biomarkers and bind to them, and the detection antibodies stain the microbeads which are excited when introduced with a laser. The excitation gives fluorescence which is detected in flow cytometry.

Prior to the analysis of body fluid samples, the buffer solution (PBS) was run through the flow cytometer to calculate the negative detection of biomarkers.

Data Acquisition –

The FCS files generated from the flow cytometer, MACS Quant 10, were analyzed to calculate median fluorescence intensities for specific biomarkers in the given mixtures using third-party software FlowJo. The median fluorescent intensity (MFI) values are indicators of the amount of a particular marker present in the samples. The MFI values were subsequently analyzed statistically at 0.05 level of significance using Graphpad Prism. However, the MFI values below 30 were considered as negative detection for biomarkers, as they correspond to the MFI values of the buffer solution.

RESULTS

GMCSF, EpCAM & Uromodulin were present in significantly higher concentrations in respective blood, saliva, and urine samples with median fluorescence intensity (MFI) ranging between 90-50. The specific biomarkers show negative values of MFI ranging between 5-20 when analyzed for non-specific samples that is, a test of EpCAM in blood or urine and so on (as shown in **Table 2**).

Table 1: Inclusion And Exclusion Criteria

S. No.	Body Fluid Combinations	Ratio of the Combinations
1	Blood:Saliva	1:3, 1:5, 1:1, 3:1, and 5:1
2	Blood:Urine	1:3, 1:5, 1:1, 3:1, and 5:1
3	Saliva:Urine	1:3, 1:5, 1:1, 3:1, and 5:1

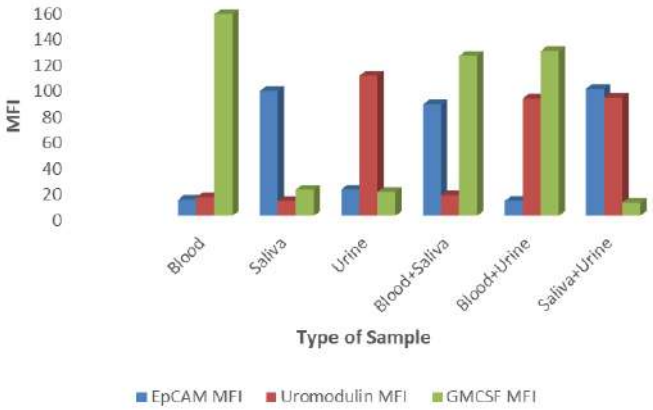
Figure 2: Overview of the steps involved in the examination of pure body fluids using cytometric bead array (CBA) flow cytometry.

Table 2: **MFI values of EpCAM, Uromodulin, and GMCSF in saliva, urine, blood, and their binary mixtures in the ratio of 1:1,** indicating the presence of specific biomarkers in corresponding body fluid samples with positive detection ranging between 80-150 and negative detection ranging between 5-20.

Type of Sample	EpCAM MFI	Uromodulin MFI	GMCSF MFI
Blood	12.53	14.33	155.877
Saliva	96.447	11.227	20.339
Urine	20.44	108.119	18.332
Blood+Saliva	86.114	15.661	123.441
Blood+Urine	11.334	90.554	127.449
Saliva+Urine	98.224	91.447	10.226

In mixtures with binary combinations of body fluids in the ratio of 1:1, each combination demonstrated positive detection, with MFI values ranging between 80 – 130, of the specific biomarkers corresponding to the body fluids present in the sample. For example, Uromodulin and EpCAM were present in a mixture of saliva and urine. On the other hand, specific biomarkers showed negative detection with MFI values ranging between 5-20 for the absence of a body fluid in a given mixture as shown in

Figure 3. **Presence of EpCAM of epithelial origin, Uromodulin of urinary origin, and GMCSF of blood origin in pure body fluid samples and in their binary combinations in the ratio of 1:1.** Positive detection of specific biomarkers in corresponding body fluid samples is demonstrated by median fluorescence intensities ranging between 80-150.



In the case of binary combinations of body fluids with varying ratios, each combination resulted in the positive detection of the specific biomarkers with significantly higher MFI values as shown in **Tables 3, 4,** and **5**. The positive MFI values of the body fluid with lower volume in the mixture range between 40 – 80. For example, in the 5:1 mixture of blood and urine, where the volume of blood is five times that of urine, the MFI value of Uromodulin (a specific biomarker of urine) is 46. Moreover, the positive MFI values of the body fluid with higher volume in the mixture range between 100 – 240. For instance, in the 5:1 mixture of blood and saliva, in which the volume of blood is five times that of saliva, the MFI value of GMCSF (a specific

Table 3. MFI values of GMCSF and EpCAM in blood to saliva mixed body fluid samples in varying ratios of 5:1, 3:1, 1:1, 1:3 and 1:5. The median fluorescence intensities show positive detection of GMCSF and EpCAM in samples where the proportion of blood and saliva are five times lower than the other body fluid in the mixture.

Type of Sample	Blood to Saliva Mixtures				
Ratio of mixture	05:01	03:01	01:01	01:03	01:05
GMCSF	243	179	157	98	73
EpCAM	54	69	95	109	121

Table 4. MFI values of GMCSF and Uromodulin in blood to urine mixed body fluid samples in varying ratios of 5:1, 3:1, 1:1, 1:3 and 1:5. The median fluorescence intensities show positive detection of GMCSF and Uromodulin in samples where the proportion of blood and urine are five times lower than the other body fluid in the mixture.

Type of Sample	Blood to Urine Mixtures				
Ratio of mixture	05:01	03:01	01:01	01:03	01:05
GMCSF	239	200	160	107	84
Uromodulin	46	62	103	126	149

Table 5. MFI values of EpCAM and Uromodulin in saliva to urine mixed body fluid samples in varying ratios of 5:1, 3:1, 1:1, 1:3 and 1:5. The median fluorescence intensities show positive detection of EpCAM and Uromodulin in samples where the proportion of saliva and urine are five times lower than the other body fluid in the mixture.

Type of Sample	Blood to Urine Mixtures				
Ratio of mixture	05:01	03:01	01:01	01:03	01:05
GMCSF	131	116	110	91	61
Uromodulin	53	71	109	132	157

biomarker of blood) is 243.

The overall data suggest that positive detection of the specific biomarkers through flow cytometry is possible in the case of pristine body fluid samples and also in the case of mixtures of body fluids in binary combinations, in varying proportions.

DISCUSSION

With the recent developments in the analysis of body fluids, the role of proteins in the detection and identification of body fluids has been explored extensively.[23] Emerging techniques such as spectroscopy, epigenetic approaches, RNA profiling, etc. have utilized biomarker proteins for analyzing body fluids. Flow cytometry, an emerging technique in the domain of single-cell analysis, has already established itself as an essential tool in the fields of clinical pathology, microbiology, virology, biotechnology, etc. The ability of this technique to detect biomarkers in very small quantities of samples provides a promising avenue for its inclusion in the forensic domain.

This study was aimed to assess the utilization of flow cytometry for the identification of body fluids based on the detection of protein biomarkers. The specific protein biomarkers used in this study that is, GMCSF for blood, EpCAM for saliva, and Uromodulin for urine are membrane-bound glycoproteins. GMCSF is present in the serum, in the extracellular matrix as well as on the membranes of cells of hematopoietic origin and is responsible for their proliferation and differentiation and also plays a vital role in the presentation of antigens by the cells.[16,17] Due to its role in the immune system, GMCSF has been extensively used in therapeutic studies, drug analysis, etc. in the biomedical domain.[16-17] EpCAM is a transmembrane protein mostly present on healthy epithelial cells. Due to its role in cell-cell adhesion, as well as in the proliferation, differentiation, regulation of the cell cycle, etc.

EpCAM is widely applied for the detection of carcinomas conditions as well in various diagnostic and therapeutic purposes.[18-19,24] Uromodulin is a specific glycoprotein with restricted renal expression. Abundantly secreted by the epithelial cells in the loop of Henle, Uromodulin is not only utilized for diagnostic purposes in the biomedical field but has also been used as a biomarker by various forensic professionals for the identification of urine.[20-21]

These protein biomarkers, already used in the biomedical

domain, were utilized in this study to detect the presence of blood, saliva, and urine in pure as well as in mixed body fluid samples, using cytometric bead array (CBA) flow cytometry. The MFI values indicating the concentration of biomarkers present in the samples, in terms of fluorescence intensities help in the detection of individual body fluids. The results indicate that the fluorescence signal of all three biomarkers demonstrated positive detection in samples with respective corresponding body fluids. Furthermore, in samples with binary combinations of body fluids in different proportions resulted in the positive detection of the specific biomarkers in mixtures containing their corresponding body fluids. For instance, GMCSF and EpCAM gave positive detection in a binary mixture of blood and saliva and negative detection of Uromodulin. Moreover, the positive detection of these biomarkers in mixtures where the volume of one body fluid was 3-5 times more than the other, validates not only the specificity of these biomarkers but also the potential of flow cytometry for their detection in such low quantities.

CONCLUSION

In conclusion, the aggregated data suggests that both pure body fluid samples and mixtures of body fluids in binary combinations, in different proportions, are capable of yielding good results for the identification of particular biomarkers using flow cytometry. Future research should utilize this technique for the analysis of other forensically relevant body fluids such as vaginal secretions, semen, sweat, etc. In addition, further research is also required to assess the maximum number of body fluids that can be mixed to have positive detection of specific biomarkers through flow cytometry. Also, the inclusion of more than one biomarker for a body fluid could increase the potential of flow cytometric detection and identification of body fluids.

Solving these queries will certainly strengthen the position of flow cytometry in the area of forensic research and analysis.

Conflict of Interest: The authors show no conflict of interest.

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A Case Report

The Puzzled Skeletal Remains That Fizzled Out!

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ABSTRACT

Medical students invariably use the human skeleton to learn the anatomy of the human body. But after use these bones are often disposed off carelessly along with garbage or in deserted public places, only to be discovered later by someone or the other.

Discovery of unknown skeletal remains poses a great challenge to the police and forensic surgeons and attracts widespread media attention and causes public frenzy. This paper discusses a case where a bag of skeletons was discovered by a random person in a vacant plot. Case was registered by the police and a detailed medico legal examination of the skeletal remains was done. The skeletal remains were found to be those used as a study material in the medical field, disposed carelessly after use. Throwing away human remains is illegal and is punishable under 297 IPC.

There should be proper guidelines for the disposal of human remains and medical students should be educated regarding the same.

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INTRODUCTION

Anatomy is one of the basic sciences that are taught to medical students in the first year of their medical education and quite often students and teachers make use of the human skeleton to study anatomy, especially the muscle attachments, bone markings etc.[1-3] Sadly, some of these bones are simply discarded along with garbage or dumped in abandoned or desolated wastelands.[4] When unknown skeletal remains are discovered by random people, it attracts a lot of media attention, creates unnecessary panic among the general public and warrants meticulous police investigation and most importantly a structured medicolegal autopsy from forensic surgeons. A lot of manpower and time is required to determine the species origin of the bones, and if human – to establish the identity of the individual from the skeletal remains and to determine the time and cause of death.[5] We present a case where a bag of skeletons was discovered in a vacant plot and how the scrupulous medicolegal autopsy revealed the set of bones to be a part of the study material used by medical students, who carelessly discarded the bones after use.

CASE REPORT

A random person discovered some skeletal remains inside a plastic shopping/carry bag in a vacant plot of land. A case was registered by the police and a detailed medico legal examination was conducted in multiple sittings. There was a total of 65 bones which included a skull without calvarium (cleanly cut) **(Figure 1)** and without mandible, 1 sternum, 2 clavicles, 21 ribs, 1 scapula, 16 vertebra, 1 sacrum, 1 hipbone, 2 humerus, 1 radius, 1 ulna, 1 femur, 2 tibia, 2 fibula,1 patella, 8 tarsal bones and 2 small broken pieces of bone. All the bones showed human morphological characteristics and were covered with soil and roots of plants. All the bones belonged to male sex. Duplication of bones were present. A few other discrepancies were also noted, which is given below.

- The sacrum was not articulating with the left hip bone or the lumbar vertebra.
- The right sided tibia and fibula were not articulating.
- The left tibia was longer than the right tibia.
- The right fibula was longer than the left fibula.
- The right clavicle was not articulating with the right scapula.

Figure 1: Varnished Skull Which is Cleanly Cut on Top, Calvarium Missing.

Estimation of stature using Mehta and Thomas formula[6] (formula for South-Indian population) using long bones showed wide variations in values.

Based on these findings, it could be opined that the bones belonged to more than one individual. Due to the very same reason, age and stature could not be commented.

An interesting and most important finding that was observed was the number "211" **(Figure2)** written in blue ink on the head of the left humerus bone. There was red ink mark over the palatine bone, palatine process and pharyngeal tubercle of the skull bone. One of the right sided ribs showed red ink mark in the costal surface above the costal groove at the angle. One of the right sided ribs showed blue and white coloured markings in the costal surface above the costal groove. Most of the bones were coated with varnish. All these findings suggested that the bones were used for study purpose.

DISCUSSION

The unknown skeletal remains that were discovered in the vacant plot were ultimately found to be ones used for study purpose. Use of such study material for understanding anatomy are extremely useful for medical students. Learning anatomy, especially osteology by touching and feeling the bones help the students to understand and remember better. This optimizes the learning experience in the undergraduate level.[3] The sources from where the students obtain such bone sets include postmortem examinations, surgical operations, or bodies donated to the anatomy departments with the consent of the people

Figure 2: Left Humerus with Number '211' Written in Blue Ik on the Head. (White Arrow)

concerned.[4] Sometimes, cleaned, polished bone sets can also be obtained from the markets for a price. The sources of these are unclear and there is no licensing system or registration for the agents or markets selling such bone sets. Procuring human body parts for study purpose is permitted by law.[4] In India, the state Anatomy Acts contain legal provisions for the use of a dead body or part of a dead body, for therapeutic or research purposes with proper consent of the concerned authority.[7] However, there is uncertainty regarding the disposal of such bone sets after use. Many students resort to dumping the bones along with garbage or throwing the bones in barren areas. As per Bio-medical Waste (Management and Handling) Rules, 1998, there is an option of deep burial of human anatomical waste in rural areas or towns with a population of less than five lakh. In larger towns, especially medical institutions, anatomical wastes should be incinerated. Bio-medical waste" refers to "any waste which is generated during the diagnosis, treatment or immunisation of human beings or animals or in research pertaining thereto or in the production or testing of biologicals or in health camps." Human skeleton comes under the purview of this act.[8] Medical students should be taught regarding the proper disposal of human skeleton after use and every medical college should issue guidelines for the same. Careless disposal of human skeleton has several legal implications

and such individuals will be penalised as per section 297 IPC, which mentions that whoever offers any indignity to any human corpse shall be punished with imprisonment of either description for a term which may extend to one year, or with a fine or both.[2] Human tissues deserves to be treated with utmost respect.

In some institutions like the **University of the Free State (UFS)** in South Africa, Department of Basic Medical Sciences issues a complete set of unarticulated human bones to their registered anatomy students for the duration of their course. These students include first and second year medical, first and second year nursing, and also first year occupational therapy and physiotherapy students. A nominal fee is collected from the students for the same, which is reimbursed at the end of their course when they return the bone sets.[3] This system works similar to the issuance of books from a central library. It is a very practical method and the question of disposal of bones doesn't arise here, as each student is held accountable for each and every bone issued to him/her. This system also addresses the issue of difficulty in obtaining bone sets whereby students are forced to buy bone sets from unauthorized outside sources for heavy prices.

An alternative to the use of human skeleton to study anatomy is the use of three dimension printed models to study bone spatial anatomy. In this 3D printing technique, 3D printed model of almost any shape can be obtained from a 3D digital model or other electronic data source.[9] Literatures on randomized controlled studies done to assess the usefulness of 3D printed models in relation to studying anatomy of hepatic segments,[10] anatomy of external cardiac anatomy,[11] and spinal fractures[12] were perused. 3D printed models were found to be useful in all the 3 studies. 3D printed models do not have a significant cost. They last for long periods of time and can be used many times. Informed consent has to be obtained and confidentiality of the donor information should be maintained.

CONCLUSION

There should be guidelines for the proper disposal of anatomical wastes like bones used for study purposes and medical students should be educated regarding the same. Disposing bone sets along with garbage and in the open vacant areas of land calls for unwanted media attention, public frenzy and unnecessary burden on the police and forensic surgeons. Perpetrators can be penalised under section 297 IPC. Medical colleges can consider the option of providing bone sets to medical students for a nominal fee which will be refunded at the end of their course on returning the bone sets and such bone sets can again be reused for the subsequent batch of medical students, thereby avoiding the need for disposal of bones. The fact that each student is held accountable for each and every bone issued to him acts as a deterrent to the careless disposal of bones by students. An alternative option to study anatomy is the use of 3D printed models, which is increasingly being used as a teaching tool in various domains.

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A Case Report

Athlete Heart – A case report

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ABSTRACT

Sudden death in body builders has drastically increased in recent days mainly due to cardiac condition among androgenic-anabolic steroids (AASs) users. The use of anabolic steroids has been linked directly to cause impact on myocytes and endothelial cells, decreases intracellular Ca2+ levels, increases release of apoptogenic proteins, and increased collagen crosslinks between myocytes potential causing concentric left ventricular hypertrophy. This case discusses the cardiovascular effects of these drugs (AASs) in the possible long-term usage.

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Keywords: Body Building, Anabolic Androgenic Steroid, Sudden Cardiac Death.

INTRODUCTION

Androgenic-anabolic steroids (AASs) are used by athletes to build strength and lean body mass, in some cases, to enhance physical appearance.[1] AASs are given as a cocktail mix of various agents taken at once to reduce the chance of developing tolerance to any one agent,[2] this process in called 'stacking' in most of the western countries. The International Olympic Committee reports that more than 50% of positive doping cases involve steroids.[3] Abuse of AAS may increase the risk of myocardial infarction, sudden cardiac death, deranged serum lipoproteins, and cardiac hypertrophy.[4]

CASE REPORT

A 32-year-old male, body builder by profession had bilateral leg pain post workout at gym for which he was taken to nearby hospital for primary treatment. Next day, he was found unresponsive in his residence and immediately taken to nearby hospital. After examination by the casualty doctors, he was declared brought dead. No history of previous cardiac illness or other significant comorbidity. The deceased family gave history of chronic usage of AASs (Inj. Testoviron, Intramuscular) along with diuretics during his body building competition few years ago. The autopsy was conducted 9 hours after his demise, inview of the cause of death.

AUTOPSY FINDINGS

The deceased had wheatish complexion, well-built and nourished measuring 174 cm in length and weighed 91 kg. Body mass index was 30.1 kg/m2.

Internal Examination

Lung, Liver and Kidney: Congested on cut section.

Heart: Weighed 302 g; Multiple hyperemic areas were present over the heart at several places **(Figure 1-2)**. Walls were intact and valves were competent. The right and left ventricle wall thicknesses were 0.5 cm and 1.8 cm respectively. The bicuspid valve measured 10 cm and tricuspid valve measured 10 cm. All the coronaries were patent on cut section.

Microscopy Features of Heart

The left ventricular wall cardiac myocytes with thickened fibres and mild disarray, boxing of nucleus **(Figure 3)**. The right ventricular wall showed focal mixed inflammatory infiltrate.

- Right coronary artery showing atherosclerosis with 25% luminal narrowing.
- Left circumflex artery showing atherosclerosis with

Figure 1: Thickness of Left Ventricular Wall (Yellow Arrow)

Figure 2: Multiple Hyperaemic Area Present Over the Heart (Yellow Circle & Yellow Arrow)

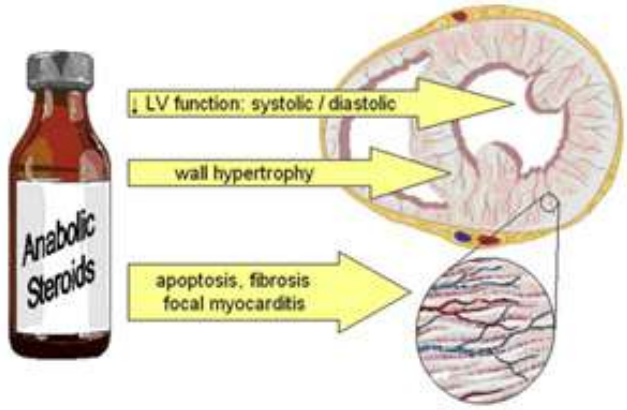

Figure 3: Thickened Fibres and Mild Disarray, with Multiple Neutrophilic Infiltrate (H&E)

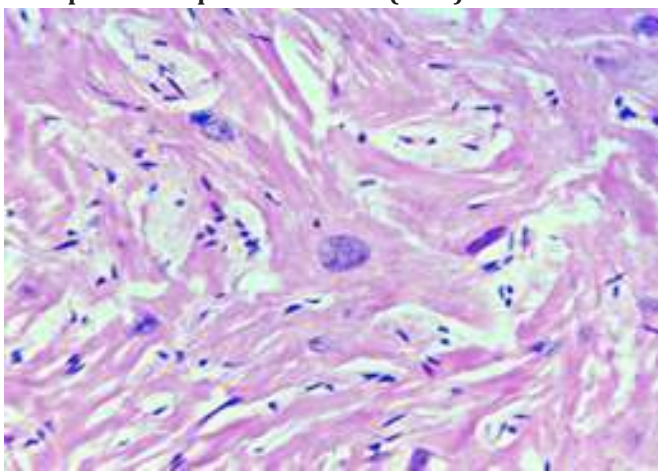

Figure 4: Thickness of Left Ventricular Wall (Yellow Arrow)

50 % luminal narrowing.

- Left anterior descending artery showing athero-sclerosis with 70 % luminal narrowing with medial calcific sclerosis.

Toxicological Examination:

The routine viscera were collected and sent to forensic science laboratory and were found negative for poisonous agents and alcohol.

DISCUSSION

Anabolic androgenic steroid are a significant class of synthetic testosterone derivatives formulated to increase the anabolic effects while reducing the androgenic effect. The primary target tissue for the anabolic effect of AASs is skeletal muscle. Anabolic steroids regulate the transcription of target genes that control the accumulation of DNA in skeletal muscle required for muscle growth. Anabolic steroids also upregulate and increase the number of androgen receptors, thus enabling increased training intensity and indirectly contributing to increased muscle size and strength in body builders.[9] AASs can be administered orally, intramuscularly, or transdermally. Among Independent AASs users and vigorously trained young athletes left ventricular hypertrophy (Athlete heart) was detected. Prolonged strength training, such as power lifting and bodybuilding, may lead to anatomical changes in the left ventricle, including increased wall thickness and LV mass.[5-6] Bodybuilders perform activities that rise stroke volume and cardiac output more than other athletes do, so these changes are more obvious in bodybuilders

than in weightlifters. In comparison with normally active individuals, weightlifters had considerably greater LV end-diastolic volume, size, and posterior wall thickness.[7] Growth hormone is essential for the physiological functioning of heart and when there is congenital growth hormone deficiency or in case of long-term usage of growth hormone leads to cardiac hypertrophy and cardiac dysfunction.[10] The LV mass index was increased in weightlifters, suggesting changes in LV shape. Contrarily, other results show that resistance training does not significantly alter the anatomy of the LV.

CONCLUSION

Our case provides evidence for the possibility of intense weight training, anabolic steroid usage and androgen sensitivity in these young men predisposed to myocardial damage and resulted in sudden cardiac death. Despite legislation to limit the empirical prescription and dispensing of these agents, these medications continue to be misused by athletes and bodybuilders. Additionally, the user must understand that the psychoactive effects of anabolic steroids can be deadly, resulting in anger, suicidal thoughts, rage, and extreme violence. We also recommend increased monitoring for AAS-related deaths that can go unnoticed and unreported in the medical literature.

Funding/Conflict of Interest: None declared.

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Case Report

Dental Perspective in the Assessment of Grievousness of Dental Injury and Age Estimation in a Sexual Assault Victim: A Case Report

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ABSTRACT :

Introduction: Forensic odontology is a branch which deals with proper handling and examination of dental evidence, and the proper evaluation and presentation of dental findings. It uses the skills of a dentist in personal identification during mass calamities, sexual assault and child abuse to name a few. The need to estimate the age of living individuals is becoming more frequent because of increasing number of crime rates by and against minors, increase in the number of immigrants without acceptable identification documents and the falsification of data to be prevented from being sentenced to a punishment. Teeth are bestowed with features of hardness and resilience against external factors such as chemicals, putrefaction and fire explosives making them a durable source of age determination. Dental age estimation has proven to be equivalent to the skeletal methods of age estimation. Further dentist's play a significant role in assessing the grievousness of the injuries sustained to the oral cavity. Hence it become a dentist's moral responsibility to act effectively in legal matters of such nature. Here we present a case suspected of sexual assault where age estimation and assessment of grievousness of injuries faced by the victim are evaluated.

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Keywords: Case Report, Demirijian's Method, Dental Age Estimation, Forensic Odontology, Schour And Massler's Method.

INTRODUCTION

Age estimation forms an integral part in human identification and plays a pivotal role in legal matters. The teeth are the most indestructible parts of the body and exhibit the least turnover of natural structure hence proving to be an asset in age estimation.[1] The methods of age estimation are divided into three categories:

1. Morphological
2. Biochemical
3. Radiological [2]

These methods have been enlisted in **Table 1**.

The methods used in this particular case include the Abridged version of Demirjian's method, The Schour and Massler method and the Harris and Nortje method. Each of these methods have proven under various studies to be as effective as skeletal methods of age estimation and providing results close to the chronological age of the patient.

Dental injuries are the most common type of orofacial injuries. However, cases of physical and sexual assault are also associated with injuries to the bony structures of the facial skeleton, the temporomandibular joint and soft tissue injuries to the face.

Moreover, the dentist's need to categorize oral injuries in court is not unusual. It is imperative for the dentist to be aware of such conditions and educate themselves about the laws pertaining to the same. Section 320 of the Indian Penal Code categorizes grievous hurt wherein the seventh point includes "fracture or dislocation of a bone or tooth. This has been enlisted in **Figure 1**.

CASE REPORT

A male patient claiming to be 16 years of age as per his Aadhar card visited the institute accompanied by a police investigator. The patient visited Mumbai with an acquaintance from his native place in search of labor. The patient gave history of sexual and physical assault by a known person which involved the patient's head being pushed onto the floor. Two days later, the patient reported the same to a shopkeeper, who helped him lodge a police complaint. The patient was asked to undergo medical and dental examination and was referred to Government Dental Hospital, Mumbai, for confirmation of

age and categorization of grievousness of dental injuries. The patient was conscious and well oriented. No significant past medical history. No significant family history.

Intraoral Examination: 11 and 12 missing, 21 was extruded slightly from its socket and was tender on percussion **(Figure 2 and Figure 3)**. Tenderness was noted along the attached gingiva and alveolar mucosa with respect to 11, 12, 21. The marginal gingiva was inflamed and mildly erythematous in the same region. No signs of erythema, ulceration, or petechiae were noted on the palate or other regions of oral mucosa **(Figure 4)**. Ellis class I fracture was seen in 22 on the mesioincisal aspect. No signs of periodontal disease.


Figure 2


Figure 3

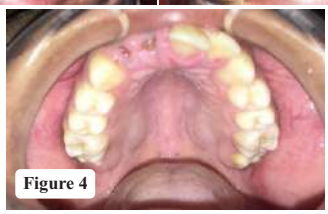


Figure 4

Dental Age Estimation Depending on Eruption Pattern of the Teeth: Complete eruption of the first and second bicuspids of all the four quadrants. Complete eruption of first and second molars of all four quadrants. Angle's Class I molar relation noted. The dental age of the patient based on the clinical findings was estimated to be approximately 13-14 years.

Digital Intraoral Periapical Radiograph with respect to 11, 12, 21 region showed empty tooth socket in relation to 11, 12 with the lamina dura being intact. The periapical area and surrounding bone appear normal. Periodontal ligament widening noted with respect to 21 **(Figure 5 and Figure 6)**.


Figure 5


Figure 6

Lateral Skull Radiograph showed completely erupted 46 with completely formed root apex, completely erupted 47 with the apex open and crown formation complete with respect to 48 with a well-defined follicular space around it **(Figure 7-8)**.


Figure 7


Figure 8

According to IPC section 320 (7), "fracture or dislocation of a bone or tooth" is considered as grievous offence. Hence, we have classified the nature of injury as grievous.

According to the chronology of eruption of teeth we can determine the dental age of the patient to be approximately 13-16 years.

The confirmation of the dental age has been carried out with the following three methods of age estimation which has proven to be apt for the desired age group and are the most commonly used methods.

Demirjian's Method (Abridged form DAECC)

The original Demirjian's method utilizes three tables at a particular time while estimating the age of a person.

The abridged form of Demirjian's method depicted tooth

Patient ID: _____ OPD number: _____
Gender: _____ Nationality: _____
Date of data collection: _____ Place of origin: _____
Dentition present: _____

Determination of score based on developmental stages of the tooth

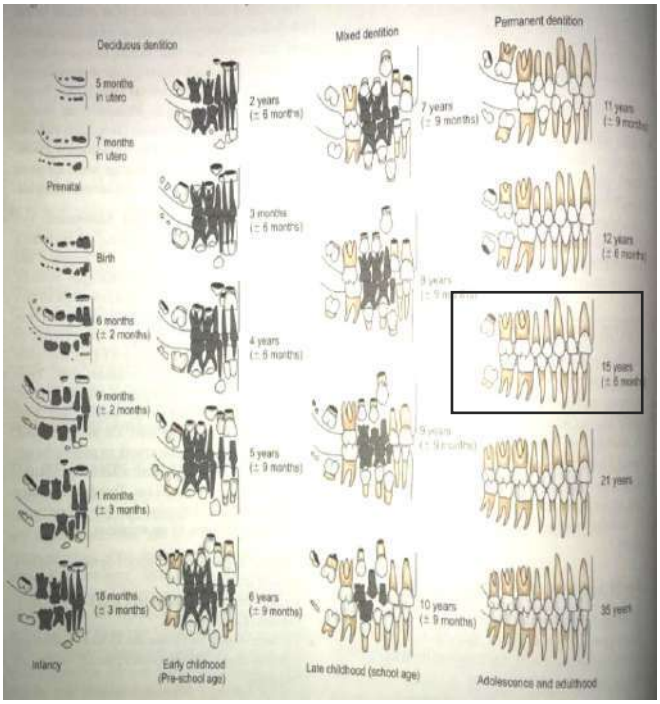
Tooth number	Developmental stages of the tooth							
	A	B	C	D	E	F	G	H
31								
CS: By/Gr				0.0/0.0	1.9/2.4	4.1/5.1	8.2/9.3	11.8/12.9
32								
CS: By/Gr			0.0/0.0	3.2/3.2	5.2/5.6	7.8/8.0	11.7/12.2	13.7/14.2
33								
CS: By/Gr			0.0/0.0	3.5/3.8	7.9/7.3	10.0/10.3	11.0/11.6	11.9/12.4
34								
CS: By/Gr		0.0/0.0	3.4/3.7	7.0/7.5	11.0/11.8	12.3/13.1	12.7/13.4	13.5/14.1
35								
CS: By/Gr	1.7/1.8	3.1/3.4	5.4/6.5	9.7/10.6	12.0/12.7	12.8/13.5	13.2/13.8	14.4/14.6
36								
CS: By/Gr			0.0/0.0	8.0/4.5	9.6/6.2	12.3/9.0	17.0/14.0	19.3/16.2
37								
CS: By/Gr	2.1/2.7	3.5/3.9	5.9/6.9	10.1/11.1	12.5/13.5	13.2/14.2	13.6/14.5	15.4/15.6

Total score

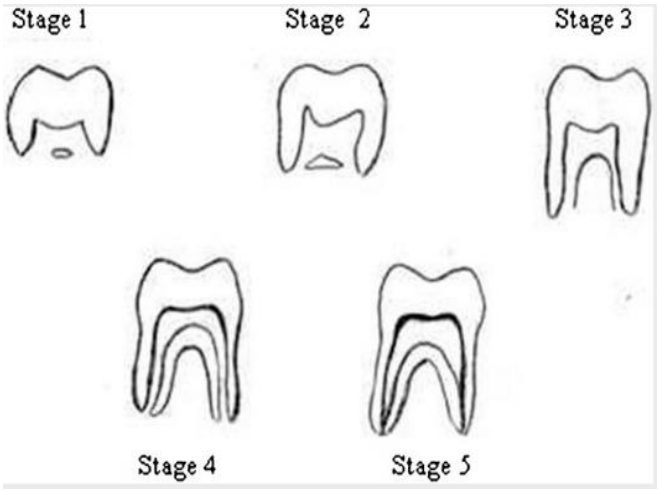
*Adapted from the scoring and calcification tables given by Demirjian.[21] OPD: Outpatient department

Table 1: Conversion of Maturity Score to Dental Age (7 Teeth): For males[3]

Age	Score	Age	Score	Age	Score	Age	Score
3.0	12.4	7.0	46.7	11.0	92.0	15.0	97.6
3.1	12.9	7.1	48.3	11.1	92.2	15.1	97.7
3.2	13.5	7.2	50.0	11.2	92.5	15.2	97.8
3.3	14.0	7.3	52.0	11.3	92.7	15.3	97.8
3.4	14.5	7.4	54.3	11.4	92.9	15.4	97.9
3.5	15.0	7.5	56.8	11.5	93.1	15.5	98.0
3.6	15.6	7.6	59.6	11.6	93.3		
3.7	16.2	7.7	62.5	11.7	93.5	15.7	98.2
3.8	17.0	7.8	66.0	11.8	93.7		
3.9	17.6	7.9	69.0	11.9	93.9	15.9	98.3
4.0	18.2	8.0	71.6	12.0	94	16.0	98.4
4.1	18.9	8.1	73.5	12.1	94.2		
4.2	19.7	8.2	75.1	12.2	94.4		
4.3	20.4	8.3	76.4	12.3	94.5		
4.4	21.0	8.4	77.7	12.4	94.6		
4.5	21.7	8.5	79.0	12.5	94.8		
4.6	22.4	8.6	80.2	12.6	95.0		
4.7	23.1	8.7	81.2	12.7	95.1		
4.8	23.8	8.8	82.0	12.8	95.2		
4.9	24.6	8.9	82.8	12.9	95.4		
5.0	25.4	9.0	83.6	13.0	95.6		
5.1	26.2	9.1	84.3	13.1	95.7		
5.2	27.0	9.2	85.0	13.2	95.8		
5.3	27.8	9.3	85.6	13.3	95.9		
5.4	28.6	9.4	86.2	13.4	96.0		
5.5	29.5	9.5	86.7	13.5	96.1		
5.6	30.3	9.6	87.2	13.6	96.2		
5.7	31.1	9.7	87.7	13.7	96.3		
5.8	31.8	9.8	88.2	13.8	96.4		
5.9	32.6	9.9	98.6	13.9	96.5		
6.0	33.6	10.0	89.0	14.0	96.6		
6.1	34.7	10.1	89.3	14.1	96.7		
6.2	35.8	10.2	89.7	14.2	96.8		
6.3	36.9	10.3	90.0	14.3	96.9		
6.4	38.0	10.4	90.3	14.4	97.0		
6.5	39.2	10.5	90.6	14.5	97.1		
6.6	40.6	10.6	91.0	14.6	97.2		
6.7	42.0	10.7	91.3	14.7	97.3		
6.8	43.6	10.8	91.6	14.8	97.4		
6.9	45.1	10.9	91.8	14.9	97.5		



number in FDI notation as well as all the eight developmental stages of each tooth, as given separately in the original tables. It included scanned copies of pictorial representation of developmental stages of all seven teeth under consideration. The scoring system of each developmental stage was also incorporated into DAECC for both boys and girls, the sum of



(Panchbhai AS. Dental radiographic indicators, a key to age estimation. Dento Maxillo Facial Radiology. 2011 May;40(4): 199-212. DOI: 10.1259/dmfr/19478385.)

which would give a final score at the end of the table.[4]

11.8 + 13.7 + 11.9 + 13.5 + 14.4 + 19.3 + 13.6 = 98.2

TOTAL SCORE: 98.2

Estimated Dental Age of the patient = 15.7 years.

Schour and Massler's Method describes 20 chronological stages of tooth development. The chart is based on histological sections and permits direct comparison with the radiographs.[5]

After the thorough analysis of the charts given my Schour and Massler, the estimated age of the patient according to this method would be 15 years +/- 6 months.

Harris and Nortje Method: The third molar corresponds to stage 1 of this method and the estimated dental age would be = 15.8+/-1.4 years On thorough clinical and radiological examination of the patient:

1. According to IPC section 320 the patient suffered grievous injury.
2. The patient's estimated dental age was 15+/- 1 year.

DISCUSSION

1. Age Estimation

Age assessment is required in circumstances such as, asylum seekers of unknown age, young people accused of criminal activities, and convicted criminals whose age is claimed to be <18 years before sentencing.[6]

A number of age estimation methods exist as have been enumerated in **Table 1**.

Over the years, many methods have been developed in order to asses which one predicts more accurately the age. **Demirjian's** method is the most widely used estimation method for dental age evaluation.[7]

The original Demirjian tables provide with a chart representing the calcification stages and separate scoring tables for boys and

girls, assigning a particular score to each calcification stage, the sum of which is then compared with the tables provided for DAE, separately for boys and girls.[8] however, this method proved to be time consuming.

The Abridged DAECC has been proven to be less time consuming and easy to interpret in the North Indian population.[4] hence, this method has been used in this particular case.

According to a study by Vivek rai et al, the Schour and Massler technique was found to be comparable and equally reliable as the skeletal age estimation methods.[9]

Further, the accuracy of the methods varies with the age group being considered. Hence, to reach a more accurate conclusion, keeping the sensitive nature of the case in mind, another method which involves the estimation of the dental age with the development of the third molar has been undertaken.

The dental age estimation by the Harris and Nortje method proved to be consistent with the other results.

The Cameriere's method is another widely used and accurate technique for age estimation. However, it involves the use of panoramic radiographs which were unavailable in this case and hence this method was not applied.

With the methods used, the dental age of the patient was estimated to be 15+/-1 year.

2. Assessment of Grievousness of Dental Injuries

Nationwide 81% of women and 43% of men reported some form of sexual harassment and/or assault in their lifetime.[10] A number of cases go unreported due to lack of knowledge amongst the victims and their families. Most sexual abuse offenders are acquainted with the victim as is seen in this case. According to reports, 52.4% of the offenders are acquainted with the victims.[10]

A large number of cases of physical or sexual assault report with injuries to the orofacial region, the most common being avulsion or fracture of the tooth as is seen in this case. Section 320 of the Indian Penal Code deals with the area of grievous hurt and has provided categories for the same.

CONCLUSION

Dental age estimation forms an integral part of forensic odontology. Numerous methods and studies have been put forward so far, but it is imperative to remember that each of these methods give an estimation of the age of the individual under consideration.

This case enlightens us about the prevalence of sexual assault on male victims and the seriousness of the crime.

The Parliament of India passed the 'Protection of Children Against Sexual Offences Bill (POCSO), 2011' regarding child sexual abuse on 22 May 2012, making it an Act.

Moreover, the prevalence of child labor due to poverty in India is significant. The very reason the victim in this case was coerced into leaving home was with the opportunity of labor in another city.

An amalgamation of government policies, public awareness and education can help prevent such crimes.

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A Case Report

Unconventional Suicide Notes at Scene of Crime: Case Report

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ABSTRACT

Introduction: Suicide notes are significant in forensic investigations as they can provide profound insights into the intellectual capacity of individuals who have taken their own lives. Those who experience suicidal ideation or tendencies tend to leave behind a variety of explanations for their actions, such as apologies to their loved ones, reasons for their decision, or as a final chapter of their life.

Results: This case study examines a real-time crime scene where suicide notes were discovered on the floor and wall using unconventional writing instruments such as blood and colored pencil respectively. This presented an additional challenge during the investigation and examination process. The handwriting on the floor and wall was compared with specimen handwriting samples from the suspect to determine authorship. Through examination helped in establishing the authorship for both the questioned (unconventional) and specimen (conventional) handwriting samples of the suspect.

Conclusion: This article highlights the challenges and issues encountered in crime scene investigation and handwriting analysis. The author asserts that a correlation can be established between unconventional (questioned) and conventional (admitted/specimen) handwriting samples, which can be utilized as evidence in a court of law.

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INTRODUCTION

Suicide notes serve as the last message of individuals who have attempted or committed suicide, providing valuable insight into their thought processes leading up to the act. Research suggests that the prevalence of the suicide notes ranges from 5% to 43% and are typically written on paper with a pen or pencil. However, Document Examiners face a greater challenge when encountering suicide notes written with unconventional writing instruments on surfaces such as walls, floors, skin, and other unexpected areas. Throughout history, there have been numerous cases in which suicide notes were found on surfaces including stomachs, palms, bedsheets, tables, balloons, doors, mirrors, and more, written by the victim just before the attempt.[1] Throughout the history of document examination, there have been numerous reported cases where handwriting has been discovered on unconventional surfaces using unconventional writing tools.

The initial instance of a suicide case in 1981 involved a suicide note inscribed on the stomach of the deceased. Despite the peculiar choice of writing surface, the distinctive characteristics of the handwriting, including its natural variations, remained relatively unchanged, thereby enabling identification and aiding in the determination of the authorship.[2] A research was carried out to investigate how the writing surfaces impact an individual's handwriting based on their position. The findings demonstrated that using abnormal substrates had a negative impact on the overall look and line quality of the author's writing, resulting in unevenness, abrupt interruptions, and indications of skipping by the writing tool.[3]

The authors reported two cases of death where suicide notes were discovered on the bodies of the deceased. They suggest that documenting similar cases could provide an opportunity to compare the handwriting characteristics of these cases.[4]

It was reported that there was a case of mass suicide, and the authors of the report discussed the distinguishing features of wall writings in comparison to ordinary writings. The authors also emphasized the significance of documenting observations about the surface nature, writing instrument used, and photographic evidence of such writings.[5]

Three more such cases were reported in which suicide notes were found on the palm of the deceased stating the reasons for committing suicide.[6]

The researchers carried out an investigation on a suicide note handwritten on the thigh of the deceased. They proposed that it is crucial to regularly practice these techniques and explore novel approaches for detecting writing on atypical surfaces.[7]

Several other studies also suggested that individuals who have used sharp metallic objects and henna (mehndi) to inscribe suicide notes on their body and palm area are indicative of suicide cases.[8,9]

A 22-year-old man was found dead in his prison cell after writing suicide note on white square bedsheet by using blue/black ballpoint pen.[10]

It is evident from existing literature that the presence of unusual suicide notes at a crime scene cannot be dismissed, and they frequently serve as crucial evidence during investi-gations. In this particular case study, handwritten notes were written using colored pencil and blood on the wall and floor of the scene respectively. These suicide notes were compared with specimen handwriting samples to verify the authorship. This study is aims to provide important insights for both crime scene investigators and document experts.

MATERIALS AND METHODS

This paper describes a case involving a 41-year-old man who attempted suicide after killing his 30-year-old wife. On thorough examination of the crime scene, handwritten notes were found in two separate locations, which were further examined to establish the authorship. One note was written on the floor using blood, where the woman's body was discovered, and a blood-smeared knife was found nearby. The other note was written on the wall of an adjacent room using a color pencil, where the male suspect was found injured. After an extensive search of the room, a colour pencil was found lying nearby the wall with a partially broken nib and traces of paint like substance adhering to the nib region. The unusual writing instruments and surfaces of the notes created significant challenges for documentation and examination at the crime scene. The handwriting on both the surface were observed to be partial in nature, with many incomplete letter formations. Additionally, photographing a note on a contrasting background was challenging due to issues with exposure, focus, color balance, and reflections.

Photography

The quality of writing, such as its alignment, letter formation, size, and overall appearance, is affected by the nature of the writing surface. If the writing is present on an immovable object, photography is the only way to preserve it for further examination. It is important to handle this process with care in order to avoid disturbing the surface and the handwritten notes. In this case, a Nikon DSLR camera was used to photograph the crime scene. The handwritten notes on the floor and wall were photographed at a perpendicular angle, with a scale placed beneath each stroke to ensure accurate measurement. A standard 30 cm scale was used for this purpose. In addition, the exposure, white balance, and ISO were properly adjusted to capture the handwritten notes on the yellow background of the wall.

RESULTS

The handwriting samples in question were analyzed using the principles of document examination to determine their authorship by comparing them to the specimen handwriting of the suspect. The examination focused on individual characteristics of letter formation, including natural variations. Specifically, letters "H", "A", "K", "M", and "i", which were written on a floor using blood and on a wall using a color pencil, were examined in detail. **(Figure 1-5)**

Figure 1: Comparison of Letter "H"

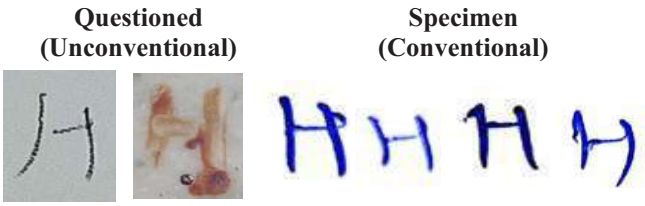


Figure 2: Comparison of Letter "A"

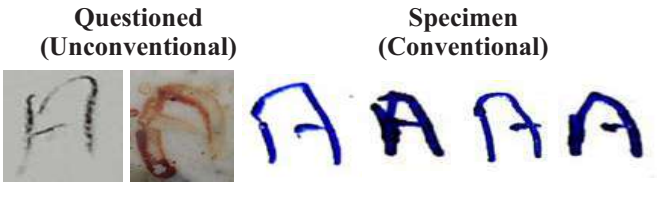


Figure 3: Comparison of Letter "K"

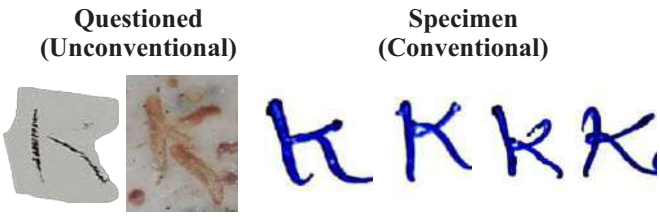


Figure 4: Comparison of Letter "M"

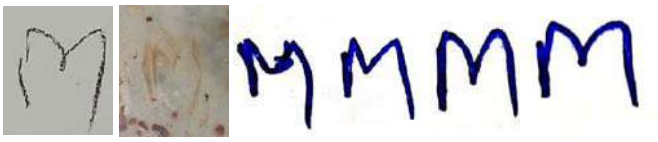
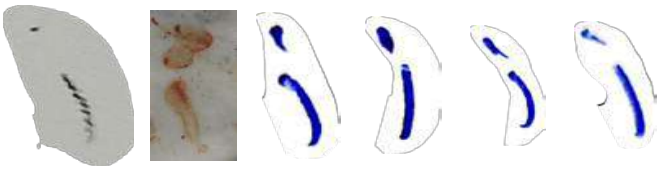


Figure 5: Comparison of Letter "i"



These letters were chosen based on their frequency, uniqueness, and ability to differentiate the author's handwriting. Various factors such as letter formation, slant, initial strokes, terminal strokes, size, and diacritics were taken into consideration during the examination and comparison. The selected letters exhibited significant handwriting features that were important for examination and comparison. After a thorough analysis, the following observations were made regarding the authorship of the handwriting samples.

Observations were made on the formation of letters "H," "A," "K," "M," and "i" on various surfaces, including walls, floors, and paper. The curvature and connection of strokes during the formation of these letters were found to be similar between the writing on the wall and paper surfaces. However, when examining the handwriting on the floor, which was written in blood, slight disturbances in letter formation were noticed. The slant of the selected letters in relation to their imaginary baseline was measured using a protractor. It was found that there were no significant differences in the slant between the wall and floor samples compared to the specimen samples. Additionally, the analysis of the initial and terminal strokes revealed that the writing on the wall and paper showed flying and tapered strokes in similar positions. In contrast, the handwriting on the floor showed blunt and hesitating initials and terminal strokes, as well as a blob of blood. This blob of blood should not be confused with shading commonly seen in regular writing when using a ballpoint pen. When examining the size of the selected letters, the writing on the floor using blood was observed to be proportionally larger than the writing on the wall using a colored pencil and the specimen writing sample. The diacritic "i" dot was also examined and found to be similar in terms of its shape and placement.

DISCUSSION

Suicide notes can provide valuable insights into the mens-rea of suicide victims as they are often written prior to the act itself. They also provide direct information about the victims, and as dying messages, can serve as a potentially valuable source of information about their psychological state at the time of the suicidal act.[11]

The use of psychological autopsy is complementary to medical autopsy in determining the motive behind a suicide. It is worth noting that in this context, suicide notes can be viewed as a crucial element of the psychological autopsy.[12]

The preceding case pertains to a unique suicide note created using blood on the floor and colored pencil on the wall. Previous records indicate that unconventional suicide notes have been discovered at crime scenes, including on the victim's skin. Notably, a recent incident involved handwriting on a wall in a regional language, drawn with charcoal. The case was resolved by analyzing distinctive characteristics of the handwriting.[13]

Examining similar cases can have a significant impact on the techniques used to analyze handwriting characteristics and features in order to establish and confirm the authorship of a note.

CONCLUSIONS

In conclusion, the case study of unconventional suicide notes at the scene of crime highlights the importance of proper crime scene investigation, photography and forensic analysis. In particular case, unconventional suicide notes was a challenge to photograph due to the differences in color contrast between the background surface and the writing instrument. At the same time when it comes to handwriting examination of such cases, forensic experts also face greater difficulties in examination. However, individualized handwriting characteristics can help to establish the authorship of an unconventional suicide note when compared to known handwriting samples. The case study demonstrates the successful use of manual methods for comparing an unconventional suicide note with specimen handwriting samples. In the future, digital methods may also be employed for similar studies. Ultimately, this case study emphasizes the need for continued innovation and

development in forensic analysis to ensure accurate and reliable results. By staying up to date with the latest technology and techniques, forensic experts can continue to make significant contributions to the justice system and help bring closure to victims and their families.

Conflict of Interest/Financial Support: None

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A Case Report

Sudden Death Due to Oesophageal Varice Rupture: Two Case Reports in An Alcoholic and A Non-Alcoholic Individual

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ABSTRACT

Of the gastrointestinal causes of sudden death, upper gastrointestinal bleeding is the most common and is usually due to the rupture of oesophageal varice, which is a complication of portal hypertension associated with cirrhosis of the liver. Here, we present two cases of sudden death due to acute upper gastrointestinal bleeding diagnosed at autopsy. One was a known alcoholic suffering from liver cirrhosis. In the other case, the individual was undergoing treatment for liver cirrhosis but was non-alcoholic with a history of consuming herb medication for a long time. Here, we highlight the importance of obtaining a detailed medical history to better correlate the autopsy findings with the disease condition.

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INTRODUCTION

The WHO defines sudden death as death within 24 hours from the onset of symptoms. 10% of sudden deaths are due to gastrointestinal causes.[1] Upper gastrointestinal bleeding is the most common among the various gastrointestinal causes of sudden death.[2]

Oesophageal variceal haemorrhage is a life-threatening portal hypertension complication associated with a mortality rate much higher than other gastrointestinal bleeding causes.[3] Also, patients with liver cirrhosis develop portal hypertension, putting them at a greater risk of developing oesophagal varices, and it has been found that over 80% of patients with liver cirrhosis risk developing oesophagal varices.[4] These case reports aim to emphasise the possibility of rupturing oesophagal varices in individuals with a known history of liver cirrhosis to prevent haemorrhagic shock from occurring, which is usually fatal.

Case 1: A 57-year-old, non-alcoholic male with a history of chronic liver disease and undergoing treatment for three years. He complained of uneasiness at home and died on the way to the hospital. An autopsy conducted revealed 2000ml of straw-coloured fluid in the peritoneal cavity, rupture of oesophageal varice at the lower end of the oesophagus **(Figure 1)**, the stomach contained about 400ml of blood and blood clots mixed with food particles, the entire small and large intestine **(Figure 2)** was filled with blood and blood clots amount to about 2000ml, the liver was shrunken and cirrhotic **(Figure 3)** with multiple nodules of varying sizes on the surface and cut section, the spleen was enlarged **(Figure 4)** weighing about 750gms. The cause of death was due to haemorrhagic shock due to rupture of oesophageal varice.

Case 2: A 40-year-old male, a known alcoholic, lived alone at home and was not responding to calls. The door was forced open, and he was found dead in a pool of blood, which brought on a suspicion of foul play. An autopsy revealed blood stains in the mouth and dried blood stains in the perianal region and the lower limbs. Internal findings revealed rupture of oesophageal varice at the oesophagogastric junction **(Figure 5)**, the stomach contained about 500ml of blood and blood clots **(Figure 6)**, the small and large intestine was filled with blood and blood clots **(Figure 7)** of about 2000ml, the liver was shrunken and cirrhotic with multiple nodules of varying sizes on the surface **(Figure 8)**, the spleen was enlarged weighing about 500gms. The cause of death was due to haemorrhagic shock due to rupture of oesophageal varice.

Figure 1: Haemorrhagic points at the lower end of the Oesophagus.

Figure 2: Blood and blood clots in the large intestine.

Figure 3: Liver cirrhosis.

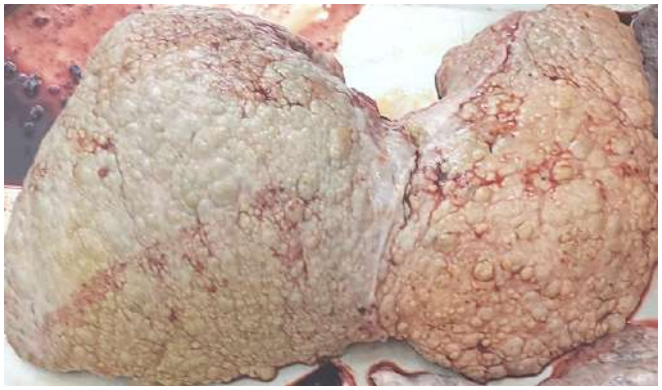
Figure 4: Splenomegaly.

Figure 5: Rupture of varice at the oesophagogastric junction.

Figure 6: Blood and blood clots in the stomach.

Figure 7: Blood and blood clots in the small intestine.

Figure 8: Liver cirrhosis.



DISCUSSION

Hemorrhage from oesophageal varices is a serious complication of cirrhosis with portal hypertension. Unfortunately, when it occurs, it is often the last of a long series of insults that damage the liver and lead to a fatal termination. The early diagnosis is difficult but must be suspected in any patientwith gastrointestinal haemorrhage, even melena.[5]

It is usually asymptomatic and is often diagnosed during autopsy.[6] Greater than half of the deaths associated with advanced cirrhosis result from variceal rupture.[1] The ruptured oesophagal varices are usually found in the lower third of the oesophagus or the oesophagogastric junction.[3]

Of these cases, one confirmed with the stereotypical type, i.e. a male, chronic alcoholic living alone with a history of liver disease, bloodstains at the death scene arousing suspicion of a violent death.[7-8] However, the other case was that of a non-alcoholic male with a history of chronic liver disease for which he was undergoing treatment. He had also undergone variceal band ligation during treatment. He was a known hypertensive and diabetic. Based on the history given by the next of kin, he was taking herb medication for a long time; however, it could not be confirmed whether that was the cause of liver disease as no detailed medical history was available. Some studies state that herb-related hepatotoxicity and liver injury can present as acute and chronic hepatitis, granulomatous hepatitis, severe cholestasis, sinusoidal obstruction, acute liver failure and progressing to cirrhosis and portal hypertension.[9] The type of herb-induced liver injury and knowledge of its natural course are important for treatment decisions and assessment.[9-10] Though liver cirrhosis is most commonly seen in alcoholics, a detailed medical history must confirm whether herb medications could also lead to this condition. As both cases were outpatient deaths, there was no detailed information about the clinical picture preceding death; therefore, conclusions were based mainly on autopsy findings, police reports and information from the relatives.

CONCLUSION

Oesophageal varice rupture is one of the common causes of sudden death in diseases related to the upper gastrointestinal system. Though commonly seen in alcoholics with a history of liver disease, it may occur in non-alcoholics with a history of consuming herb medications leading to cirrhosis; however, this should be confirmed by obtaining a detailed medical history.

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Glimpse of Annual Conference of Punjab Academy of Forensic Medicine & Toxicology

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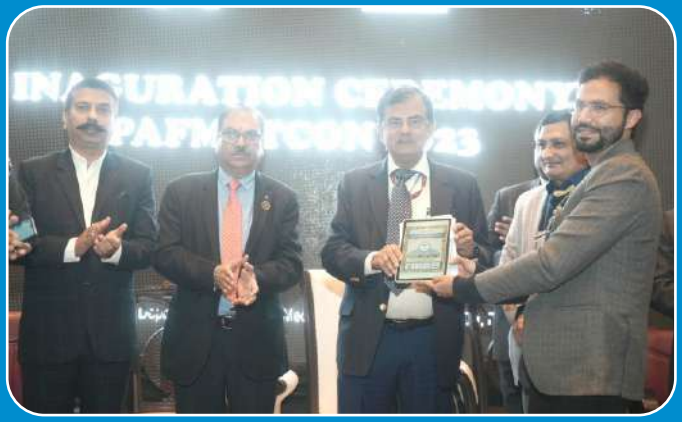

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