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Prof. (Dr.) Ajay Kumar

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From the Desk of Editor-in-Chief

It gives me immense pleasure to present this edition of our journal, which continues to serve as a platform for advancing knowledge, encouraging academic discourse, and fostering innovation in the field of Forensic Medicine and Toxicology. Each manuscript featured herein reflects the dedication, expertise, and curiosity of our contributors, addressing pressing issues and emerging trends that shape the practice and research of our discipline. As always, our commitment remains to ensure scientific rigor, ethical standards, and relevance to both academic and applied forensic settings.

In this issue, we bring together diverse articles that not only enhance our understanding of technical and investigative aspects but also highlight the social, legal, and public health implications of forensic science. It is my hope that these contributions will inspire further inquiry, collaboration, and practical application among our readers. I extend my sincere gratitude to our authors, reviewers, and editorial team for their invaluable efforts, and to our readership for their continued engagement and trust in our publication. My special thanks go to Joint Editor Dr. Harvinder Singh Chhabra and Assistant Editor Dr. Rattan Singh for their unwavering support and sincere efforts in the publication and release of this issue. Together, we move forward in our shared mission to expand the frontiers of forensic knowledge.

Thank you for being a part of this journey.

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Editorial

Artificial Intelligence and the Future of Research Proposal Writing in Forensics

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INTRODUCTION

In today's world, the Forensic Medicine and Toxicology stand at a crucial point where newer investigative technologies are growing more rapidly and in a sophisticated manner. Today, Artificial Intelligence have entered every walk of life, be it from diagnosis to treatment to research writing, algorithm generation etc. This is transforming not only laboratory procedures and analytical workflows but also the very process by which research proposals are conceived, structured, and refined. The integration of AI into concept proposal imagination to proposal writing offers immense promise—yet warrants thoughtful scrutiny, particularly in a healthcare discipline where rigour, accuracy, and ethical accountability are put up at the highest pedestal.

Key Components of a High-Quality Forensic Research Proposal: In forensics or any other branch of science, a research proposal is way more than an academic exercise; it is a concept which is developed by the researcher and he tries to help the society by improvising the research and proceed towards translational research. This thought of research helping the mankind with the help of Artificial Intelligence is growing by leaps and bounds. To achieve this goal and on this journey, the key components of research remains the same, which are:

• **Clear Title and Background:** A clear concept of Title and background of the study will have a direct bearing on the outcome of the study. AI will help in refining the title and the main goals of the Study. This will help in situating the study within the realm forensic practice, legal frameworks, or investigative needs.

• **Problem Statement:** The use of Artificial Intelligence can really identify the gaps in current forensic techniques, evidence interpretation, or system-level processes, thus giving the researcher a clear problem statement to be addressed for that Topic. It can be visa versa also, as problem statement given to AI can suggest the Aim and objectives of the study. The developed Aims and objectives needs to satisfy the needs of the population and population based determinants.

• **Research Questions/Objectives:** A good and properly framed research question has half work done towards the project. The research question should be Explicit, measurable, and relevant to forensic applications.

• **Literature Review:** With the help of AI the process of literature review can be done very fast, accurately, and with excellent command on current scientific, legal, and methodological developments. All these have a great impact on the quality of research undertaken.

• **Methodology:** Justifying design choices, sample types, sample size calculations , validation protocols, analytical instruments required to be used, and ethical considerations related to human subjects or sensitive data can be elaborately written and step by step methodology developed for the study.

• **Expected Outcomes and Impact:** AI in research writing can easily highlight implications for casework reliability, courtroom admissibility, or policy reform.

• **Timeline and Feasibility:** Ensuring that proposed forensic analyses can be conducted within realistic constraints and as per the suggested protocols of the proposal.

• **Budget and Resource Justification:** Addressing the costs associated with specialised equipment and validated workflows with budget justifications can be done easily with the help of AI.

AI can support many of these components, but cannot replace the discipline-specific insight essential for sound forensic inquiry. The use of AI has refined the art of proposal writing and has improved the outcome of the research proposals. These AI tools have become valuable assistants in the proposal development process, aiding forensic researchers in:

- Conceptualising study designs, such as validation frameworks for new analytical methods.
- Mapping literature trends, especially across rapidly evolving areas like digital forensics or forensic genomics.
- Refining the clarity, logic, and cohesion of a proposal's narrative.
- Ensuring alignment between research questions, methodological choices, and anticipated outcomes.
- Producing multiple drafts efficiently, allowing researchers to spend more time on scientific reasoning and less on linguistic polishing.

These advantages are significant, particularly in resource-limited forensic laboratories or academic programs with heavy casework demands. Yet AI should complement-not overshadow-the scientist's expertise, critique, and ethical responsibility. Specialised academic AI platforms such as PaperPal, Writefull, and Scite Assistant have quickly become valuable allies for forensic scholars. Unlike generic AI applications, these tools are designed with research writing conventions in mind and offer:

- Context-sensitive editing that respects scientific terminology and methodological descriptions.
- Structured templates for proposals, abstracts, ethics applications, and validation studies.
- Citation support rooted in academic sources rather than invented references.
- Higher reliability and fewer hallucinations compared to general-purpose models.
- Compliance checks for clarity, scientific tone, and journal-level expectations.

For authors in forensic science-where precision of language and methodological clarity are directly tied to evidentiary integrity-such specialised tools offer a measurable advantage.

The researcher needs to be cautious of the AI generated content and should invest more time in cross verifying the data generated by the AI software. Certain precautions are to be kept in mind when using Generic Gen-AI Tools (ChatGPT, Claude, Gemini) as it can result in Risk of Fabricated References like AI-generated articles, case studies, or forensic methods that do not exist. This fabricated reference can easily slip through if not carefully verified by the researcher. Sometimes there is risk of Oversimplification of Forensic Principles and Generic models may lack the nuance required to accurately capture complex forensic workflows, standards or legal considerations. The use of AI in research can result in Ethical and Academic Integrity Concerns. It may result in Uncritical acceptance of AI-generated text, and may violate institutional guidelines on plagiarism or undisclosed AI use. Uploading case details, sensitive datasets, or unpublished forensic techniques can compromise confidentiality and chain-of-custody expectations. AI suggestions may appear polished but lack scientific validity or fail to meet discipline-specific standards for validation and admissibility.

As such, forensic researchers should use general-purpose AI tools for brainstorming, draft refinement, or conceptual structuring-but the scientific content must always originate from the researcher. Now a days majority of the review articles are AI generated and the researcher has very little to contribute to the article. It has now become very difficult to check plagiarism of the review articles as humanised form of the AI generated articles are being submitted to the editors of the Journals for publication.

CONCLUSION: AI as a Partner, Not a Proxy

In forensics or any other branch of science, where the consequences of research ripple outward into new evidence, legal decisions, public trust, and investigative accuracy, the integration of AI into proposal writing must be both strategic and principled. AI offers speed, clarity, and organisational support, while purpose-built academic tools provide discipline-appropriate assistance. Yet the core of forensic research-scientific rigour, critical judgment, and ethical accountability has to remain inherently human. As the field embraces AI, the most compelling forensic research proposals will be those that harness AI technology thoughtfully, maintaining transparency and responsibly towards the society and the researcher. This will ensure that proposals and innovation strengthens, and not compromises the integrity of forensic science research.

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Original Research Paper

Rapid & Non-Invasive Screening of Cannabis in Human Hair and Nails Using Attenuated Total Reflectance FTIR: A Pilot Study

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ABSTRACT

Introduction: Cannabis is one of the most commonly abused substances globally. Rapid, non-invasive detection of cannabis use is crucial for screening purposes in forensic and workplace testing. Keratinized matrices like hair and nails serve as good samples for detecting past cannabis use. Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy is a fast, cost-effective, non-destructive, and less invasive method for detecting cannabis exposure from hair and nail samples. The primary objective of this study is to validate keratinized matrices as an effective medium for cannabis detection and to assess the detectability and reliability of ATR-FTIR. The study was conducted in Jhansi, India. Hair and nail samples were collected from 16 volunteers and analyzed alongside a standard cannabis reference sample. Distinct absorption peaks characteristic of major cannabinoids, such as tetrahydrocannabinol (THC) and cannabidiol (CBD), were consistently observed in samples from cannabis users, particularly in nail matrices. In contrast, these signals were absent in samples from non-users. These findings demonstrate that ATR-FTIR has the potential to be an effective method for detecting past cannabis use using hair and nails in resource-limited contexts.

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INTRODUCTION

Drug abuse or substance abuse refers to the inappropriate use of illegal drugs or prescribed medicine causing negative impact on health and daily functioning. Drug abuse is affecting over 190 million individuals globally, and the cases among those under age 30 are on a constant rise, necessitating urgent, youth-centered public health strategies and preventive interventions.[1-2]

Cannabis is one of the most commonly and widely used narcotic/deliriant substance over the world including India.[3] After alcohol and nicotine, Cannabis is the third most popular psychoactive substance.[4-6] According to National Drug Dependence Treatment Centre, All India Institute of Medical Sciences (AIIMS), New Delhi, there are around 31 million people abusing cannabis in India with at least 0.25% of Indians being cannabis dependent.[5]

The three main species of cannabis are Cannabis sativa, Cannabis indica, and Cannabis ruderalis. Its psychoactive resin, whether raw or refined, is known as cannabis resin.[7-9] The primary active constituents of cannabis are cannabinoids, among which Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN) are the most prominent.[4-5] THC causes euphoria and alters sensory perception.[10-11]

CBD, on the other hand, is non-intoxicating and has been extensively researched for its potential therapeutic benefits, which include anti-inflammatory, anxiolytic, and neuroprotective properties.[9,12] CBN, a breakdown product of THC, has minor euphoric properties and may cause relaxation.[13-14] The makeup and strength of these cannabinoids might differ based on the cannabis strain and extraction technique.[7]

1.1 Toxicokinetic Profile of Cannabis:

THC is largely metabolised in the liver by cytochrome P450 enzymes including CYP2C9, CYP2C19, and CYP3A4.[15-18] It is initially transformed into the active metabolite 11-hydroxy-THC (11-OH-THC), followed by the inactive 11-nor-9-carboxy-THC (THC-COOH), which is frequently tested for in drug screening or analysis.[15-17] There are approximately 100 distinct THC metabolites, the majority of which are hydroxylated or carboxylated, making them more water-soluble for elimination.[19]

The majority of THC and its metabolites are excreted as glucuronide conjugates in faeces (65-80%) and urine (20-25%). Because THC is very fat-soluble, it can get sequestered in fat tissues, resulting in a gradual release back into the circulation, extending its detectability in chronic abusers. The elimination half-life of THC-COOH in urine is around 30 to 60 hours, however, this can be prolonged in habitual cannabis users.[15-17,20]

Cannabinoids, particularly THC and its metabolites, are deposited into keratinised tissues such as hair and nails primarily via the bloodstream. This absorption into hair and nails occurs gradually and consistently, making these keratinised tissues dependable long-term indicators of prior cannabis usage. As hair and nails grow slowly, studying them gives a history of abuse spanning months, allowing forensic and clinical toxicologists to analyse drug exposure over long periods of time using less invasive, non-destructive and qualitative testing methods such as ATR-FTIR.[21-25]

MATERIALS AND METHODS

2.1 Place of Study: This cross-sectional study was conducted for a period of 2 weeks including the collection & analysis of the samples at the Innovation centre, Bundelkhand University, Jhansi, India.

2.2 Sample Collection: Hair and fingernail samples were collected from 16 volunteers aged 20–30 years in the Jhansi region of Uttar Pradesh, India. Among them, 15 self-identified as cannabis users, and one served as a control. All samples were obtained following strict ethical protocols, with informed and signed consent from each participant. A standard cannabis sample was also acquired for reference spectral comparison.

The hair samples were harvested as close as possible to the scalp with the help of sanitized scissors for avoiding external contamination. Fingernails were collected using a clean and sanitized nail clippers with proper care. Then each sample was placed into an individual Ziplock bags and labelled with proper and unique number. All the samples were collected by taking proper precautions to avoid contamination.

2.3 Sample Preparation: Samples underwent a standardized decontamination protocol to minimize external contamination. This involved sequential washing with mild detergent, acetone, methanol, and distilled water. Following air-drying at room temperature, specimens were finely sectioned using sterilized scissors or nail clippers.

2.4 Instrumental Specifications: The study was carried out using a Perkin Elmer SPECTRUM TWO FTIR spectrometer with a diamond ATR crystal for better sample contact. The instrument had a 4 cm^{-1} resolution and a scanning range of 4000-450 cm^{-1}, using a MIR TGS (Mid-Infrared, Triglycine Sulfate) detector. Data gathering and processing were handled using NIOS2 software.

The Perkin Elmer Spectrum Two FTIR was calibrated according to the manufacturer's instructions, and a background spectrum was recorded before analysing each batch to verify accuracy and avoid environmental interferences. The introduction of samples to the spectrometer is done by placing the biological matrices directly onto the ATR crystal. The analysis of each sample was repeated three times to ensure reproducibility, verify consistency and to improve accuracy.

2.5. Spectral Data Processing and Interpretation: Spectral data was analysed by applying baseline correction and standardisation using the software to eliminate background noise and standardize intensity. Key absorption peaks associated with cannabis-related chemicals were subsequently identified by comparing the processed spectra to conventional reference profiles.

Following the collection of spectral data, each biological sample was matched to a cannabis standard to find specific distinctive peaks. These included the O-H stretch around 3280 cm^{-1}, C-H aliphatic stretches between 2919 and 2850 cm^{-1}, aromatic C=C stretch near 1615 cm^{-1}, and C-O stretches between 1234 and 1039 cm^{-1}. These peaks relate to functional groups identified in cannabis compounds, including cannabinoids and their metabolites.

The existence and strength of these peaks were carefully examined to distinguish between samples from cannabis users and non-user. User samples generally showed clear and strong signals at these key frequencies, which corresponded closely to the cannabis reference spectrum. In contrast, non-user data exhibited weak or no signals in

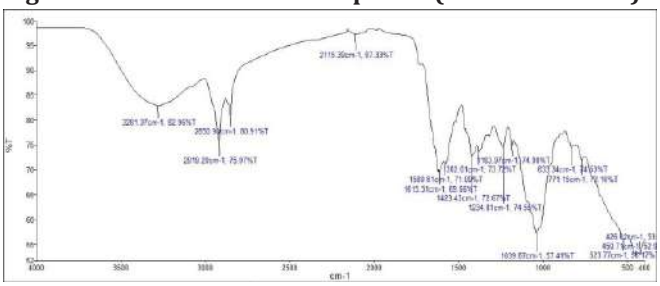
these areas.

RESULTS

3.1 Analysis of Standard Cannabis Sample: The standard cannabis sample was analysed with ATR-FTIR spectroscopy to create a baseline to determine key functional groups related to cannabinoids. The resultant spectrum revealed multiple different absorption peaks, each of which corresponded to typical chemical bonds seen in substances such as THC and CBD.

A significant O-H stretching vibration at 3281 cm^{-1} was observed, which typically associates with hydroxyl groups found in alcohols and phenols functional groups that are found in CBD **(Figure 1)**.[26]

Figure 1: Standard Cannabis Spectra (Positive Control)



The spectrum revealed prominent C-H stretching peaks within 2919 and 2850 cm^{-1}, suggesting the presence of aliphatic CH_2 and CH_3 groups, which are frequent in organic hydrocarbons and indicative of cannabinoid structures. A sharp peak near 1615 cm^{-1} was observed, suggesting aromatic C=C stretching. This indicates that there are of aromatic ring systems shared with both THC and CBD. Strong C-O stretching bands were detected within 1234 and 1039 cm^{-1}, indicating the existence of ether and alcohol connections, which are characteristic of cannabinoids.[18,27]

These clearly defined peaks acted as diagnostic markers for cannabis-related chemicals while also providing a valid spectral reference. This reference was then utilised to test against biological samples (hair and nails) for the presence of cannabis, enabling quick and non-invasive screening.

3.2 Hair Sample Analysis: Hair specimens collected from cannabis users showed obvious infrared absorption patterns that closely matched those of the reference cannabis spectrum. Most samples showed a clear C-O stretching peak within 1234 and 1236 cm^{-1}, indicating the presence of cannabinoids like THC and CBD. This peak, positioned in the fingerprint area of the spectrum, acted as an accurate chemical indicative of cannabis exposure.[28,29]

O-H stretching vibrations at 3280 cm^{-1} were frequently observed in user samples. These represent hydroxyl groups found in phenolic compounds such as cannabidiol (CBD). C-H aliphatic stretching peaks at 2920 cm^{-1} and 2850 cm^{-1} were prevalent, indicating the presence of alkyl chains, a hallmark component of cannabis.[29-31]

(Figure 2 & 3)

Figure 2: ATR-FTIR Sample Spectra of Hair Sample

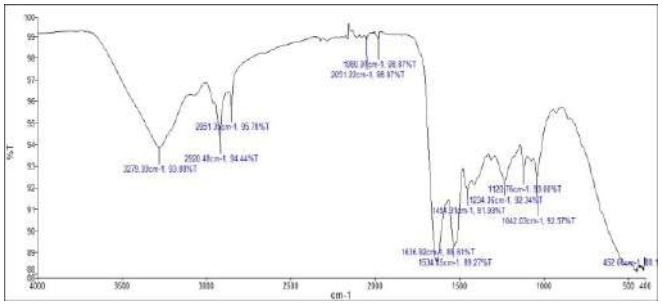
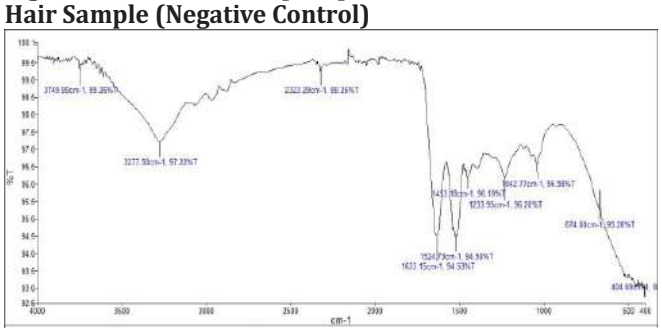


Figure 3: ATR-FTIR Sample Spectra of Non-User Hair Sample (Negative Control)



In particular, some samples, especially S4, S6, S9, S12, and S13; showed multiple well-resolved peaks in these locations, indicating the existence of cannabinoids. However, the non-user sample showed very feeble or non-existent signals at these critical points, demonstrating the protocol's capacity to distinguish between cannabis users and non-users. These findings corroborate the effectiveness of ATR-FTIR in detecting cannabinoid-related chemicals in hair and support its usage as a non-invasive screening method.

3.3 Nail Sample Analysis: Nail samples indicated substantial indications of cannabis usage. The majority of user nail specimens showed C-O stretching peaks at 1036-1043 cm^{-1} and 1235 cm^{-1}, indicating the presence of cannabinoids such as CBD and THC. O-H stretching vibrations (3280-3287 cm^{-1}) and C-H aliphatic stretches (2920 and 2850 cm^{-1}) were frequently observed. Certain user samples, including S5, S7, S8, S9, S11, and S13; showed exceptionally strong and well-defined peaks in these locations, closely matching the cannabis reference spectrum. **(Figure 3 & 4)**

Figure 4: ATR-FTIR Sample Spectra of Nail Sample

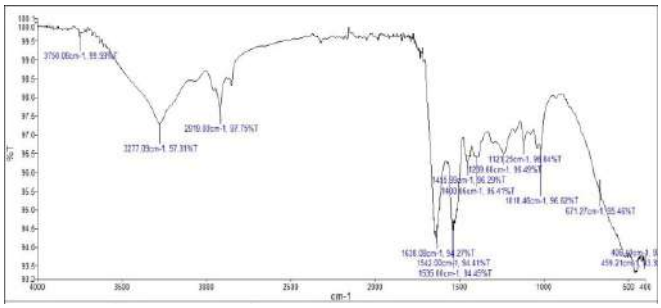
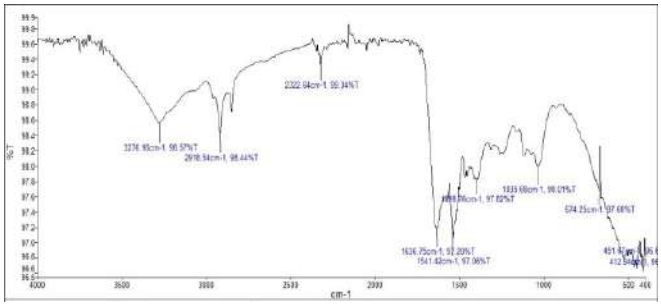


Figure 5: ATR-FTIR Sample Spectra of Non-User Nail Sample (Negative Control)



DISCUSSION

The non-invasive detection of cannabis in human hair and nails using ATR-FTIR spectroscopy is a highly valuable tool in forensic toxicology. The purpose of this work was to assess the efficiency of ATR-FTIR as a quick, cost-effective, and non-invasive approach for identifying cannabis metabolites in stable biological matrixes like hair and nails.

The findings are consistent with previous research like M. Cobo-Golpe 2025, indicating the use of hair and nails as dependable biomarkers for prolonged drug consumption.[25] The spectral analysis of cannabis metabolites, especially THC and CBD, demonstrated distinct absorption bands, which supported prior investigations on the use of ATR-FTIR in forensic investigations.[32]

Nails exhibited more distinct but consistent spectral features than hair samples, implying that nails could serve as a more resilient biological matrix for long-term cannabis exposure. The keratinised nature of nails is believed to limit external contamination and interference from cosmetic items, increasing their dependability for forensic and clinical testing.

The comparative observation from the results shows the indication of their unique wavelengths from IR spectral range enabling the identification of the components with the standard peaks which helps in the interpretation of the peaks differentiating between users and non-users' samples based on the presence and intensity of peaks in key spectral region. As the method is non-destructive in nature it preserved the integrity of the sample for potential re-analysis.

4.1 Spectral Analysis and Functional Groups: The ATR-FTIR spectra of the standard cannabis sample (SD) showed distinct peaks at 3281 cm^{-1} (O-H stretch), 2919-2850 cm^{-1} (C-H stretch), and 1234-1039 cm^{-1} (C-O stretch), as shown in the above **Table 1, 2**; which correspond to the functional groups of THC and CBD based on a study by Aubrey Barney, 2025.[33] These peaks were additionally observed in user specimens, indicating the existence of cannabis metabolites.

Particularly, nail samples showed greater and more consistent spectral correlations to the standard, indicating that nails may provide a more stable matrix for long-term detection than hair. Similar findings were reported by Alessandro Palmeri, 2000wherein thick keratin structure of nails served as a key biological matrix for retention of drugs in chronic abusers. [34] According to Palmeri and Cobo-Golpe; hair samples maybe effective, but their peak intensity varied, which can be attributed to extrinsic variables such as applications of cosmetics and environmental pollution.[35]

In the present study, hair samples S4, S6, S9, S12, and S13 had strong peaks, whereas others had weak signals. This discrepancy highlights the difficulties of hair analysis, particularly the impact of melanin content and hair

Table 1: Comparative Observation of the Recorded wave Numbers by Perkin Elmer ATR-FTIR Spectrometer.

Matrix	Key Wavenumbers (cm^{-1})	Functional Groups Identified	Cannabis Marker Compounds
Cannabis Standard	3281 cm^{-1}, 2919 cm^{-1}, 2850 cm^{-1}, 1615 cm^{-1}, 1234 cm^{-1}, 1039 cm^{-1}	O–H, C–H, C=C, C–O	THC, CBD
Hair (users)	3280 cm^{-1}, 2920 cm^{-1}, 2850 cm^{-1}, 1615 cm^{-1}, 1234 cm^{-1}, 1042 cm^{-1}	O–H, C–H, C=C, C–O	THC, CBD, Alkanes
Nails (users)	3287 cm^{-1}, 2920 cm^{-1}, 2850 cm^{-1}, 1615 cm^{-1}, 1235 cm^{-1}, 1043 cm^{-1}	O–H, C–H, C=C, C–O	THC, CBD, Alkanes

Table 2: Representation of the Characteristics and Functional Group Present in the Hair and Nail Samples of Users by Perkin Elmer ATR-FTIR Spectrometer.

S.No	Sample	Hair Matrix Wave Number cm^{-1}	Composition with Functional Group	Nail Matrix Wave Number cm^{-1}	Composition with Functional Group
1	S1	1236.53 cm-1	C-O Stretch (CBD & THC)	1235.12 cm-1	C-O Stretch (CBD & THC)
2	S2	2850.39 cm-1	C-H Stretch (Alkanes)	2920.11 cm-1	C-H Stretch (Alkanes)
3	S3	2919.52 cm-1	C-H Stretch (Alkanes)	1043.05 cm-1	C-O Stretch (CBD & THC)
4	S4	3280.11 cm-1 2850.42 cm-1	O-H; C-H Stretch (CBD & Alkanes)	2920.51 cm-1	C-H Stretch (CBD &Alkanes)
5	S5	1234.37 cm-1	C-O Stretch (CBD & THC)	2919.40 cm-1, 2850.68 cm-1	O-H; C-H Stretch (Alkanes)
6	S6	2920.54 cm-1 1233.77 cm-1	C-H; C-O Stretch (CBD, THC & Alkanes)	1040.83 cm-1	C-O Stretch (CBD & THC)
7	S7	1234.34 cm-1	C-O Stretch (CBD & THC)	3287.12 cm-1, 1038.43 cm-1	O-H; C-O Stretch (CBD & THC)
8	S8	1234.35 cm-1	C-O Stretch (CBD & THC)	3282.30 cm-1, 1038.18 cm-1	O-H; C-O Stretch (CBD & THC)
9	S9	3280.08 cm-1 1233.65 cm-1	O-H; C-O Stretch (CBD & THC)	3280.11 cm-1, 1036.51 cm-1	O-H; C-O Stretch (CBD & THC)
10	S10	2920.72 cm-1	C-H Stretch (Alkanes)	1041.77 cm-1	C-O Stretch (CBD & THC)
11	S11	1236.16 cm-1	C-O Stretch (CBD & THC)	2918.54 cm-1, 1035.68 cm-1	C-H & C-O Stretch (CBD, THC & Alkanes)
12	S12	2920.03 cm-1 1233.28 cm-1	C-H; C-O Stretch (CBD, THC & Alkanes)	1039.01 cm-1	C-O Stretch (CBD & THC)
13	S13	2920.48 cm-1 1234.36 cm-1	C-H; C-O Stretch (CBD, THC & Alkanes)	3281.32 cm-1, 1039.43 cm-1	O-H; C-O Stretch (CBD & THC)
14	S14	1042.28 cm-1	C-O Stretch (CBD & THC)	1040.41 cm-1	C-O Stretch (CBD & THC)
15	S15	2920.70 cm-1	C-H Stretch (Alkanes)	1038.99 cm-1	C-O Stretch (CBD & THC)
16	S16	3277.50 cm-1, 2323.29 cm-1, 1233.95 cm-1, 1042.77 cm-1	C-H; O=C=O; C-N Stretch & N- H bending; C-O, C-C Stretch (Alkyne & CO2, Amide III band; polysaccharides or glycoproteins)	3277.09 cm-1, 1542.00 cm-1, 1239.66 cm-1	C-H; C-F; N-H, C-N stretch (Alkyne; Fluorine compound; Amide II band; Amide III band)

hygiene.[25,34,36] In contrast, the nail samples (e.g., S5, S7-S9, S11, S13) showed more consistent and powerful peaks, which is consistent with previous studies that advice nails as a superior matrix due to their resilience to external contamination and longer detection window.[25]

The non-user sample (S16) had no significant spectral matches, demonstrating the method's discriminatory efficacy. This observation is pivotal for forensic applications because it demonstrates ATR-FTIR's capacity to distinguish between users and non-users without disruptive sampling.[37]

Aubrey Barney, 2025 was also stated that ATR-FTIR spectroscopy has various benefits over conventional approaches such as GC-MS and LC-MS/MS. The approach also needs little sample preparation, which reduces analysis period and costs, a key advantage in resource-constrained environments.[33,38-40] Furthermore, ATR-FTIR's surface sensitivity makes it perfect for identifying trace metabolites in keratinised matrixes.[33,39,40]

4.2 Implications for Forensic and Public Health Applications: The study's findings have significant implications for legal investigations and public health monitoring. According to Kuwayama, 2022 and Usman, 2019; segmental analysis of hair and nails could provide a drug use timeline, which could help with criminal investigations or workplace inspection.[35,41] The method's non-invasive nature makes it more convenient to use.[42]

Furthermore, detecting long-term cannabis usage aids attempts to combat substance abuse epidemics. In India, where cannabis use is culturally accepted but officially

prohibited,[4] Vandelli PF states that ATR-FTIR might improve monitoring and treatment programs.[43]

4.2 Limitations

Despite the strengths of this study, certain limitations were encountered that may have a profound influence on this study like lack of quantitative data, confirmatory analytical techniques, threat of external contamination from chronic use of shampoo, cosmetics and environmental pollution, limitations of the technique in identifying the bulk material composition of the metabolites and lack of comparative biomatrix validation.

4.3 Future Directions

Future research in this area should focus on the comparative analysis between other controlled substances. Further research over a large sample size can aid in the standardization of the methods for easy analysis. Automation, machine learning integration, and portable devices may make this method more suitable for on-site forensic investigations and routine screenings.

CONCLUSION

ATR-FTIR spectroscopy has proven to be a viable and non-invasive method for identifying cannabis usage in human hair and nails. This approach identifies users from non-users by comparing major cannabinoids' spectral signatures, particularly THC and CBD, with nails displaying greater resolution and fewer external interferences than hair. This emphasises the use of nails as a stable, long-term matrix for drug detection.

The approach provides quick, cost-effective, and non-invasive analysis suitable for forensic screening, especially in resource-constrained environments. As cannabis usage increases, particularly among young individuals, ATR-FTIR can play an important role in public health evaluations, criminal investigations, and routine occupational drug tests.

Conflict of interest/Financial: Nill

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Original Research Paper

Standardizing Postmortem Coronary Angiography in Routine Autopsies Using Barium Contrast Agent

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ABSTRACT

Introduction: Coronary artery disease remains one of the leading causes of death globally, with the assessment of coronary narrowing being a particularly challenging and meticulous task. To address this, we conducted a coronary angiography study on 55 subjects using a barium sulfate and water mixture, forming a paste-like consistency ideal for radiographic imaging. After administering the barium contrast agent, we took detailed radiographs of the subjects' hearts. These radiographs allowed us to meticulously examine the coronary arteries for any narrowing or abnormalities in their pathways. Our goal was to enhance the detection and understanding of coronary artery disease during routine autopsies by providing clearer and more precise imaging. When comparing radiological findings with histopathological results using Hematoxylin and Eosin stain, stenosis was observed in 31 samples. Histologically, 26 samples showed coronary stenosis, while radiological examination detected stenosis in 11 samples. Agreement in estimating stenosis severity occurred in 13% of cases, with radiography underestimating in 71% and overestimating in 16% of cases. This method is effective for detecting clear cases of stenosis and is both user-friendly and time-efficient. This simple method, usable even in remote areas with basic X-ray equipment, significantly improves the accuracy and efficiency of postmortem coronary assessments, providing valuable insights into coronary pathologies.

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INTRODUCTION

Accessing the coronary artery system and evaluating its patency and anatomy during autopsies is notoriously challenging. Meticulous examination is crucial for understanding the heart's coronary system's anatomical, physiological, and pathological structures. Sudden cardiac death, defined as death from cardiovascular causes within an hour of symptom onset, is predominantly caused by coronary artery disease, which accounts for nearly 80% of these cases.[1-2] Two widely recognized methods for artery dissection are longitudinal sectioning and serial transverse sectioning. However, each has its limitations: longitudinal sectioning can dislodge thrombi, potentially missing critical diagnoses, while serial transverse sectioning is often ineffective for examining calcified vessels.[3,4] Monroe J.

Schlesinger pioneered using radiographic techniques to study the coronary system. He injected lead agar into the coronary arteries and performed X-rays on sectioned, flattened hearts, providing detailed visualization of the arteries' status and distribution.[5] Dr. Schlesinger encountered occupational hazards due to excessive lead exposure, resulting in lead toxicity. Following his death, researchers introduced a safer alternative using barium

agar dye, replacing the previous lead-based formula.[6] Similar to other techniques, postmortem coronary angiography has a learning curve and can be challenging without proper guidance and resources. Initially, there was a recommendation to use a mixture and pressure gauge for injecting contrast agents into the coronary arteries.

However, given the limited supplies and infrastructure in some locations, there is a need for a simpler and alternative method that requires minimal infrastructure. This method is crucial in scenarios where organ mutilation is avoidable and prompt reporting is essential. Our study aims to capture coronary artery images using postmortem coronary angiography in a cost-effective and time-efficient manner. These findings were then compared with those from gross and histopathological techniques to affirm the method's feasibility and reliability.

MATERIAL AND METHODS

The study was conducted during routine medico-legal autopsies, with consent obtained from next of kin or relatives. We examined 55 hearts from subjects free of known cardiac pathologies. Hearts showing cardiac rupture, traumatic injury, or structural heart diseases like congenital defects or patent proximal coronary artery ostium were excluded. Subjects had an average age of 37.5 years, ranging from 15 to 82 years. The study was conducted after prior approval from the Institutional Ethics Committee, All India Institute of Medical Sciences, Jodhpur, Rajasthan, bearing Certificate reference Number: AIIMS/IEC/2018/804.

The heart was dissected from the lungs and major vessels using standard procedures, followed by a thorough gross inspection.[7] The heart chambers were gently flushed with water to clear out blood and postmortem clots, ensuring the flow direction mimicked normal blood circulation. Following this, the heart's weight was measured, and the positions of the coronary ostia were identified. To facilitate access, the aorta was trimmed approximately 2 to 3 cm above the ostia.

The patency of the proximal coronary ostia was assessed. If any coronary obstruction was found, attempts were made to cannulate the arteries using intravenous cannulas of varying sizes. Specimens where obstruction persisted despite cannulation were excluded from the study. Hearts that couldn't undergo immediate radiography were kept in a saline bath at room temperature until X-rays could be conducted.

Preparation of Catheters: Intravenous catheters of different sizes were selected based on the coronary artery lumen diameter, with a preference for the 16-gauge catheter. The tip of each catheter was heated to create a slightly wider lip, ensuring secure fixation within the coronary artery ostium.

Preparation of the Contrast Agent: Microbar HD, containing Barium Sulphate powder, was utilised. The powder was diluted with water to achieve a paste-like consistency of 3 grams per millilitre.

Cannulation: Two syringes were filled with the Barium Sulphate paste and then connected to the intravenous catheters. The contrast media was injected carefully until it flowed from the catheter tips, ensuring no air bubbles were present in the syringes or catheters. Fat and pericardial tissue around the coronary arteries, near the ostia, were gently removed using blunt dissection, avoiding damage to the arterial walls. This allowed easy retraction of the tissues when tying the string around the proximal coronary arteries.

The string was passed beneath each artery approximately 0.5 to 1 cm from the ostium using blunt dissection. The catheter tips were cautiously inserted into each coronary artery ostium individually. Once the catheters were advanced approximately 1 to 2 cm, the strings were securely tied around the coronary arteries to secure their position. The contrast agent was then injected slowly into the coronary lumens until resistance was felt. Subsequently, the syringes were removed, and the cannula caps were tightened to prevent leakage of the contrast agent and facilitate the preparation and preservation of the specimen.

Radiograph: The heart, with attached intravenous cannulas, was positioned on the specimen table in its anatomical orientation, ensuring the cannulas did not obstruct the X-ray view. Two plain X-rays were taken at 40W for 4 milliseconds each: one in the anatomical position and another after rotating the heart backwards. These radiographs were recorded and subsequently analysed for observed findings. The assessment of coronary artery narrowing involved capturing DICOM images of the heart through radiography **(Figure 1-4)**, followed by analysing these images using specialised software.

Stenosis calculations were performed by comparing the diameter of the most narrowed segment of the coronary artery to the average diameters of normal segments immediately above and below the stenotic area. This

method allowed for the calculation of the percentage of stenosis observed in the coronary arteries.

Figure 1: Extravasation of contrast agent near the Ostia's / orifices of the coronaries.

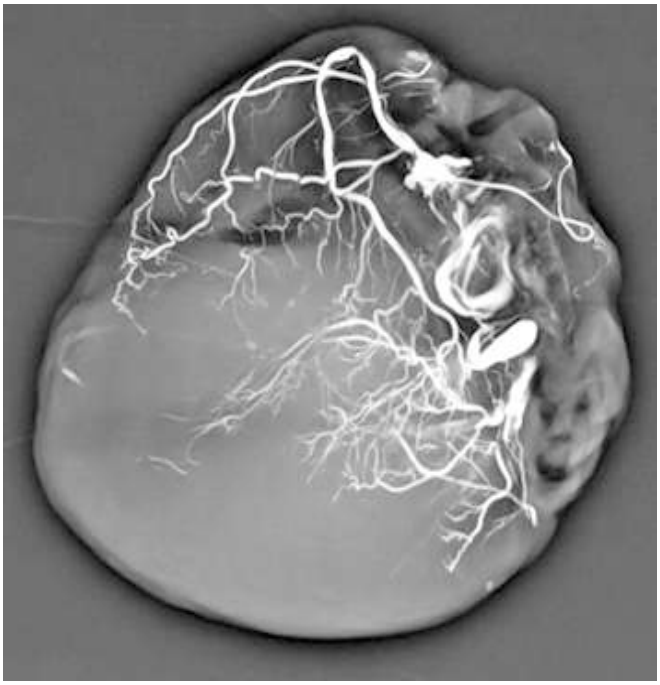


Figure 2: Proper instillation of contrast agent using fixed cannulas along with caps in all the arteries.

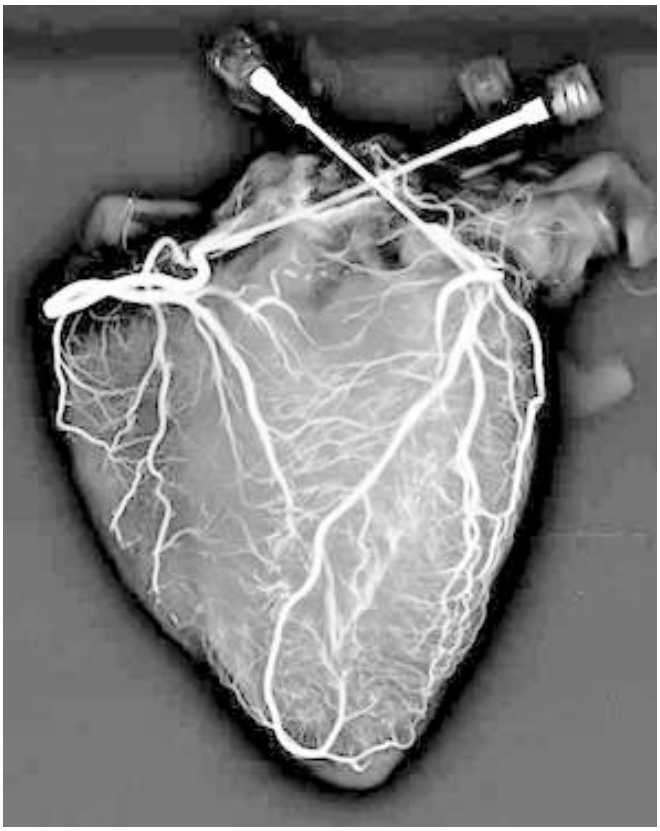


Figure 3: Extravasation of contrast agent into the surrounding tissues.

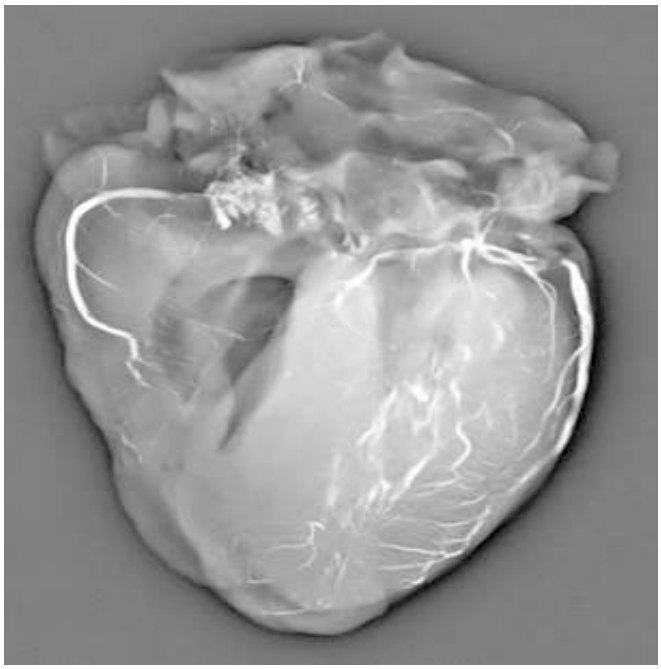
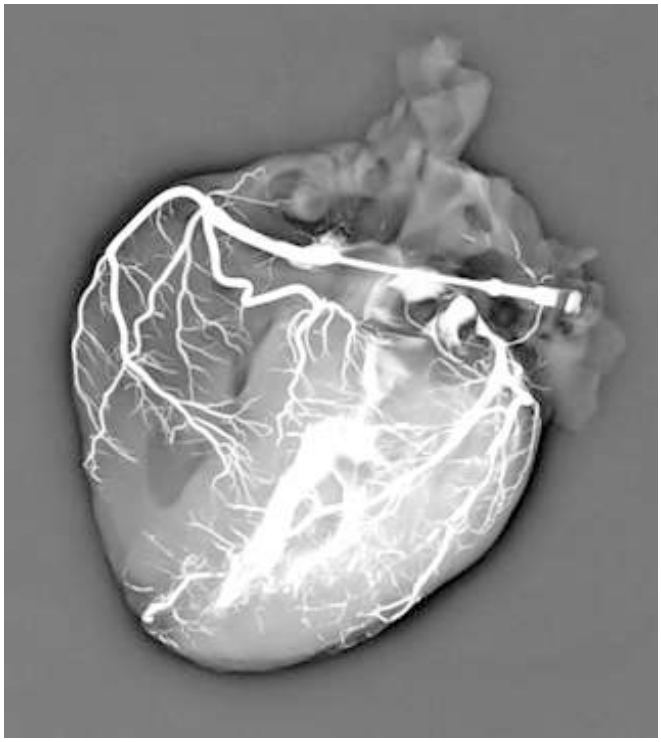


Figure 4: Extravasation of contrast agent forming patches.



Gross Examination: Following the X-ray procedure, the gross dissection of the coronary vessels proceeded systematically along their paths, with sections taken at 3 mm intervals until the vessels extended deep into the myocardium. Observations and findings were recorded accordingly.

Histopathological Examination: Areas showing any narrowing or stenosis observed on radiography were preserved in formalin. These formalin-fixed samples underwent histopathological examination using routine stains like Hematoxylin & Eosin and Elastic Verhoeff-van Gieson.

Scoring: The obtained samples were categorised according to the scoring system established by Oberman et al. (1974):[8]

Grade 1 = Less than 50% obstruction.

Grade 2 = 50% to 70% obstruction.

Grade 3 = More than 70% obstruction.

Grade 4 = Complete obstruction.

The threshold of 70% obstruction has been identified as critical and is often referred to as the "gold standard" for clinical significance.[9-11] The scoring system is uniformly applied across all methods to facilitate straightforward interpretation of the results. The findings were compared based on the number of cases showing clinically significant changes using any of the described methods. Radiological examination findings were then compared with those obtained through routine autopsy methods, namely gross dissection and histopathology.

RESULTS

Samples examined using various techniques demonstrated an increased prevalence of coronary stenosis, correlating with age. Even the younger population exhibited changes associated with coronary atherosclerosis, particularly prominent in histopathological examinations.

In our study, we analysed a total of 165 samples, each yielding distinct findings based on the techniques employed. Radiographic findings were correlated with those obtained from gross dissection and histopathological examinations, detailed as follows.

When comparing radiological findings with those from gross dissection, we identified stenosis in a total of 26 samples. Grossly, changes related to coronary stenosis were observed in 16 samples, whereas radiological examination revealed stenosis in 11 samples. The agreement in estimating the degree of coronary stenosis or obstruction between both methods was found in 3.8% of the samples, underestimated by radiography in 57.7%, and overestimated in 38.5% of the samples.

When comparing radiological findings with histopathological findings using Hematoxylin and Eosin stain, we identified stenosis in a total of 31 samples. Histologically, changes related to coronary stenosis were observed in 26 samples, whereas radiological examination revealed stenosis in 11 samples. The agreement in estimating the degree of coronary stenosis or obstruction between both methods was found in 13% of the samples, underestimated by radiography in 71%, and overestimated in 16% of the samples.

The results are based on clinically significant findings, specifically identifying cases with more than 70% obstruction or stenosis. While the radiological technique demonstrated lower efficacy compared to routine methods overall, instances with clear findings showed positive results with postmortem coronary angiography, confirmed by routine methods.

DISCUSSION

Evaluating coronary arterial anatomy and patterns is crucial but challenging. Traditional methods like serial horizontal and longitudinal sectioning each have their limitations. Postmortem coronary angiography was introduced as a potential solution to overcome these difficulties.[3] It was assumed that postmortem coronary angiography could assist in assessing coronary obstructions and documenting the distribution and patency of coronary arteries, which could later serve as evidence in court for cases of sudden death.

Radiological studies could be invaluable for identifying pathological lesions that might be missed during a simple gross examination in a postmortem setting. Additionally, small collateral vessels, often overlooked during gross dissection, could be more accurately appreciated using this method.[12-13] Traditionally, radiological techniques in forensic practice have been primarily used for detecting fractures and bullet fragments.[14-16] However, the scope of postmortem angiography is extensive, encompassing the evaluation of congenital heart defects in neonatal fatalities by providing detailed views of vasculature and cardiac anomalies.[17-18]

Additionally, this method holds potential for use in epidemiological studies, broadening its forensic applications.[19] However, this technique does not offer functional information about blood flow direction and pressure. A final assessment should always be made following a complete dissection. During the procedure, we encountered several technical difficulties that required time and experience to resolve, ensuring more accurate contrast images of the coronary arteries. These challenges,

along with the methods we used to overcome them, are detailed below:

- Initially, attempted to use cardiac catheters, but found them unsuitable due to the difficulty of inserting them into the ostia and their collapsible nature. During the injection of the contrast agent, these catheters often dislodged from the ostia because of increased backpressure in the coronaries. This resulted in the spillage of the contrast agent, creating artefacts in the results and reducing the internal pressure in the coronaries **(Figure 1)**, thereby compromising the accuracy of the findings.

  We opted to use 16-gauge intravenous cannulas, which are widely available in healthcare centres. These cannulas offer several advantages: they do not collapse under normal conditions, are easily accessible, require no special training to use, and maintain their position within the ostia when secured properly. This stability helps prevent spillage caused by backpressure during the injection of the contrast agent. Additionally, the provided caps allow for control of any leakage. Their small size, ease of availability, and low cost make them an ideal choice for use in remote areas **(Figure 2)**.

- Using Barium Sulphate as a contrast agent has both advantages and disadvantages. One key advantage is its ability to flush out postmortem clots, reducing artefacts. Additionally, Barium Sulphate can penetrate smaller vessels and capillaries, making it suitable for micro-angiography and producing high-contrast images. The water-based solution does not interfere with subsequent gross dissection and histopathological examination, preserving the integrity of the samples for further analysis.[20] The drawbacks of using Barium Sulphate as a contrast agent include extravasation when dissolved in water, inability to visualise microcirculation when dissolved in gelatin or agar, and potential precipitation-induced artefacts **(Figure 3)**. However, these challenges can be managed and mitigated through the methodologies outlined earlier, along with practice and experience.[21] Other methods, such as casting and water-based dyes, have their own issues, including time-consuming procedures, limited availability, and difficulties in perfusing into the coronary arteries.

  Achieving the correct consistency of the contrast agent was crucial. Initially, the ratio of contrast agent to water was incorrect, leading to diffusion of the contrast agent into the heart walls during radiography, resulting in an opaque shadow over the entire heart and obscuring coronary structures. With continued practice, we refined our method to achieve a paste-like consistency of 3 grams per millilitre of Barium Sulphate powder in water. This consistency allowed us to retain the contrast agent within the coronary arteries without dispersion into the cardiac muscle, enabling clearer visualisation of the coronary structures.

- Despite numerous attempts, our main concerns included the risk of contrast agent backflow or extravasation, which sometimes led to the formation of small contrast patches that could act as artefacts **(Figure 4)**. Some authors have employed differently colored dyes to distinguish between the pathways of the right and left coronary arteries, but this approach demands additional preparation and resources to establish separate standards for each dye.[5] In our study, we opted for a single contrast agent and simplified the assessment of its pathway within the coronary arteries.

  The findings from the obtained samples were individually assessed. Postmortem coronary angiography revealed significant obstruction in four cases, with corresponding findings observed in these cases using histopathological examination. However, in some instances where histopathological methods identified significant findings, radiological examination did not detect notable changes. Overall, angiography findings correlated well with histological findings. Determining the precise location of occlusion or stenosis proves challenging during gross examination and can be easily overlooked during dissection, highlighting the value of angiography in identifying these issues, which are subsequently confirmed by histopathological analysis.

- Postmortem coronary angiography serves primarily for four types of investigations: detailed analysis of vascular patterns and anatomical variations specific to organs, observations of pathological and physiological changes, examination of alterations resulting from unnatural causes, and experimental testing of new contrast agents.[22]

The study employed postmortem coronary angiography to investigate findings related to coronary arteries, utilising Barium Sulphate as a contrast agent in water and a 16-gauge cannula. A learning curve was necessary to become

proficient in this method. During the initial stages, mastering the correct application of the contrast agent was a gradual learning process. Similarly, various cannulation techniques were experimented with, and conclusions were drawn after several trial-and-error attempts.

In our study employing postmortem coronary angiography, results from samples were compared with those obtained through gross dissection and histopathological examination. Cases showing abnormalities on angiography were further examined and found to exhibit significant changes upon closer inspection. Thus, while not providing definitive evidence, angiography can be valuable in suggesting potential findings of coronary stenosis. Other methods remain equally crucial, as there is a significant risk of overlooking findings associated with coronary pathways during gross and histopathological examinations. These methods may miss a comprehensive assessment of the entire coronary pathway, making it a challenging task to trace accurately.

Numerous techniques have been developed for performing postmortem coronary angiography, primarily involving the injection of semisolid media, either alone or in combination with substances such as barium sulphate or lead oxide.[4,23-24] However, these techniques necessitate pressure regulators, extensive preparation time, and specialised expertise, limiting their availability in many postmortem institutions. In 1987, Schultz TC introduced a method using barium sulfate that eliminates the need for pressure regulators and semisolid injection media.[21] Our aim is not only to integrate this method into routine practice but also to establish a simple, cost-effective, and accessible approach that requires minimal time and effort. This method should be applicable in suspected cases to provide conclusive evidence. Nevertheless, it's important to note that radiological techniques cannot replace standard autopsy procedures.

CONCLUSION

Our approach's innovation lies in its simplicity and cost-effectiveness, utilising readily available items even in small hospital settings. We advocate for the adoption of radiological techniques, especially in cases of suspected sudden death. This method could pave the way for comprehensive body angiography in the future, as gross examination alone cannot achieve detailed vascular examination without altering the surroundings.

Limitation

The lack of histopathological correlation and the study's comparatively small sample size are limitations that could limit how broadly the results can be applied. The quality of imaging facilities and operator skill, which can differ depending on the context, are key factors in postmortem coronary angiography accuracy. Image interpretation may also be impacted by technical artefacts, postmortem changes, and the lack of antemortem clinical data. To confirm and improve the diagnostic value of this approach, more research with standardised procedures, bigger sample sizes, and comparison with traditional autopsy results is needed.

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Original Research Paper

Analysis of the Relationship between Radiological Age Determination, Total Fusion Score Distribution, Age Distribution From X Rays of Knee and Pelvis

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ABSTRACT

Introduction: Determining age is crucial in various civil and criminal proceedings, such as identification, consent, criminal accountability, medical assessments, will validity, reaching adulthood, kidnapping, sexual assault, illegal abortion, sports, and more.[1] The human bones grow from various ossification centers, and through radiological examination of these epiphyseal centers, we can estimate an individual's approximate age.[2] In this study, we focused on the age group of 17 to 19 years, analyzing their knee and pelvic joints.

Aim and Objectives: To evaluate the determination of radiological age through the distribution of total fusion scores and the assessment of age from knee and pelvis images in relation to birth certificates for individuals aged 17 to 19 years using digital X-rays.

Materials And Methods: This observational cross section study was carried out at the Department of Forensic Medicine, Government medical college, Kota. It included 100 participants of both sexes who gave consent for the study and fulfilled the inclusion criteria.

Results: In this study, the participant pool was intentionally balanced with 50 male and 50 female subjects, ensuring equal representation. The statistical summary disclosed a mean age of 17.94 years. The standard deviation of 0.83 signifies a moderately close grouping of age values in relation to the mean, indicating limited variability.

Conclusion: The present study gives important evidence supporting the accuracy and utility of radiological age estimation using epiphyseal fusion scoring for knee joint and pelvis in Indian adolescents. Geographical boundaries, narrow age range, moderate sample size, and reliance on radiographs limit the generalizability and depth of the findings.

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INTRODUCTION

Estimation of age is one of the main tasks of a forensic practitioner, especially in third world countries, where many births take place in rural settings, without the benefit of the expert supervision of a trained obstetrician. Such births are poorly recorded or more often not recorded at all in terms of exact dates. Although there are several laws making birth registration mandatory, such as the Birth and Death Registration Act, 1969, many births still go unregistered.

Age estimation is also important in various civil and criminal proceedings, including identification, consent, criminal liability, clinical assessment, the validity of a will, reaching the age of majority, abduction, rape, criminal abortion, and athletics.[3-4] In the absence of proper documentation to determine a person's age, forensic experts must rely on population data related to growth and development. This data helps in the construction of a biological profile that reflects the developmental status of the individual whose age is unknown.

The profile is created through the analysis of ossification centers, dental assessment, and secondary sexual characteristics.[5] However, dental assessments and secondary sexual characteristics are less significant in age determination for individuals over 16 years, as the eruption of teeth is nearly complete by this age, and there

are no further developmental changes in secondary sexual characteristics.[6] In individuals older than 12 years, the most notable changes, in chronological order, are observed in the appearance and fusion of ossification centers in various bones. Currently, research is concentrating on developing multi-factorial methods for age estimation, where a combination of techniques can yield the most precise age estimate with minimal error.[7]

Numerous ossification centers exist within the bones. The emergence and fusion of certain centers in conjunction with others in the same bones provide a foundation for age estimation. The long bones of the lower limb are crucial for age assessment in both living individuals and skeletal remains.[8-9]

The bones of the human skeleton develop from various ossification centers. Ossification progresses from these centers until the bone is fully developed. These changes can be studied through radiological examination and this information can be used to determine age. Therefore, it is possible to determine the approximate age of a person by radiologically examining the bones until ossification is complete.[10] The age of appearance and fusion of the epiphyses of bones varies. Variation in the age of appearance and fusion of ossification is mainly due to various factors such as climate, heredity, race, nutrition, dietary habits, gender and socio-economic status of the population.[11-12]

Ossification is seen earlier in the tropical countries and in females. Medico-legally 18 years of age holds a special place such as this age is, age for marriage for girls, majority or adulthood (juvenile act), age of consent for sexual relationship (POCSO ACT), age for casting vote, age for legally driving a vehicle, consent for act which cause death or grievous hurt (Sec. 87 IPC).[13]

In the current study, we used the 17-19 year old age group as our study population. Although the hand and wrist regions have received the most attention in age estimation, radiological analysis of the epiphyses of long bones can overcome the shortcomings of non-adult skeletal collections and allow researchers to obtain contemporary information on epiphyseal union. The knee joint and pelvis represent an anatomical region that may also provide new information and can be used in combination with the above well-established techniques to maximize age estimation accuracy.[14]

In this study, the knee joint and pelvis were chosen for several reasons. It is an anatomical region that can be easily radiographed at low radiation doses, has no underlying anatomical structures. Last but not least, the knee joint provides information for three epiphysis simultaneously: the distal femur, proximal tibia, and proximal fibula. The pelvis provides information for the other three epiphysis: the iliac crest, ischial tuberosity, and head of the femur.

In 1964, a survey committee recommended to the Government of India that a regional study be conducted to address the problem of age determination.[1] The Indian population differs widely from Western populations in genetic, dietary, socioeconomic, and ethnic factors. While some studies have been conducted in the Indian population, there is little published literature for this age group (17–19 years) in the Rajasthan population.[15] The objective of this study is to determine the correlation of the lower end of the femur, the upper end of the tibia and fibula, and the pelvis to birth certificates using digital X-rays or skiagrams.

MATERIALS AND METHODS

This study was carried out at the Department of Forensic Medicine and Department of Radiology, Government medical college, Kota, after obtaining due clearance from research, ethical and review board of Government medical college, Kota. It was an observational, cross sectional study conducted on 100 -participants, equally distributed across males and female, in the age group of 17-19 years of age with reliable document of date of birth.

Inclusion Criteria:

- Subjects belonged to Rajasthan by origin.
- Only Subjects who had documentary evidence of age in the form of birth certificate issued by Nagar Nigam & competent authority and Birth certificate.
- Subjects who gave their consent for participation in the study.

Exclusion Criteria:

- Subjects with Severe malnutrition.
- Subjects with Chronic illness.
- Subjects with Endocrinal disorders.
- Subjects with deformities of limbs and pelvis.

Method of Collection of Data: After obtaining informed consent from the subjects satisfying the inclusion criteria, the general physical examination was conducted to access the health status and rule out any deformities to select the subjects after applying exclusion criteria.

Materials: Digital X ray machine, Data collecting

instrument, X-ray film, Lead marker, lead apron, 8 x 10 inches rigid cassette, View box, Weighing machine and height measuring scale, Performa

Sampling Method:

- Stratified Sampling based on age.
- Sample size – 100 (50 Boys+50 girls)

Method: After taking informed consent explaining the details of the study, the details pertaining to clinical, dental examination for age estimation was documented in the proforma. The participants were subjected to Digital X-ray examination of pelvis, and knee joints. The Radiographs were then studied by the radiologist who was blinded, for appearance and fusion of ossification centres.

Statistical Analysis: The data was tabulated in Microsoft Excel Worksheet and statistically analysed at 95% confidence limits. The collected qualitative data was expressed in groups, diagrams, proportion and percentages and analysed using SPSS version 16 software. The quantitative data was expressed in linear and standard deviation and analysed. Continuous data was expressed in form of proportion and percentages differences and it was analysed using chi square test. $P<0.05$ was considered significant.

RESULTS

Table 1: Age Distribution

Statistic	Value (Years)
Mean	17.94
Median	18.00
Minimum	17.00
Maximum	19.00
Standard Deviation	0.83

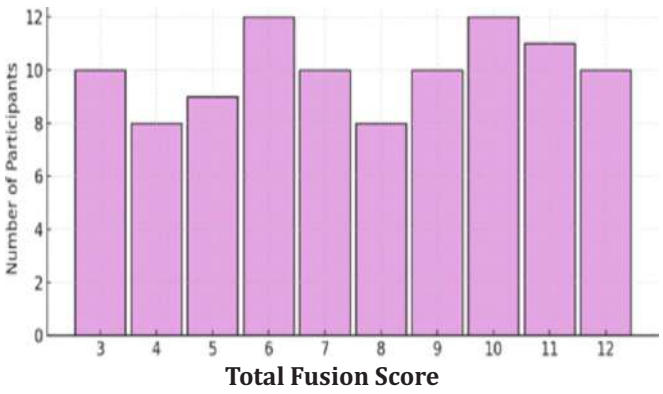
Age distribution analysis is essential to understand the characteristics of the study population and ensure appropriate representation across different age groups. A total of 100 participants were assessed, and their documented ages were analyzed for distribution patterns. The statistical summary revealed a mean age of 17.94 years, indicating that most participants were close to the legal limit of 18 years, which is of particular forensic relevance. The mean age was calculated as 18.00 years, which coincides with the midpoint of the age range under study. This suggested a relatively symmetric distribution of ages within the selected group.

The total fusion score serves as an important overall index for evaluating skeletal maturity. In this study, it was

Table 2: Total Fusion Score Distribution

Statistic	Value
Mean	7.64
Median	8.00
Minimum	3.00
Maximum	12.00
Standard Deviation	2.88

Figure 1: Histogram Showing the Total Fusion Score Distribution



calculated by summing the fusion status at six epiphyseal centers-the distal femur, proximal tibia, proximal fibula, iliac crest, ischial tuberosity, and femoral head. Each center was assigned a score from 0 (not fused) to 2 (completely fused), with a maximum possible score of 12. Thus, the total fusion score reflects the overall degree of epiphyseal closure, which is an indicator of chronological age. The statistical summary of the total fusion score reveals a mean of 7.64 and a median of 8.00, with scores ranging from 3 to 12. The standard deviation of 2.88 indicates moderate variability in skeletal maturity among participants. The presence of several individuals with a maximum score of 12 confirms complete fusion. The distribution pattern is informative. Participants with fusion scores between 3 and 5 represent early- to mid-stage fusion. These are likely younger individuals or individuals with delayed ossification, typically seen in males or in whom nutritional or hormonal factors affect bone growth. Those with scores between 6 and 9 reflect active fusion stages-typically transitional skeletal maturity. The upper limit of 10 to 12 indicates fully mature epiphyses, suggesting that these individuals are biologically mature. This wide distribution of fusion scores enhances the internal validity of the study. A narrower distribution would reflect uniformity and limit the applicability of findings across different skeletal maturity stages. The present pattern supports robust statistical comparisons across age groups and genders. It

also provides fertile ground for examining correlations between fusion scores and other clinical parameters such as documented age, radiological age, or height and weight. From a forensic perspective, the total fusion score provides a standardized, objective means of assessing maturity.

In this study, the participant group was intentionally balanced with 50 male and 50 female subjects, ensuring equal representation. This symmetrical distribution is consistent with the study's objective of radiological age estimation and ensures that both sexes are equally evaluated in the analysis of epiphyseal maturity. Gender-related differences in skeletal maturity are well documented in the medical and forensic literature. Women typically exhibit epiphyseal fusion earlier than men, especially during adolescence. Therefore, ensuring equal gender distribution in a study focused on radiological age estimation is important to eliminate bias and increase the generalizability of results.

DISCUSSION

The objective of this prospective observational study was to determine the relationship between documented age and radiological age based on the fusion of epiphyseal centers in the knee and pelvis of Indian adolescents aged 17 to 19 years. By examining six fusion centers-the distal femur, proximal tibia, proximal fibula, iliac crest, ischial tuberosity, and head of the femur-the study attempted to establish a valid, region-specific, and gender-sensitive model for skeletal age estimation, especially at the valid age limit of 18 years. The results revealed a high degree of agreement between radiological and chronological age, with an average radiological age of 18.24 years and documented age of 17.94 years. The close distribution confirmed the accuracy of the radiological assessment, with 60% of subjects showing no age difference. Our study demonstrated a strong correlation between radiological and documented age, supported by a highly significant chi-square value ($p < 0.00001$) and high classification accuracy (87%) within a 95% confidence interval (79.0%–92.2%).

These findings are consistent with those of Rao et al. (2022) , who reported a Pearson correlation of 0.91 and a classification accuracy of 90.7% using the same fusion centers.[16]

Similarly, Kumar et al. (2023) proposed a logistic model predicting age≥18 years with 92.3% accuracy using radiographs of the knee and pelvis.[12] These studies confirm that radiological ossification status, when interpreted using a multi-epiphyseal approach, provides a reliable, legally defensible estimate of chronological age.

Our approach used a total fusion score system evaluated across six centers, which minimized errors due to biological variation and increased reliability. Multi-center scoring yielded better results than single-joint assessments. Khan et al. (2016)[17] proposed a similar scoring model with a total score out of 24, where a score ≥20 was 96.3% predictive of age ≥18. Similarly, Chaudhary et al. (2017) developed a fusion index that accurately classified adolescents in 92.8% of cases.[15] These comparisons confirm that combining pelvic and knee joint radiographs significantly increases the accuracy of age estimation.

Early maturation in women: Consistent with global and Indian literature, our data confirmed earlier skeletal fusion in women compared to men. In women, fusion was completed approximately 0.8 to 1.0 years earlier at all fracture sites. Deshmukh et al. (2012)[18] also demonstrated similar trends, with distal femur fusion occurring at 17.2 years in women and 18.5 years in men. This gender-based maturity difference, as observed in several studies including Chatterjee et al. (2008)[14] and Roy et al.,[16] is important for forensic applications, especially when evaluating borderline cases where legal adulthood must be determined with accuracy and objectivity.

CONCLUSION

In summary, this study examines the effectiveness of radiological age estimation using epiphyseal fusion scoring in Indian adolescents which is mean of 7.64 and a median of 8.00, with scores ranging from 3 to 12. The standard deviation of 2.88 indicates moderate variability in skeletal maturity among participants. Recognizing these limitations is essential to understanding the limitations of the research and highlights the need for future studies that are more diverse and long-term, leading to improvements in forensic age estimation methods in India.

LIMITATION OF OUR STUDY

1. Geographic and Population Limitations: The sample does not represent the wide variety in ethnicity, genetics, environmental conditions, and nutritional habits seen in different stages and regions of adult life.

2. Sample Size Limitations: Larger, multi-center studies are needed to ensure that variations are not due to random fluctuations or limited diversity within the participant group.

3. Cross Sectional Design Limitation: it does not track

the progression of ossification in the same individual over time.

4. Lack of hormonal and biochemical correlation skeletal maturity.

Conflict of Interest/Source of Funding : Nill

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Original Research Paper

Confirmation of Snakebite through Renal Changes Observed at Autopsy Utilizing Mukhopadhyay's Score

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ABSTRACT

Introduction: Snakebite envenomation represents a significant global health concern with approximately 81,000-138,000 annual deaths worldwide, disproportionately affecting South Asia and sub-Saharan Africa. Postmortem diagnosis of snakebite remains challenging when clinical history is ambiguous, or bite marks are absent. Venom-induced acute kidney injury produces characteristic histopathological changes that persist after death. The Mukhopadhyay's scoring system evaluates specific renal histopathological findings to provide an objective framework for postmortem snakebite diagnosis, particularly valuable in resource-limited settings where advanced diagnostic techniques are inaccessible.

Objective: To determine the renal changes at autopsy in snake bite victims using the diagnostic criteria Mukhopadhyay's Score (MS).

Materials and Method: This observational cross-sectional study was conducted at Government Villupuram Medical College (Tamil Nadu) from January 2022 to December 2024. 82 cases were examined after obtaining informed consent from their families. Sociodemographic data and samples for gross and histopathological examination were collected. Renal findings were scored using Mukhopadhyay's criteria based on lesion type, distribution, and severity. Statistical analysis examined associations between demographic characteristics, MS scores, and DF distribution.

Results: The study population had a mean age of 45 years, with most of the victims aged >50 years. Haemotoxic envenomation accounted for 58.54% of fatalities. Chi-square analysis revealed significant associations between age >50 years and both MS Score (p=0.012) and envenomation type (p=0.003). Gross examination of both the kidney showed hemorrhage in medulla, while histopathology picture demonstrated progression of renal damage from minimal congestion to diffuse tubular necrosis corresponding with increasing MS scores (0 to 2).

Conclusion: This study confirms the utilization of Mukhopadhyay's score as an effective tool for postmortem snakebite diagnosis through characteristic renal histopathological changes. The significant association between higher age and renal damage severity provides important forensic and clinical insights. The progressive relationship between MS scores and renal pathology offers reliable diagnostic criteria applicable in resource-constrained settings such as our country.

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INTRODUCTION

Snake envenomation is an often neglected but a major public health problem worldwide, especially in tropical and subtropical areas. The World Health Organization (WHO) recognizes snakebite as a neglected tropical disease that contributes to approximately 81,000-138,000 deaths annually worldwide, with South Asia, Southeast Asia, and sub-Saharan Africa bearing the highest burden.[1] India accounts for approximately 45,000 deaths attributed to snakebite annually. However, this figure is likely higher than the exact burden due to incomplete reporting and misdiagnosis.[2]

Diagnosis of fatal snakebite remains challenging in medicolegal investigations, particularly when the victim dies prior to reaching medical facility or when the bite mark is absent or inconspicuous. Traditional diagnostic approaches rely heavily on clinical history, identification of fang marks, and detection of venom in body fluids. However, these methods have significant limitations in postmortem settings.[3] Fang marks may be difficult to identify due to decomposition, trauma, or anatomical location. Furthermore, venom detection assays are not universally available and may yield false negatives due to variations in venom composition across snake species.[4]

Renal involvement is a prominent feature of snake envenomation, particularly with viperid species. Venom-induced acute kidney injury (AKI) is a major cause of morbidity and mortality in snakebite victims.[5] The pathophysiology of snake venom-induced renal damage encompasses various mechanisms including direct cytotoxicity, hemodynamic alterations, immunologic reactions, disseminated intravascular coagulation, and rhabdomyolysis.[6] These pathological processes produce characteristic histopathological changes in renal tissue that can be assessed in postmortem.

Mukhopadhyay et al. developed a systematic scoring system based on specific renal histopathological findings for more reliable and convenient postmortem diagnostic criteria for snakebite. This scoring system, now known as Mukhopadhyay's Score, evaluates various parameters including glomerular changes (fibrin thrombi, endothelial swelling), tubular alterations (epithelial necrosis, myoglobin casts), and vascular features (fibrinoid necrosis, interstitial hemorrhage). Each parameter is assigned a weighted score, with the cumulative total providing a quantitative assessment of the likelihood of snakebite envenomation.[7]

The application of Mukhopadhyay's Score in forensic practice offers several advantages. It provides an objective framework for diagnosis when traditional markers are ambiguous or unavailable. The scoring system accommodates the spectrum of pathological changes associated with different venomous species, recognizing that not all parameters will be present in every case.[8] Additionally, the method utilizes routine histopathological techniques that are accessible in most forensic laboratories, making it a practical tool for resource-limited settings.

Despite these advantages, validation studies reveal certain limitations. The specificity of renal changes may be compromised in cases with pre-existing kidney disease or other conditions that cause similar histopathological features.[9] Moreover, the timing between envenomation and death influences the development and recognition of characteristic findings, with very rapid deaths potentially showing minimal changes.

Recent advances in immunohistochemistry and molecular techniques have enabled the detection of venom components in tissue samples, potentially complementing the histopathological approach.[10] These methods offer increased specificity but require specialized equipment and expertise. However, in a resource-limited setting these methods may not be feasible. Hence, to address this gap we conducted a study with the objective of determining the renal changes at autopsy in case of snake bite victims using the autopsy proven diagnostic criteria Mukhopadhyay's Score (MS).

OBJECTIVE:

To confirm a case of snake bite from renal changes at autopsy using the autopsy proven diagnostic criteria Mukhopadhyay's Score (MS).

MATERIALS AND METHODS

Study Design and Setting

This was an observational, cross-sectional autopsy-based study conducted at the Department of Forensic Medicine, Government Villupuram Medical College and Hospital, Tamil Nadu, over a period of 3 years, i.e., from January 2022 to December 2024.

Study Population

A total of 82 medico-legal autopsy cases with a suspected history of snakebite were included in the study. The inclusion criteria comprised of deceased individuals with clinical or circumstantial evidence of snakebite (e.g., witnessed envenomation, fang marks, or clinical diagnosis prior to death), who underwent complete medico-legal autopsy during the study period. Informed consent was obtained from the legal next of kin or family members of the deceased prior to the inclusion of cases in the study.

Data Collection

After obtaining informed consent from the family members of the victims, data was collected used a structured questionnaire. Sociodemographic details including age, sex, occupation, and rural/urban residence were recorded from hospital records and family interviews. Circumstantial evidence such as time and

location of bite, type of snake bite and time since death were also documented.

Gross Examination and Histopathological Examination

On gross examination of kidney during autopsy, parameters such as dimensions, weight, morphological changes, cortical thickness at the level of hilum, capsular adherence, cortical-medullary distinction, and presence of congestion or petechial hemorrhages were recorded.

The tissue samples were processed using standard paraffin embedding techniques for microscopic examination. Sections of 3–5 µm thickness were stained with hematoxylin and eosin (H&E) and examined under light microscopy. The renal lesions were evaluated and scored using Mukhopadhyay's scoring criteria.[7] This scoring system evaluated the presence, distribution, and severity of lesions in the glomeruli, tubules, interstitium, and vasculature. Each component was assigned a score based on defined parameters, and a total Mukhopadhyay's Score (MS) was calculated for each case with score 0 as no minimal congestion and score 5 as Diffuse cortical necrosis. Further, Discriminant Function analysis was performed using 3 variables as predictors of the type of snakebite - score (MS), cortical thickness (C) and weight of the kidneys in grams (W). The only predictor for distinguishing between haemotoxic and neurotoxic snakebite was Mukhopadhyay's Score.

The formula used to calculate DF was:

DF=1.05 x MS – 1.78

DF score < - 0.21 was categorized as neurotoxic variety while > - 0.21 as haemotoxic variety.

Data Analysis

Data was compiled and analyzed in MS Excel and SPSS vs 17. Descriptive statistics were shown in terms of count and percentages. Mukhopadhyay's Scores, and Discriminant Function analysis were examined and described. Chi-square test was done to establish association between the factors. A p-value of <0.05 was considered statistically significant.

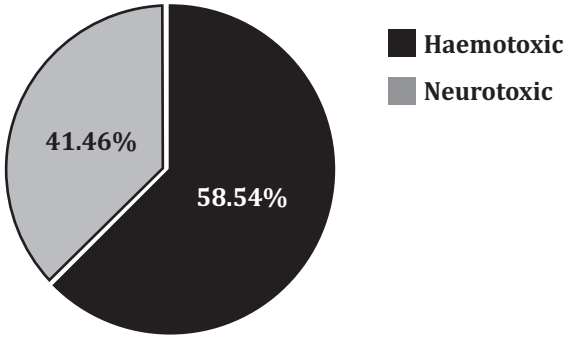
RESULTS

In the present study we examined 82 fatal snake bite cases. Out of which majority of the subjects belonged to the age group of >50 years (47.56%) followed by 31-50 years age range (26.83%). The mean age of the study subjects was 45 years, with the minimum age to be 1.5 years and maximum age was 90 years. Whereas a higher proportion of the cases were males (62.2%) while female was only 37.8%. **(Table 1)** It was reported that most of the victims were bitten by Haemotoxic type of snake (58.54%) than Neurotoxic type (41.46%) **(Figure 1)**.

Table 1: Sociodemographic Characteristics of the Snake Bite Victims (N=82)

	Category	N	(%)
Age Category (in years) [Mean age = 45 years]	<30	21	25.61
	31-50	22	26.83
	>50	39	47.56
Gender	Male	51	62.2
	Female	31	37.8

Figure 1: Type of Snake Bite (N=82)



The real damage due to snake envenomation was described using Mukhopadhyay score (MS score) and Discriminant Function analysis (DF). **Table 2** shows the distribution according to the respective scores. Majority (41.5%) showed minimal congestion (MS Score 0) whereas moderate damage with focal tubular necrosis (MS Score 2) and severe renal damage with diffuse tubular necrosis (MS Score 3) were reported in 34.1% and 24.4% autopsy samples respectively. The discriminant function (DF) scores corresponded with the MS scores with majority (41.5%) victims having a DF score (-1.78), followed by MS score 3 and DF score 1.37 **(Table 2)**. On chi-square analysis shown in **(Table 3)**, age >50 was found to

Table 2: Distribution According to the Mukhopadhyay Score (MS) and Discriminant Function Analysis (DF) Categories (N=82)

	Score Category	N	(%)
MS Score	0	34	41.5
	2	20	24.4
	3	28	34.1
DF	-1.78	34	41.5
	0.32	20	24.4
	1.37	28	34.1

have significant association with MS score (p=0.012) and type of snake bite (0.003) **(Table 4)**.

Table 3: Association of Sociodemographic Factors with MS Score (N=82)

Sociodemographic Factors	MS Score			p value
Age	0	2	3	0.012*
<30	14	4	3	
31-50	11	3	8	
>50	9	13	17	
Gender	0	2	3	
Male	20	14	17	0.702
Female	14	6	11	

**p value < 0.05 are considered to be statistically significant.*

Table 4: Association of Sociodemographic Factors with type of Snake Bite (N=82)

Sociodemographic Factors	Type of Snake Bite		p value
Age	Haemotoxic	Neurotoxic	0.003*
<30	7	14	
31-50	11	11	
>50	30	9	
Gender	Haemotoxic	Neurotoxic	
Male	31	20	0.596
Female	17	14	

**p value < 0.05 are considered to be statistically significant.*

Older individuals (>50 years) were more likely to have severe renal damage (MS Score 3) while younger individuals (<30 years) were more frequently presented with minimal damage (MS Score 0). Similarly, haemotoxic bites were more common in older individuals (>50 years group whereas neurotoxic snake bites were more prevalent in younger subjects (<30 years group). Gender was neither significantly associated with MS score nor type of snake bite. On examination of the renal autopsy samples of the snake bite victims, the gross cross-section demonstrated hemorrhage in cortex and medulla of both the kidneys **(Figure 2)**. On histopathological examination of the same, the Mukhopadhyay's Scores (MS) corresponded with the histopathological findings of renal damage. An MS Score 0 corresponded with Minimal congestion, MS Score 2 with focal tubular necrosis and MS score 3 with diffuse tubular necrosis. These findings suggest that the Mukhopadhyay scoring system provides an effective method for confirming snakebite through characteristic renal changes observed at autopsy, with clear correlations between age, type of envenomation, and severity of renal damage.

Figure 2: Gross cross-section of Specimen Showing Haemorrhage in Cortex & Medulla of Both Sides of Kidneys.

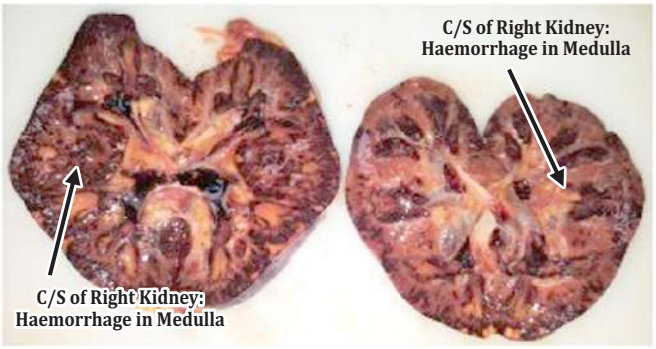


Figure 3: Photomicrograph of Kidney Showing Minimal Congestion (MS Score 0)- H&E 10X (Left) and H&E 40 X (Right)

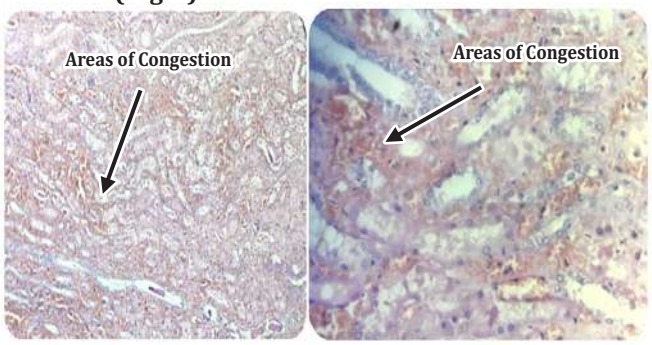


Figure 4: Photomicrograph of Kidney Showing Focal Tubular necrosis (MS Score 2)- H&E 10X (Left) and H&E 40 X (Right)

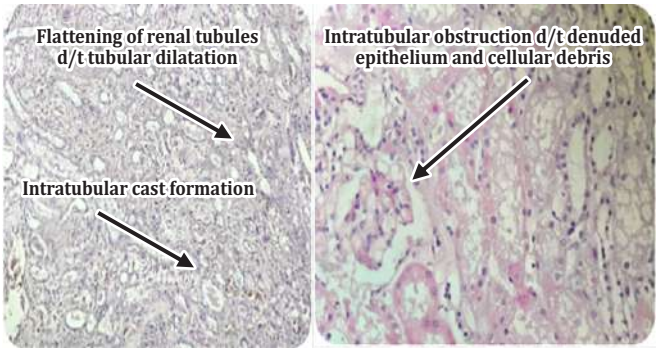
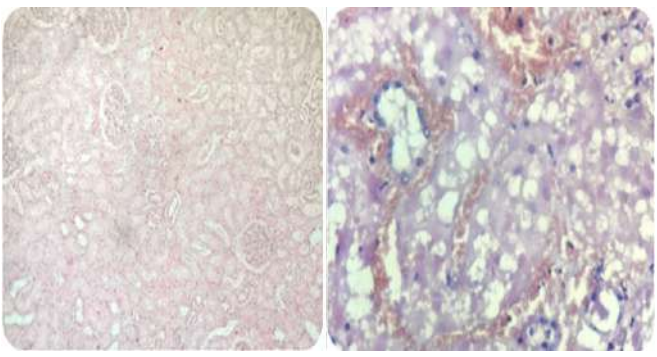


Figure 5: Photomicrograph of Kidney showing Diffuse Tubular Necrosis (MS Score 3) - H&E 10X (Left) and H&E 40 X (Right)



DISCUSSION

The present study conducted at a tertiary care facility in Villupuram district, North Tamil Nadu aimed to confirm snakebite envenomation through renal changes observed at autopsy using the Mukhopadhyay's scoring system. Our study analyzed 82 snake bite victims, on sociodemographic parameters histopathological changes, and association between these demographic factors with MS score and envenomation types.

The sociodemographic data revealed a mean age of 45 years with predominance in the >50 years age group (47.56%), followed by 31-50 years (26.83%) and <30 years (25.61%). Males constituted 62.2% of cases compared to 37.8% females. These findings align with a study conducted by Mohapatra et al., who reported a similar age distribution with peak incidence in the fourth and fifth decades of life and male predominance of 59.4% in their study of 142 snakebite cases.[2] Singh P et al reported a higher incidence of cases in the fourth (30.56%) and third decade (25.93%).[14] Similarly, Nayak et al. study reported that 65.3% male victims who worked as agricultural workers were more vulnerable, indicating the role of occupation in gender predisposition of snake bite.[11] On the contrary, Bhalla et al. study found a higher incidence in a younger age group (21-30 years), which might be due to regional variations in outdoor activities and occupational patterns and their relation with snake bite.[12] The male preponderance observed in our study corresponds with findings by Saravu et al., who reported 69.8% male victims in their 10-year retrospective analysis of snakebite cases.[13] Also similar findings with 67.59% and 70.7% in male victims were reported in two other studies by Kautilya D et al and Singh P et al.[14-15] This gender disparity can be attributed to occupational exposure, particularly in agricultural settings where men traditionally handle outdoor activities involving higher risk of snake encounters. The World Health Organization's highlights this gender disparity, estimating that 59-71% of snakebite victims globally are males.[1]

On analysis using the Mukhopadhyay scoring system, it was observed that predominantly, 41.5% with minimal congestion (MS Score 0) followed by 34.1% of cases with diffuse tubular necrosis (MS Score 3) and 24.4% had focal tubular necrosis (MS Score 2). These findings are comparable with the Mukhopadhyay et al. original study, which established this scoring system through analysis of 87 autopsy cases and reported similar distribution patterns of renal damage on histopathology.[7]

The discriminant function (DF) scores in our study showed perfect correlation with MS scores, validating scoring pattern proposed by Mukhopadhyay.[7] A study by Chaudhary et al. similarly utilized the MS scoring system in their study of 39 suspected snakebite deaths and found it reliable for post-mortem diagnosis, reporting 33.3% cases with MS Score 3, which almost corresponds to our findings.[16] Albuquerque et al. documented that approximately 29% of snakebite victims developed acute kidney injury with histopathological characteristics consistent with MS Score 2 and 3, which aligns with our combined percentage of 58.5% for these categories.[17] The reliability of renal histopathological examination in post-mortem diagnosis of snakebite has been further supported by the study conducted by Sinha et al. , who reported tubular necrosis in 36.7% of fatal snakebite cases, similar to our finding of 34.1% for MS Score 3 (diffuse tubular necrosis).[18]

Our study demonstrated a statistically significant association between age and severity of renal damage given by MS score (p=0.012), with individuals >50 years showing greater susceptibility to severe renal damage (MS Score 3). This finding can be explained by the age-related decline in renal reserve and impaired recovery mechanisms in older individuals. Isbister et al. study highlighted that advanced age was an independent risk factor for severe nephrotoxicity following envenomation, with 2.8-fold increased risk in patients >50 years, supporting our observations.[3] Similarly, Athappan et al. in their analysis of 1,548 snakebite cases, found that age >40 years was associated with a higher incidence of acute kidney injury (odds ratio 2.1; 95% CI: 1.5-3.1) while Pinho et al. reported that patients >55 years had a 3.3 times higher risk of developing severe acute kidney injury following Bothrops envenomation compared to younger individuals, consistent with our finding of age-dependent vulnerability.[19-20]

A significant association was observed between age and type of snake bite (p=0.003), with haemotoxic bites being more prevalent in older victims (>50 years), whereas neurotoxic bites were more common in younger subjects (<30 years). This finding may be interpreted in the context of environmental and behavioral factors. A study by Joseph et al. found that the species of snake responsible for envenomation varied with geographical location and seasonal activities, which might indirectly influence age-specific exposure patterns.[21] Warrell et al study noted that in South Asian countries, older individuals working in

agricultural fields were more likely to encounter vipers (predominantly haemotoxic), while younger individuals in peridomestic environments had higher exposure to kraits and cobras (predominantly neurotoxic).[22] The study conducted by Rahman et al. reported that occupational activities influenced snake species encounters, with agricultural workers (typically older) having higher exposure to terrestrial haemotoxic species, while domestic activities (more common among younger individuals) increased the risk of encounters with neurotoxic species. These observations provide context to our finding of age-specific patterns in envenomation types.[23] The clinical relevance of this finding is substantial in our study, as it suggests that age could be considered a predictive factor for the type of snake envenomation when the snake species is unknown, potentially guiding early management strategies before confirmatory tests.

The progression from minimal congestion to focal and diffuse tubular necrosis documented in our photomicrographs provides clear morphological evidence for snake venom-induced nephropathy. The gross findings of hemorrhage in both cortex and medulla are consistent with observations by Sitprijaet et al., who described similar macroscopic changes in their review of snake venom nephrotoxicity.[5] Our microscopic findings corroborated with the findings reported by Mittal et al., who documented predominant acute tubular necrosis in 31.8% of fatal snakebite cases, similar to our finding of 34.1% cases with MS Score 3.[24] A study by Kolhe et al. described the spectrum of histopathological changes in renal biopsies for snakebite victims, focusing on progression from minimal changes to extensive tubular necrosis, validating the Mukhopadhyay's grading system for the diagnosis.[25] The acute tubular necrosis pattern observed in our study is consistent with findings of Rathnayaka et al., where they documented proximal tubular epithelial degeneration and necrosis as the hallmark microscopic change.[26] These similarities between our autopsy findings and experimental models further validate the Mukhopadhyay scoring system as a reliable and adequate method for post-mortem diagnosis of snakebite.

Clinical and Forensic Implications of our Study:

The findings of our study have significant implications for both clinical management and forensic diagnosis. The established relationship between age and severity of renal damage suggests that older individuals require more aggressive supportive care and earlier intervention. Additionally, the association between age and type of envenomation could inform clinical suspicion regarding the suspected snake species involved when this information is unavailable.

From the forensic perspective, the utilization of the Mukhopadhyay scoring system offers pathologists a structured and convenient as well as cost effective approach to confirm snakebite envenomation in cases where external evidence is minimal or absent. This is particularly relevant in decomposed bodies or in cases where the circumstantial evidence is unclear. As noted by the study conducted by Sharma et al., integration of histopathological evidence with circumstantial findings significantly improves accuracy in determining the cause of death in suspected snakebite cases.[27] Thachil et al. also emphasized that in tropical countries, unusual causes of death including snakebite are often underreported due to lack of specific post-mortem diagnostic criteria.[28] Our study addresses this gap by providing validated diagnostic tool through the MS scoring system.

Limitations:

Although this study highlights important findings, few limitations are acknowledged. The retrospective nature of the analysis limited our ability to correlate findings with clinical or laboratory parameters. Future prospective studies integrating ante-mortem clinical data with post-mortem findings would enhance the diagnostic utility of the MS scoring system. The identification of snake species was based on circumstantial evidence and morphological characteristics of bite marks without confirmatory venom detection tests. Incorporation of immunoassays for venom detection would increase diagnostic precision in future studies. Our classification of envenomation types into haemotoxic and neurotoxic categories is a simplification of the complex venom profiles that may include additional pathophysiological mechanisms. Hence, future research should explore clearer classification incorporating mixed envenomation patterns and their corresponding histopathological visualization.

CONCLUSION

Our study confirms the utility of the Mukhopadhyay scoring system in post-mortem diagnosis of snakebite envenomation through characteristic renal histopathological changes. The significant associations between age and severity of renal damage, as well as between age and type of envenomation, provide valuable insights for both clinical management and forensic

diagnosis. The clear progression of histopathological changes from minimal congestion to diffuse tubular necrosis. This tool can be utilized for improved, prompt and accurate diagnosis in resource-limited settings where snake envenomation remains a significant public health challenge, specifically where ancillary testing methods are unavailable.

CONFLICT OF INTEREST/FUNDING: Nill

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Original Research Paper

Histopathological Changes in Lungs, Liver, Kidneys, and Spleen in Thermal Burn Deaths in Relation to Total Body Surface Area and Survival Duration (≤72 hours): An Autopsy-Based 1-Year Cross-Sectional Study in Prayagraj, UP.

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ABSTRACT

Introduction: Thermal burns remain a major cause of preventable mortality and produce progressive microscopic changes in vital organs. Correlating these alterations with the extent of burns and post-injury survival duration may improve the interpretation of burn deaths in forensic autopsies.

Aims & Objective: To assess histopathological changes in lungs, liver, kidneys and spleen in fatal thermal burn cases and correlate these findings with total body surface area (TBSA) involvement and post-burn survival duration.

Materials and Methods: This cross-sectional study was conducted on 84 medicolegal autopsy cases that died within 72 hours of sustaining thermal burns. Sections from lungs, liver, spleen and kidneys were examined using routine histopathology. TBSA was categorized into 0–30%, 31–60%, 61–90% and >90%. Survival duration was grouped as up to 24 hours, 24–48 hours and 48–72 hours. Demographic variables (age and gender) and available clinical/investigative information were noted.

Results: Most victims were females (83.3%) and belonged to the 21–40-year age group. The most pronounced histopathological alterations occurred in cases with 61–90% TBSA. In the lungs, alveolar congestion and edema predominated in deaths within 24 hours, whereas alveolar fibrin strands and widened septa were frequently observed after 48 hours. In the liver, hepatocytic congestion and portal inflammation were common in early deaths, progressing to focal hemorrhage and infarction/necrosis in prolonged-survival cases. Marked splenic congestion and hemorrhage were observed in early deaths, while infarction and follicular hyperplasia became more evident with increasing survival time. Renal changes shifted from interstitial edema and vascular congestion in early deaths to acute tubular necrosis and tubular/glomerular degeneration after 48 hours.

Conclusion: Thermal burns produce characteristic, time-dependent and TBSA-dependent histopathological changes in visceral organs. Severe burns (61–90% TBSA) and prolonged survival (48–72 hours) are associated with maximal tissue damage. These findings support the forensic interpretation of burn deaths and may assist in distinguishing antemortem burn responses during autopsy.

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INTRODUCTION

Thermal burns are a major public health problem and continue to contribute significantly to accidental mortality worldwide. The extent of tissue destruction depends on the intensity and duration of heat exposure and may range from superficial epithelial injury to deep coagulative necrosis involving multiple organ systems.[1]

Burn injuries that are sustained during life trigger systemic inflammatory and vascular responses that can induce morphological alterations in several visceral organs. In contrast, postmortem burns do not elicit such vital reactions, making histopathology an important

medicolegal tool to distinguish antemortem injury from postmortem exposure.[2]

Although the skin is the primary site of thermal trauma, internal organs are frequently affected due to systemic inflammatory response, hypovolemia, shock and sepsis. Among these, the lungs, liver, kidneys and spleen are particularly vulnerable and may demonstrate morphological evidence of physiological stress and progressive organ dysfunction.[3] Evaluating these visceral changes at autopsy can assist in identifying the cause of death and assessing the survival interval following burn injury.

Previous studies have investigated visceral histopathology in burn deaths; however, most have been limited to individual organs or lacked correlation between total body surface area (TBSA) involvement and survival duration.[2] There is limited literature examining the early post-burn period (≤72 hours), a critical window during which vital reactions are most pronounced and forensically meaningful.[4]

The present study evaluates histopathological changes in the lungs, liver, kidneys and spleen in autopsy-confirmed thermal burn deaths and correlates these alterations with TBSA and post-burn survival duration (up to 72 hours). Establishing such correlations may enhance the interpretation of burn-related deaths, particularly in distinguishing antemortem burn responses and understanding the pathophysiological sequence leading to death.

MATERIALS AND METHODS

This cross-sectional autopsy-based study was conducted in the Department of Forensic Medicine and Toxicology in collaboration with the Department of Pathology, Moti Lal Nehru Medical College, Prayagraj. A total of 84 medicolegal autopsy cases of thermal burn deaths received within 72 hours of sustaining injury were included over a one-year study period (31 July 2023 – 30 July 2024).

Inclusion Criteria: Autopsy-confirmed thermal burns, death within 72 hours of sustaining burn injury, adequate preservation of visceral organs for histopathological examination.

Exclusion Criteria: Chemical, electrical and lightning burns, unidentified bodies or bodies in advanced decomposition, cases with known chronic smoking history.

Data Collection: Demographic details (age and gender), total body surface area (TBSA) percentage, survival duration after burn injury and clinical information available in medical case records were documented. Circumstantial information (accidental, suicidal or homicidal) was recorded when available from police inquest papers or hospital records.

TBSA and Survival Classification: Cases were categorized according to: TBSA: 0–30%, 31–60%, 61–90% and >90%, Survival duration: up to 24 hours, 24–48 hours and 48–72 hours.

Sampling and Histopathology: Representative tissue samples from lungs, liver, kidneys and spleen were collected during autopsy. All samples were fixed in 10% buffered formalin, processed routinely and stained using hematoxylin and eosin (H&E). Histopathological findings were recorded independently for each organ according to prespecified morphological parameters (e.g., congestion, edema, fibrin deposition, necrosis, hemorrhage and degenerative changes).

Study Outcome Measures: The primary outcome was the pattern of histopathological changes in visceral organs in relation to: TBSA percentage, post-burn survival duration.

Ethical considerations - The study received approval from the Institutional Ethics Committee (ECR/922/inst/UP/RR-22) of Moti Lal Nehru Medical College, Prayagraj. Written informed consent was obtained from the legal next of kin for research use of autopsy materials. The study adhered to the ethical principles of the Declaration of Helsinki.

RESULTS

A total of 84 thermal burn death cases were included in the study, comprising 14 males (16.67%) and 70 females (83.33%). The maximum number of cases were from the age group of the 21–30 years and 31–40 years, each contributing 26 cases (30.95%), followed by 41–50 years (15 cases; 17.86%). The least affected age group was 0–10 years with 3 cases (3.57%) **(Table 1)**.

With respect to the nature of burn, accidental burns constituted the overwhelming majority (78 cases; 92.86%), whereas 6 cases (7.14%) were suicidal in manner. No homicidal burn was reported during the study period. All male cases were accidental, whereas all suicidal burn cases occurred among females. **(Table 2)**

In the histopathological findings of the lungs, Cases with 61–90% TBSA consistently showed the highest frequency of all pulmonary histopathological findings across all

Table 1: Distribution of Cases Based on the Age of the Deceased (N=84)

Age Group	Number of Cases		Total
	Male	Female	
0-10 Years	0 (0%)	3 (03.57%)	3 (3.57%)
11-20 Years	0 (0%)	6 (07.14%)	6 (7.14%)
21-30 Years	3 (03.57%)	23 (27.38%)	26 (30.95%)
31-40 Years	4 (04.76%)	22 (26.19%)	26 (30.95%)
41-50 Years	3 (03.57%)	12 (14.29%)	15 (17.86%)
>50 Years	4 (04.76%)	4 (04.76%)	8 (09.52%)
Total	14 (16.67%)	70 (83.33%)	84 (100%)

Table 2: Distribution of Cases Based on Nature of Burn (N=84)

Nature of Burn	Number of Cases		Total
	Male	Female	
Accidental	14 (16.67%)	64 (76.19%)	78 (92.86%)
Suicidal	0 (0%)	6 (07.14%)	6 (07.14%)
Total	14 (16.67%)	70 (83.33%)	84 (100%)

survival groups. Alveolar congestion and alveolar edema were especially concentrated in those surviving up to 24 hours. Alveolar fibrin strands and widened septa appeared mainly in cases surviving beyond 48 hours, particularly within the 61–90% TBSA category. Dilated blood vessels were most notable in early survival (≤24 hours), again predominantly in 61–90% TBSA cases **(Table 3)**.

Hepatic histopathological changes showed a strong association with both burn extent and survival time. Hepatocytic congestion, portal inflammation and focal hemorrhage occurred most frequently among individuals with 61–90% TBSA and survival up to 24 hours. Infarction/necrosis did not appear in early survival groups but became evident only in prolonged survival (48–72 hours), mainly in the same TBSA category. Dilated and congested hepatic sinusoids were seen predominantly among cases with 61–90% TBSA and survival between 24–48 hours **(Table 4)**.

In the spleen, histopathological alterations also showed a strong link with the extent of burns and duration of survival. The group with the most cases is the 61-90% TBSA with survival up to 24 hours, showing congestion in 23 cases and haemorrhage in 22 cases. This indicates frequent vascular disturbance and bleeding in the spleens of patients with moderate to severe burns in early survival. The second most affected group is the 61-90% TBSA with survival 24-48 hours, where congestion appeared in 15 cases and necrosis/infarction in 4 cases, alongside 6 cases with haemorrhage and 2 with follicular hyperplasia. In the 31-60% TBSA group with survival up to 24 hours, 4 cases exhibited congestion and haemorrhage but no infarction or follicular hyperplasia. Other groups including those with lower TBSA or longer survival showed fewer findings

Table 3: Histopathological Changes in Lungs in Relation to TBSA % of Burn and Survival Period (N=84).

Microscopic Findings	0-30% TBSA and Survival Period Up to 24 hours	0-30% TBSA and Survival Period 24-48 hours	0-30% TBSA and Survival Period 48-72 hours	31-60% TBSA and Survival Period Up to 24 hours	31-60% TBSA and Survival Period 24-48 hours	31-60% TBSA and Survival Period 48-72 hours	61-90% TBSA and Survival Period Up to 24 hours	61-90% TBSA and Survival Period 24-48 hours	61-90% TBSA and Survival Period 48-72 hours	>90% TBSA and Survival Period Up to 24 hours	>90% TBSA and Survival Period 24-48 hours	>90% TBSA and Survival Period 48-72 hours
Alveolar Congestion	0	0	0	4	0	0	26	15	5	5	0	0
Alveolar oedema fluid	0	0	0	4	0	0	23	18	1	3	0	0
Alveolar fibrin strands	0	0	2	0	0	3	1	3	14	0	0	0
Widened and inflamed septa	0	0	0	0	1	2	2	18	11	0	0	0
Dilated blood vessels	0	0	0	4	1	0	24	18	2	4	0	0

Table 4: Histopathological Changes in Liver in Relation to TBSA % of Burn and Survival Period (N=84).

Microscopic Findings	0-30% TBSA and Survival Period Up to 24 hours	0-30% TBSA and Survival Period 24-48 hours	0-30% TBSA and Survival Period 48-72 hours	31-60% TBSA and Survival Period Up to 24 hours	31-60% TBSA and Survival Period 24-48 hours	31-60% TBSA and Survival Period 48-72 hours	61-90% TBSA and Survival Period Up to 24 hours	61-90% TBSA and Survival Period 24-48 hours	61-90% TBSA and Survival Period 48-72 hours	>90% TBSA and Survival Period Up to 24 hours	>90% TBSA and Survival Period 24-48 hours	>90% TBSA and Survival Period 48-72 hours
Alveolar Congestion	0	0	0	4	0	0	20	6	2	2	0	0
Alveolar oedema fluid	0	0	0	4	0	2	19	18	11	2	0	0
Alveolar fibrin strands	0	0	0	3	0	0	14	15	2	2	0	0
Widened and inflamed septa	0	0	0	0	0	2	0	4	16	0	0	0
Dilated blood vessels	0	0	0	0	0	0	8	16	8	1	0	0

overall **(Table 5)**.

In the kidneys, histopathological changes were primarily concentrated in severe burns and varied with survival duration. Interstitial edema and vascular congestion were most prominent in cases surviving up to 24 hours, almost entirely within the 61–90% TBSA category. Acute tubular necrosis was uncommon in early deaths but peaked in the 24–48 hours group. Tubular degeneration and glomerular degeneration appeared late (48–72 hours), and were concentrated mostly among cases with 61–90% TBSA. **(Table 6)**.

Across all organs, histopathological changes were most pronounced in cases with 61–90% TBSA, while survival beyond 48 hours was associated with late features such as fibrin deposition, infarction and tubular/glomerular degeneration.

DISCUSSION

Distribution of cases based on the age of the deceased – The highest incidence of burn deaths was seen in the 21–30 and 31–40 years age groups (30.95% each), reflecting greater vulnerability among young and middle-aged adults. Similar age predominance has been reported by Gupta et al.[5] and Malini N. et al.,[6] where most cases occurred between 20–40 years. Although Ali et al.[7] noted predominance in children under 12 years in Pakistan, most Indian studies consistently identify early adulthood as the most affected age group. In the study of Kaur, Amandeep & Pradhan, Monisha (2019),[11] males were more with ratio of 1.40.

Distribution of Cases Based on Nature of Burn: Accidental burns constituted the majority of deaths (92.86%), followed by suicidal burns (7.14%). The pattern corresponds with previous Indian studies, including Pujari and Kedare[2] and Ambade and Godbole,[8] which also reported accidental burns as the leading cause. Suicidal burns were seen exclusively among females in the present study, a trend supported by findings from Srinivas et al.[9] In the study of Gupta S et al.,[12] 94.8% cases of burn were accidental death while 5.2% were of suicidal death.

Histopathological changes in lungs in relation to TBSA % of burn and Survival Period– In the present study, alveolar congestion and alveolar edema were the most frequent pulmonary findings, especially in cases with 61–90% TBSA and survival up to 24 hours. This suggests an early inflammatory response and circulatory compromise following burn injury. Alveolar fibrin strands and widened septa were predominantly observed in those who survived 48–72 hours, indicating progression to acute respiratory distress and fibrinous exudation over time. Similar patterns have been described by Sarkar et al.,[4] who reported alveolar fibrin strands and septal inflammation as common late findings in burn deaths with longer

Table 5: Histopathological Changes in Spleen in Relation to TBSA % of Burn and Survival Period (N=84)

Microscopic Findings	0-30% TBSA and Survival Period Up to 24 hours	0-30% TBSA and Survival Period 24-48 hours	0-30% TBSA and Survival Period 48-72 hours	31-60% TBSA and Survival Period Up to 24 hours	31-60% TBSA and Survival Period 24-48 hours	31-60% TBSA and Survival Period 48-72 hours	61-90% TBSA and Survival Period Up to 24 hours	61-90% TBSA and Survival Period 24-48 hours	61-90% TBSA and Survival Period 48-72 hours	>90% TBSA and Survival Period Up to 24 hours	>90% TBSA and Survival Period 24-48 hours	>90% TBSA and Survival Period 48-72 hours
Congestion	0	0	0	4	0	0	23	15	2	2	0	0
Infarction/ Necrosis	0	0	0	0	0	2	0	4	11	0	0	0
Haemorrhage	0	0	0	4	0	0	22	6	0	2	0	0
Follicular Hyperplasia	0	0	0	0	0	0	0	2	8	0	0	0

Table 6: Histopathological Changes in Kidneys in Relation to TBSA % of Burn and Survival Period (N=84).

Microscopic Findings	0-30% TBSA and Survival Period Up to 24 hours	0-30% TBSA and Survival Period 24-48 hours	0-30% TBSA and Survival Period 48-72 hours	31-60% TBSA and Survival Period Up to 24 hours	31-60% TBSA and Survival Period 24-48 hours	31-60% TBSA and Survival Period 48-72 hours	61-90% TBSA and Survival Period Up to 24 hours	61-90% TBSA and Survival Period 24-48 hours	61-90% TBSA and Survival Period 48-72 hours	>90% TBSA and Survival Period Up to 24 hours	>90% TBSA and Survival Period 24-48 hours	>90% TBSA and Survival Period 48-72 hours
Interstitial Oedema	0	0	0	3	0	0	12	8	3	1	0	0
Acute Tubular Necrosis	0	0	0	0	0	0	3	18	5	0	0	0
Tubular Degeneration	0	0	0	0	0	2	0	9	11	0	0	0
Glomerular Degeneration	0	0	0	0	0	0	0	1	4	0	0	0
Vascular Congestion	0	0	0	4	0	0	19	14	3	2	0	0

survival durations. Pujari and Kedare[2] also identified congestion in almost all cases dying within 24 hours and increased edema in prolonged survival. The findings in the present study therefore align with the established evolution of burn-related pulmonary injury from vascular congestion to fibrinous damage with increasing post-burn interval.

Histopathological changes in liver in relation to TBSA % of burn and Survival Period – Hepatocytic congestion, portal inflammation and focal hemorrhage were major hepatic findings in cases with 61–90% TBSA and early survival (≤24 hours). Infarction and necrosis, however, appeared predominantly in individuals who survived 48–72 hours, indicating that severe ischemic and hypoxic changes

develop in the later stages of burn-related systemic deterioration. Pujari and Kedare[2] documented hepatic congestion in early deaths and progressive necrosis in prolonged survival, supporting the temporal pattern observed in the present study. Prasad et al.[10] similarly noted sinusoidal dilatation and necrosis in burn deaths surviving beyond 48 hours. Therefore, the hepatic findings follow a predictable timeline from congestion to necrosis with increasing survival interval.

Histopathological changes in spleen in relation to TBSA % of burn and Survival Period – Splenic congestion and hemorrhage were most frequently noted in cases with 61–90% TBSA and survival up to 24 hours, suggesting acute circulatory overload in the early stage. Infarction/necrosis and follicular hyperplasia appeared mainly in the 48–72-hour group, indicating delayed structural and immunological alterations. Although comparative literature on splenic morphology in burn autopsies is limited, available studies consistently demonstrate congestion as the predominant early finding and infarction/necrosis as a late manifestation associated with prolonged survival. The present findings therefore contribute valuable additional evidence in an area with relatively limited published research.

Histopathological changes in kidneys in relation to TBSA % of burn and Survival Period – Renal involvement in burn deaths showed a distinct temporal pattern. Interstitial edema and vascular congestion were most prominent within the first 24 hours, particularly in cases with 61–90% TBSA, reflecting acute circulatory and fluid imbalance. Acute tubular necrosis, tubular degeneration and glomerular degeneration increased progressively with longer survival (24–72 hours), signifying ischemic–toxic injury as shock and sepsis advance. Studies by Pujari and Kedare[2] and Prasad et al.[10] also identified renal congestion as an early feature and acute tubular necrosis as a dominant late feature, consistent with the progression observed in this study.

Limitations: The present study did not include biochemical and clinical variables, which could have provided deeper insight into systemic responses. Multicentric studies with larger sample sizes are recommended.

CONCLUSION

Thermal burns, a prevalent and potentially devastating type of injury, are characterized by tissue damage resulting from exposure to excessive heat sources. Thermal burns in the present study most commonly affected young to middle-aged adults, with accidental burns forming the overwhelming majority of cases. Histopathological examination of the lungs, liver, spleen and kidneys revealed a clear and predictable pattern of visceral injury that correlated strongly with both total body surface area (TBSA) involvement and post-burn survival duration. Early deaths (≤24 hours) predominantly showed vascular congestion and oedema, while prolonged survival (48–72 hours) was associated with fibrin deposition, parenchymal degeneration, necrosis and other advanced morphological changes. The most severe alterations were consistently observed in cases with 61–90% TBSA. These findings confirm that multiorgan histopathology provides valuable medicolegal information for distinguishing antemortem burn reactions and approximating survival period in fatal burn cases. Incorporating systematic internal organ analysis into routine forensic autopsies can significantly strengthen the interpretation of burn deaths.

Incorporating structured multiorgan histopathological examination into routine autopsy practice can enhance the scientific accuracy of cause-of-death formulation and strengthen the forensic interpretation of burn-related deaths. Broader adoption of this approach across medicolegal centres, supplemented by clinical and biochemical correlation wherever available, has the potential to improve uniformity of reporting, reduce subjectivity in postmortem assessments and ultimately support more reliable judicial decision-making.

Conflicts of Interest/Source of Funding: Nill

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Original Research Paper

Knowledge and Attitude of Current Medico-Legal Practices: A Study among Medical Students, Interns, and Resident Doctors In a Tertiary Care Hospital

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ABSTRACT

Introduction: Despite being a vital part of clinical practice, medico-legal responsibilities remain overlooked in both undergraduate and postgraduate training. This may compromise both, the safety of patient and legal accountability. This study aimed to assess awareness and attitudes toward medico-legal issues among medical students, interns, and post graduate residents.

Materials and Method: This cross-sectional, questionnaire-based study, was conducted at Christian Medical College, Ludhiana. Medical students, interns, and residents completed a validated questionnaire consisting of 11 awareness items and 5 attitude items related to medico legal issues. Data were collected anonymously and analysed using the Statistical Package for the Social Sciences (SPSS, version 26.0) and R software (version 4.4), applying descriptive statistics and chi-square tests. A p-value of less than 0.05 was considered statistically significant.

Results: Females had a higher representation in our study (57.5%) compared to male participants. Moderate awareness was seen in 52.9% of participants, while 27.1% had high and 20% had low awareness. Attitudes were mainly positive (85%), followed by neutral (10%) and negative (5%). Female participants showed higher awareness and more positive attitudes than males ($p < 0.05$). Qualification levels and awareness levels did not show significant correlation ($p = 0.289$). Awareness and attitude depicted a strong association ($p < 0.001$).

Conclusion: While attitude levels were mostly positive, moderate awareness predominated and negative attitude was associated with less medicolegal knowledge. These findings highlight the need for structured, experience based learning related to medico-legal matters through workshops, case-based discussions, and simulated court settings. The strong association found in this study between knowledge and attitude indicates that improved knowledge can lead to a more positive attitude towards medico legal aspects of healthcare.

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INTRODUCTION

The practice of medicine encompasses a spectrum of legal, and ethical responsibilities, and the absence of some degree of medico-legal awareness in physicians can put the patient at risk.[1] As a bridge to the knowledge gap, educational initiatives can involve different things ranging from forensic skills laboratories to simulation-based workshops that demonstrate improvements in knowledge and confidence.[2]

The Competency-Based Medical Education (CBME) framework, implemented by the National Medical Commission (NMC), highlights the importance of practical assessments in education that link theoretical learning with clinical responsibilities mitigating the issues arising due to the traditional aspects of knowledge centred education.[3] In support of this, Palimar et al. indicated that medical students viewed the ethics and communication modules positively and appreciated structured, experiential learning methods.[4]

In our study, "awareness" means the extent to which

people are aware of the medico-legal issues, and "attitude" refers to how they view or think about these issues; these variables can also affect their behaviour and practice as professionals.[5]

International guidelines, such as Good Medical Practice from the General Medical Council, mandate that doctors must have a thorough understanding of their medico-legal obligations to uphold their professional standards, ensure patient safety, and maintain legal accountability.[6-7] In India, researches done in Maharashtra and parts of northern India, have also demonstrated the deficiencies in knowledge, attitude and practice related to medico-legal matters, which signifies the need for bringing change to the medical curriculum.[8-10] Jha et al. also observed that medical students often had a poor understanding of the various aspects of medical law and medical ethics, which was majorly due to less emphasis provided on medico-legal education and training in the undergraduate levels.[11]

Many systematic reviews have highlighted the insufficient emphasis on medical ethics and law, which has left students unprepared to address medico legal issues in clinical practice.[12] Additionally, ethical sensitivity and gender-sensitive practice are considered key areas of professional competence, which shape decision-making in complex medico-legal scenarios and promote patient-centred care.[6]

The decision in Jacob Mathew vs. State of Punjab (2005) reinforces doctors' ethical and legal responsibility and the relevance of medico-legal knowledge in differentiating civil from criminal responsibility.[13] Against this backdrop, the current study seeks to examine and compare the knowledge and attitudes of medical students, interns, and resident doctors regarding medico-legal concerns in Christian Medical College Ludhiana, as well as to identify opportunities for curriculum improvement.

AIMS & OBJECTIVES:

1. Assess the awareness levels of medico-legal issues among medical students, interns, and residents.
2. Assess their attitude toward medico-legal responsibilities.
3. Determine how awareness levels, attitude, and the baseline parameters are associated

MATERIALS AND METHOD

This was a questionnaire based cross-sectional study which was started following approvals from Research committee and ethics committee vide letter no. (IECBMHR/202508-402/Apprvl-Student-Proj/CMC&H) of the institution and was conducted between 6th August 2025 and 10th September 2025 at Christian Medical College, Ludhiana. A structured questionnaire was designed to evaluate the awareness and attitude of participants' regarding medico-legal issues. The questionnaire was developed after a literature review and improved with expert input to ensure validity of the questionnaire. It consisted of an awareness section and an attitude section: the awareness section, consisted of 11 items with a maximum score of 22, and the attitude section, consisted of five items with a maximum score of 25. For the awareness section, scores more than 16 indicated high awareness, scores between 12 and 16 indicated moderate awareness, and scores below 12 indicated low awareness. In the attitude section, scores between 16 and 25 were considered positive, those from 11–15 were neutral, and scores below 10 were negative **(see Annexure 1)**.

The pre-validated questionnaire, was distributed through Google Forms, after obtaining informed consent, and responses were collected anonymously to reduce reporting. The data was entered in Microsoft Excel and then analysed using the Statistical package for the social sciences (SPSS, version 26.0) and R software (version 4.4; R foundation for statistical computing, Vienna, Austria). Descriptive statistics were used to summarize the results. The Associations between participant characteristics, awareness and attitude levels were evaluated using the chi-square test. p value < 0.05 was considered statistically significant.

INCLUSION CRITERIA: Undergraduate medical students (Bachelor of Medicine, Bachelor of Surgery [MBBS] Phase 3 Part I and Phase 3 Part II), interns, and postgraduate residents.

Exclusion Criteria: Any participant who was on a long term leave during the study.

Sample Size: The sample size was calculated using OpenEpi version 3 software. Based on the prevalence of knowledge regarding medico-legal problems among medical students (66.27%) as reported in an earlier publication, with a 95% confidence interval and an absolute precision of 6%, the required sample size for the present study was estimated to be 240.

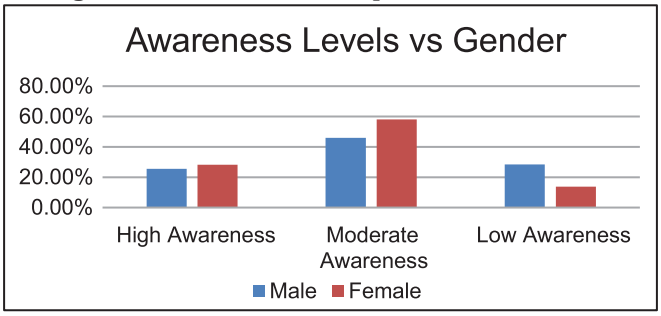
RESULTS

The data presented in **Table 1** shows that majority of participants were females (57.5%). Overall, 62.5% had passed the Forensic Medicine professional examination.

Table 1: Baseline Characteristics of Study Population.

GENDER	FREQUENCY	(%)
MALE	102	42.5
FEMALE	138	57.5
PASSED FORENSIC MEDICINE PROFESSIONAL EXAM	**FREQUENCY**	**PERCENTAGE**
YES	150	62.5
NO	90	37.5
QUALIFICATION	**FREQUENCY**	**PERCENTAGE**
MBBS 3rd year	90	37.5
MBBS 4th year	60	25
INTERNSHIP	45	18.75
POST GRADUATE RESIDENT	45	18.75

Figure 1: Bar Graph Comparing Awareness Levels Among Male and Female Participants.



With respect to qualification, 37.5% were MBBS Phase 3 Part 1 students, followed by Phase 3 Part 2 (25%), interns (18.75%), and postgraduate residents (18.75%).

Majority (52.9%) of participants demonstrated a moderate level of awareness (52.9%) while 27.1% participants had a high level and 20% had a low level of awareness.

Positive attitude was predominant among 85% of the participants. Neutral attitude was observed in 10% of the participants whereas only 5% demonstrated a negative attitude.

Figure 2: Bar Graph Comparing Attitude Levels Among Male and Female Participants.

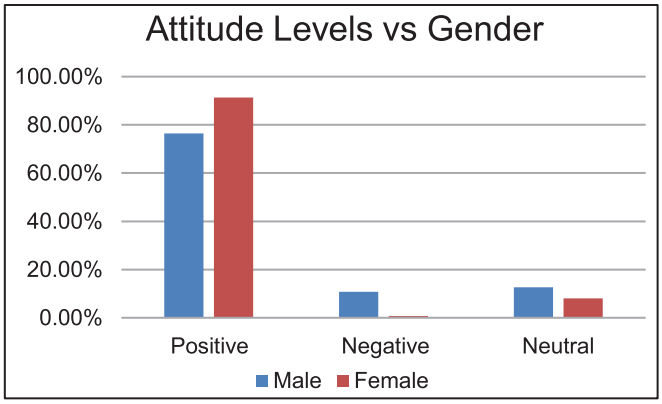
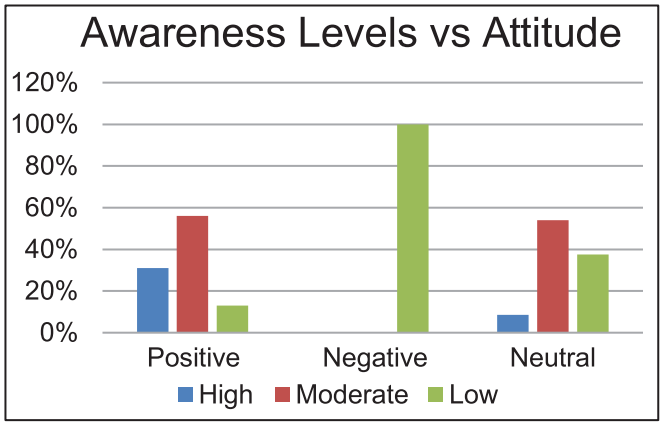


Figure 3: Distribution of Attitudes Across Different Awareness Levels.



The mean awareness score was 14.41 with a standard deviation (SD) of 3.19 and a median score of 14 (interquartile range [IQR]: 12–16). The mean attitude score was 19.13 with a standard deviation of 3.56 and a median score of 20 (IQR: 19–21).

As seen in **Figure 3**, High awareness was observed among 25.5% of male participants, , Moderate awareness was seen in 46% of male participants, and 28.5% showed low

Table 2: Distribution of Awareness Levels and Attitude Grades, Along with Descriptive Statistics, Among Study Participants (n=240).

Variable	Category	Frequency (n)	(%)	Mean ± SD	Median (IQR)
Awareness Level	**High**	65	27.1		
	Moderate	127	52.9		
	Low	48	20.0		
	Total	240	100	14.41 ± 3.19	14 (12–16)
Attitude Grade	**Positive**	204	85.0		
	Neutral	24	10.0		
	Negative	12	5.0		
	Total	240	100	19.13 ± 3.56	20 (19–21)

Figure 4: Distribution of Awareness Levels Across Qualifications.

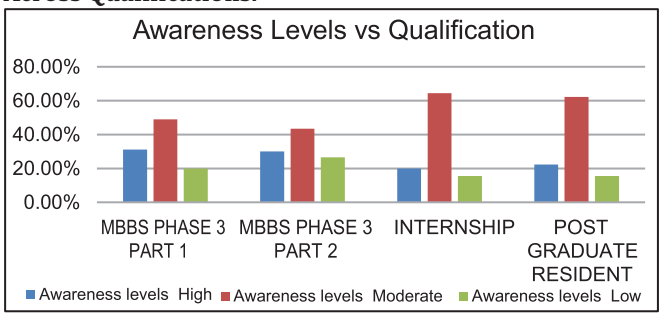
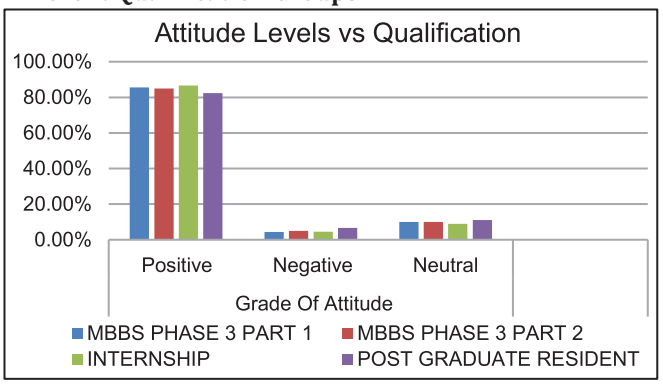


Figure 5: Comparison of Attitude Levels Among Different Qualification Groups.



awareness. In comparison, 28.2% of females had high awareness, 58% moderate awareness, and only 13.8% depicted low awareness levels. Females made up 57.5% of study population whereas 42.5% of the study population comprised of males . It was observed in the results that moderate awareness was more prevalent among females, while low awareness was more prevalent among males. This difference in awareness levels between genders was statistically significant ($p < 0.05$).

Females demonstrated a much higher proportion of positive attitudes (91.3%) compared to males (76.5%). Whereas, negative (10.8%) and neutral attitudes (12.7%) were more common among males, while females showed very low levels of negative (0.7%) and neutral attitudes (8%). This difference was statistically significant ($p < 0.001$).

A strong association was observed between awareness levels and attitude levels ($p < 0.001$). The majority participants that demonstrated a positive attitude had high awareness (31%), while 56% demonstrated a moderate awareness and low awareness was seen in 13% of participants with positive attitude. Among those with a neutral attitude 8.5% had high awareness, 54% had moderate awareness, and 37.5% had low awareness. Among those with a negative attitude, everyone (100%) had low awareness levels signifying the strong correlation between attitude and awareness levels.

When awareness levels were compared across different qualification groups, the majority in each group demonstrated a moderate level of awareness. For MBBS Phase 3 Part 1, 31.1% had high, 48.9% moderate, and 20% low awareness. In Phase 3 Part 2, 30% had high, 43.4% moderate, and 26.6% low. Among interns, 20% had high, 64.5% moderate, and 15.5% low awareness, while in postgraduate residents, 22.3% had high, 62.2% moderate, and 15.5% low., No statistical significance was observed between association of qualification and awareness levels ($p = 0.289$).

As seen in **Figure 7**, Positive attitude was predominantly observed among all groups. In MBBS Phase 3 Part I, 85.6% had a positive attitude, 10% negative, and 4.4% neutral. Among participants of Phase 3 Part II, 85% had a positive attitude, 10% negative, and 5% neutral. Among interns, 86.6% had a positive attitude, 8.9% negative, and 4.5% neutral. Majority (82.3%) of the post graduates had a positive attitude, 11.1% negative, and 6.6% had neutral attitude levels.

DISCUSSION

This study assesses the medico-legal awareness and attitudes among undergraduate students, interns, and postgraduate residents. Positive attitude was highly prevalent amongst the study population with 85% of the study population having a positive attitude. The majority of the study population demonstrated moderate awareness, a minority of the study population achieved high awareness and the remaining study population remained in the low-awareness category. These findings underscore persistent gaps in medico-legal training and highlight opportunities for curriculum reform.

The prevalence of moderate awareness among the study population is in consistence with the study by study by Rani et al., in which interns and postgraduate residents in Andhra Pradesh demonstrated only limited competency in medicolegal responsibilities such as documentation, injury reporting, and consent processes.[1] These inadequacies in the implementation of the curriculum are most likely due to structural restrictions in Indian medical education. Medico-legal training is primarily lecture-based and a part of the preclinical years, with little correlation during clinical rotations. Furthermore, evaluation frameworks rarely test medico-legal competencies, which leads to prioritisation of examinable clinical subjects over medico legal aspects of the profession, with no hands on

experience of cases in emergency especially in private hospitals.[3]

On an international level, the integration of medico-legal education longitudinally in the curricula is increasingly done. Arbel et al. highlighted that in countries such as the United Kingdom and Australia, medical law and professional responsibility are embedded into the curricula across multiple years and reinforced through case-based discussions, simulations, and competency-based evaluations.[12] This disparity implies that India's reliance on lecture-based teaching and infrequent exposure may be insufficient to produce legally competent graduates, emphasising the importance of structured experiential learning.

The study revealed statistically significant gender differences in awareness and attitudes. Female participants demonstrated moderate to high levels of awareness more frequently than the male participants. This finding was indirectly similar to a study conducted by Vemulapati et al. who in her study found higher gender-awareness scores among female undergraduates compared to their male counterparts.[14] In our study, a larger share of female participants showed a positive attitude compared with males. Similar trends were reported by Mediboina et al., who found that female medical students tended to take a more active interest in ethics-related learning than their male counterparts.[6] Differences in how students are taught, their learning styles, or the mix of participants in each group might help explain this pattern. These findings suggest the value of gender-aware teaching methods that give all students equal opportunities to learn and participate.

Correlation between awareness levels and attitudes were found to be highly statistically significant. Participants with positive attitude expressed higher knowledge levels than those with neutral or negative attitude. This finding is in consistence with findings from a cross-sectional study in Maharashtra, which indicated a correlation between lower awareness among interns and reduced confidence in medico-legal practices.[8] This finding depicts the correlation between awareness levels and attitude as people with a positive attitude tend to be more eager in learning the subject than those with a negative outlook. This inter-relationship is crucial for designing the curriculum. Integrating teaching strategies, such as case-based discussions and simulations, can improve the attitude towards the medico legal education due to more involvement of the students compared to the conventional lecture methods.

Interestingly, there was no correlation of awareness levels and attitude with progression of academic year. There was a predominance of moderate awareness levels across all groups, and statistical analysis confirmed the absence of significant associations. This contrasts with findings reported by Modi and Singh, who in their study observed higher medico-legal competence among postgraduate trainees, which was due to structured exposure to medico legal cases during advanced training.[3]

There may be various reasons behind this variation. Tasks such as medico legal report, post mortem report, and autopsy participation are often delegated to senior consultants and not to the interns and residents, preventing practical exposure at junior levels. Postgraduate programs tend to emphasise more on clinical competence over medico legal aspects.

Sutay et al. demonstrated how introducing a forensic skills laboratory for MBBS Phase III students improved documentation practices and awareness, depicting the impact of a simple practise making a difference in low resource settings.[2] Thus, we can also make use of various methodologies like forensic skill labs, case based discussions and mock court sessions to improve medico legal skills in clinical practise.

Due to the variations in training in different institutions, A nationwide uniform approach is of utmost importance. The lack of standardization leads to uneven preparedness among graduates. Including workplace based assessments and OSPE stations that focus on medico-legal scenarios, and embedding these skills into national exit exams, could help establish a minimum expected level of competence. International guidelines, such as those from the General Medical Council which stress documentation, consent, and safeguarding offer practical models to draw from.[7] While direct adoption may not suit the Indian context, adapting competency-based evaluations and hands-on learning methods is both realistic and likely to make training far more effective.

Limitations and Future Directions

There were certain limitations to the study. The Self-reported design can lead to recall and social desirability biases, whereas due to the cross sectional study establishment of causal relationships is not possible. The single centre study design also tends to limit the applicability of the study. The relatively large sample size and inclusion of participants from different training levels

improve the study's internal validity. Future research could adopt longitudinal or intervention based study designs to check the effectiveness of structured modules such as e-learning and integrated case-based teaching. Mixed-methods studies may also help explore gender differences and the influence of institutional culture, offering deeper insights to guide the development effective medico-legal education programs.

CONCLUSION

Moderate awareness levels were largely prevalent amongst the study population; qualification level did not have a significant impact on the awareness levels whereas gender influenced both awareness and attitude. A significant association was observed between attitude and knowledge levels highlighting the need for practical oriented, and competency-based education. The study highlighted how implementation of a structured training and a uniform educational framework can help future doctors uphold ethical standards and follow the legal responsibilities leading to an improvement in the standards of health care.

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Annexure 1: Questionnaire and Scoring Framework

KNOWLEDGE		
1. In cases where there is criminal suspicion involving living or deceased individuals, should the physician start the treatment before the police authorities are notified through an official process ?	YES NO DO NOT KNOW	2 0 0.25
2. Is notifying the police in cases of criminal suspicion a necessary legal obligation with associated legal responsibilities ?	YES NO DO NOT KNOW	2 0 0.25
3. In such cases, should the physician inform the victim's relatives before reporting to the police?	YES NO DO NOT KNOW	0 2 0.25
4. Can healthcare professionals face pressure or stress from relatives or others that may hinder them from reporting suspected criminal cases ?	YES NO DO NOT KNOW	2 0 0.25
5. Can healthcare institutions implement their own protocol for handling cases of sexual and physical abuse ?	YES NO DO NOT KNOW	0 2 0.25
6. Do the photographs taken by medical staff prior to referral to forensic medicine specialists help in the management of physical or sexual assault victims ?	YES NO DO NOT KNOW	2 0 0.25
7. Could photographic documentation help protect medical staff from future legal implications?	YES NO DO NOT KNOW	2 0 0.25
8. Is obtaining informed consent from the relatives also essential before taking photographs in such cases ?	YES NO DO NOT KNOW	0 2 0.25
9. Is comprehensive documentation in medico-legal cases—including detailed wound descriptions, measurements, timing, and photographic evidence—considered complete ?	YES NO DO NOT KNOW	2 0 0.25
10. Can partially completed medico legal reports be submitted to the police authorities in high profile cases ?	YES NO DO NOT KNOW	0 2 0.25
11. Are there significant legal consequences or penalties to arise from medico-legal reports presented in court ?	YES NO DO NOT KNOW	2 0 0.25

ATTITUDE		
12. Patient privacy and confidentiality should be strictly maintained when handling medico-legal cases in the workplace.	• Strongly Disagree • Disagree • Undecided • Agree • Strongly Agree	1 2 3 4 5
13. It is important for healthcare professionals to maintain punctuality during their working hours.	• Strongly Disagree • Disagree • Undecided • Agree • Strongly Agree	1 2 3 4 5

ATTITUDE		
14. Additional training programs focused on the management of medico-legal cases would be beneficial.	• Strongly Disagree • Disagree • Undecided • Agree • Strongly Agree	1 2 3 4 5
15. Do you think that your knowledge regarding medico-legal cases is adequate.	• Strongly Disagree • Disagree • Undecided • Agree • Strongly Agree	1 2 3 4 5
16. You are interested in attending regular training sessions on topics such as writing medico-legal reports in the Emergency Room.	• Strongly Disagree • Disagree • Undecided • Agree • Strongly Agree	1 2 3 4 5

TOTAL SCORE = 22 (KNOWLEDGE)

RANGE OF SCORES (KNOWLEDGE)	AWARENESS LEVELS
>16	HIGH AWARENESS
12-16	MODERATE AWARENESS
<12	LOW AWARENESS

TOTAL SCORE = 25 (ATTITUDE)

RANGE OF SCORES (ATTITUDE)	AWARENESS LEVELS
16-25	POSITIVE ATTITUDE
11-15	NEUTRAL ATTITUDE
<10	NEGATIVE ATTITUDE

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Original Research Paper

Knowledge and Attitude Toward Medical Negligence and Ethical Practices Among Undergraduate Medical Students in a Tertiary Care Teaching Hospital, Chennai, Tamil Nadu

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ABSTRACT

Introduction: Medical practice involves more than treating patients; it also requires doctors to understand ethics, legal responsibilities, and their professional duties. Despite this, several studies indicate that medico-legal and ethical training at the undergraduate level remains inadequate. With increased patient awareness and greater scrutiny of healthcare services, it becomes important for medical students to develop the knowledge and confidence required to handle medico-legal responsibilities.

Aims and Objectives: To assess undergraduate medical students' knowledge and attitudes regarding medical negligence and ethical practices, and to identify areas in which medico-legal education requires strengthening.

Materials and Methods: A cross-sectional questionnaire-based study was conducted among Phase II–IV MBBS students and interns at Chettinad Hospital and Research Institute, Chennai. A structured and validated tool assessing demographic details, medico-legal knowledge, ethical attitudes, and perceptions of curriculum adequacy was administered to 258 participants. Data were analysed descriptively.

Results: The mean age of respondents was 21.1 years, with the highest participation from Phase III students (62.6%). A little over half (60.5%) identified all essential components of medical negligence, while less than half correctly recognised the Bolam Test (44.2%) or the applicable landmark case for criminal negligence (48.8%). Only 4.7% identified negligent omission correctly. Many students were worried about legal issues, with 65% saying they feared litigation, and about 70% admitting they felt hesitant to deal with medico-legal cases. While just over half 52% felt the curriculum prepared them well. Most still found documentation and consent difficult to handle. Students also showed a strong preference for learning through simulations and case-based activities rather than traditional methods

Conclusion: Undergraduate students showed only a basic knowledge of medico-legal concepts, with more difficulty in areas such as documentation, types of negligence, and legal judgments. Many also reported feeling anxious about legal consequences and tended to approach clinical situations defensively. These findings point to the need for more structured, practical medico-legal training in the undergraduate curriculum to help future doctors feel more confident and better prepared.

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INTRODUCTION

Medical ethics and medico-legal principles play an essential role in responsible clinical practice. They guide how doctors safeguard patient welfare, obtain informed consent, and meet their professional responsibilities. The healthcare environment has undergone significant changes in recent years, with patients becoming increasingly aware of their rights and more likely to

question clinical decisions. As a result, doctors are increasingly expected to justify their actions not only from a clinical perspective but also in terms of ethical and legal standards. Allegations of medical negligence defined as a failure to provide an appropriate standard of care that results in harm, have become more frequent. This shift highlights the need for clearer and more practical medico-legal training during undergraduate education.[1]

Studies from different regions of India show that many medical students and young doctors lack sufficient understanding of basic medico-legal concepts. Dash SK[1] reported that although clinicians acknowledged the value of ethical practice, their knowledge of the NMC Code of Ethics and the role of ethics committees was limited. Rai JJ[2] found notable differences between interns and residents in their understanding of informed consent, vicarious liability, and related responsibilities. Dey M[3] reported that even though many students believed they took adequate precautions in medico-legal situations, significant gaps remained in their understanding of consent, documentation, and medico-legal responsibilities. Varghese AM[4] also noted that more than 60 percent of interns had poor awareness of medico-legal principles.

Further evidence supports these concerns. Reddy PS[5] found that many interns were unsure of the legal age for consent and had difficulty with medico-legal documentation. Similar findings have been reported among para-clinical and clinical students. Dey M[6] observed that although students claimed to take precautions while handling medico-legal work, they still showed significant gaps in their understanding of death certification and consent.

Insights from forensic autopsy studies reinforce the need for better training. Yadav A[12] reported that nearly 45 percent of alleged negligence cases were linked to incorrect treatment or procedural errors. This demonstrates the practical consequences of inadequate medico-legal preparation. Additional observations by Sreenivasan M[7] further emphasize the importance of applying medico-legal knowledge within forensic practice.

Given the increasing medico-legal expectations on new medical graduates, it is important to evaluate how well undergraduate students understand these concepts and how prepared they feel to manage their responsibilities.

OBJECTIVES

Primary Objective

- To assess the level of awareness and attitude regarding medical negligence and ethical practices among undergraduate medical students.

Secondary Objectives

- To evaluate students' knowledge related to medico-legal documentation, informed consent, and record keeping.
- To identify gaps in their medico-legal and ethical training.
- To understand students' perceptions of the need for medico-legal education within the undergraduate curriculum.

Materials and Method

Study Design

A descriptive, cross-sectional questionnaire-based study was conducted among undergraduate MBBS students at a tertiary care teaching hospital.

Study Setting

The study was carried out in the Department of Forensic Medicine, Chettinad Hospital and Research Institute (CHRI), Kelambakkam, Chennai.

Study Population

Participants included MBBS students from Phase II, Phase III, Phase IV, and interns enrolled at CHRI.

Inclusion Criteria

- MBBS students from Phase II to Phase IV and interns
- Students who provided written informed consent
- Students present during the data collection period

Exclusion Criteria

- Students unwilling to participate
- Students were absent despite three follow-up attempts

Sampling and Sample Size

With a population of approximately 1000 undergraduate students and a 5% margin of error at a 95% confidence level, the minimum required sample size was calculated as 258. To account for non-response, 290–300 students were targeted using simple random sampling.

Study Tool

A structured questionnaire was used, adapted from earlier work by Dash SK[1]

Rai JJ[2], and Varghese AM[4]. The tool included four sections:

- Demographic Details

- Knowledge of basic medico-legal concepts
- Attitudes toward ethical practice and medico-legal responsibilities
- Perceptions of the need for medico-legal education

A pilot test was carried out with 10 students, and the questionnaire was refined based on their feedback.

Data Collection Procedure

- Data were collected over four weeks.
- Questionnaires were administered either in person or digitally after obtaining informed consent.
- All responses were anonymous and
- Each participant completed the questionnaire within 10 to 15 minutes.

Ethical Considerations

Ethical approval was granted by the Institutional Ethics Committee, CHRI. Participation was voluntary, confidentiality was ensured, and no risks were posed to participants.

RESULTS

The mean age of the participants was 21.1 years, and most students were between 20 and 22 years of age. This reflects the usual age range of medical undergraduates in their clinical training years. Most respondents were from Phase III accounting for 62.6 per cent of the sample, followed by students from Phase IV, Phase II and the group of interns. Similar demographic patterns have been noted in earlier studies by Dash SK[1] and Rai JJ[2], which indicates that the sample in this study is representative.

Knowledge levels varied across different areas. While most students, about 60.5 per cent, were able to identify the three basic components of medical negligence (duty of care, breach of duty and resulting harm), many could recognise only one or two of these elements. Understanding of the concept of res ipsa loquitur was also limited, as many students associated it only with specific examples, such as a surgical instrument left inside the body. Less than half, 44.2 per cent, correctly interpreted the Bolam Test as a measure based on the standards followed by peers in the profession. There was also noticeable confusion regarding the different types of negligence. Around half of the students incorrectly described criminal negligence as a prolonged lack of care, and only 4.7 per cent correctly identified negligent omission. These gaps point to the need for clearer teaching of medico-legal terminology. Awareness of important legal cases was also limited. Fewer than half of the students correctly linked the Jacob Mathew v. State of Punjab judgment with the criteria for criminal prosecution in cases of gross negligence.

These observations are consistent with findings reported by Varghese AM[4] and Reddy PS,[5] who noted similar difficulties among interns and junior doctors.

Perceptions related to medico-legal responsibilities showed several clear trends. Nearly two-thirds of the students felt that fear of litigation influences clinical decision making, and about 70 percent said they were hesitant to handle medico-legal cases. These concerns are consistent with earlier observations made by Rai JJ,[2] Dey M,[6] and Singh J.[8] Many students also believed that defensive medical practices can be ethically justified, a pattern that is similar to international findings reported by Studdert DM.[9]

When asked about their training, students frequently mentioned their difficulty in documentation and retrieving consent from the relatives due to a lack of ability to communicate with relatives. These topics are commonly involved in medico-legal disputes, which highlights the importance of improving training in these areas. Students also showed a strong preference for simulation-based learning and case discussions. This preference supports the recommendations of Yadav R[10] and Patel S,[11] who emphasised the value of interactive and applied approaches to medico-legal education.

Insights from forensic autopsy studies further illustrate the real-world impact of inadequate medico-legal training. Yadav A[12] and Kumar P[13] reported that procedural errors and poor documentation were common findings in alleged negligence cases. These issues show how gaps in theoretical understanding can contribute to practical challenges and legal consequences.

CONCLUSION

Our study shows that while undergraduate medical students are aware of some basic medico-legal principles, many still struggle with key areas such as documentation, informed consent, and recognising different forms of negligence. A considerable number of students also shared that they feel anxious about the possibility of litigation, which in turn makes them apprehensive about handling medico-legal work confidently.

Students clearly expressed that learning through simulations, case discussions, and practical demonstrations would make them feel better equipped to

deal with real clinical situations.

Overall, these findings point to the need for more structured, hands-on medico-legal training in the undergraduate curriculum so that future doctors feel more assured, competent, and prepared to meet their ethical and legal responsibilities in practice.

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Table 1: Age-wise Distribution of Participants (N= 258).

AGE	FREQUENCY	PERCENTAGE
20	78	30.2%
21	114	44.2%
22	48	18.6%
23	6	2.3%
24	6	2.3%
25	6	2.3%

Table 2: Knowledge-Based Questions on Medical Negligence and Ethics.

S. NO.	QUESTION	OPTIONS PROVIDED	PURPOSE
1	Which of the following is an essential component for establishing medical negligence?	Duty of care / Breach of duty / Resultant damage / All of the above	Tests clarity on basic components of negligence
2	Which case set the precedent for 'standard of care' in medical negligence?	Jacob Mathew v. State of Punjab (2005) / Parmanand Katara v. Union of India / Gian Kaur v. State of Punjab / None	Assesses awareness of the landmark case
3	What is the significance of the Bolam Test in the Indian medico-legal context?	Assesses patient consent / Defines ethical boundaries / Assesses standard of care by peer practice / Determines legality	Checks understanding of the Bolam Test
4	In which of the following situations is res ipsa loquitur applicable?	Known complication / Failure to warn / Instrument left inside body / All of the above	Evaluates knowledge of res ipsa loquitur
5	Which form of negligence involves continued lack of care over time, often in chronic patients?	Criminal negligence / Vicarious liability / Gross negligence / Negligent omission	Tests knowledge of negligence types
6	Which of the following is not a defence against medical negligence?	Contributory negligence / Therapeutic privilege / Res ipsa loquitur / Volenti non fit injuria	Identifies understanding of legal defences
7	The principle of 'therapeutic privilege' allows doctors to withhold information when:	Patient is in shock / Disclosure may harm psychological well-being / Consent form signed / Emergencies only	Assesses exception to disclosure
8	Who is vicariously liable if a junior doctor commits a negligent act in a hospital?	The doctor / The patient / The hospital administration / The government	Tests knowledge of liability distribution
9	When does a medical negligence act become punishable under Section 304A IPC?	Death due to rash/negligent act / Injury, intentional / Consent not taken / Documentation absent	Checks understanding of criminal liability
10	Which court case allowed criminal prosecution of doctors for gross negligence?	Dr. Suresh Gupta v. Govt. of NCT of Delhi / IMA v. V.P. Shantha / Jacob Mathew v. State of Punjab / None	Tests awareness of the case on gross negligence

Table 3: Attitude of Students Toward Medical Negligence (N=258).

S. NO.	QUESTION/CONCEPT TESTED	MOST COMMON RESPONSE (%)	OTHER RESPONSES (%)	INFERENCE
1	Fear of litigation affects clinical decisions	Agree/Strongly Agree (~65%)	Neutral/Disagree (~35%)	The majority felt that fear of litigation influences their clinical practice.
2	Hesitated to take medicolegal cases	Yes (~70%)	No/Somewhat (~30%)	Many students showed reluctance in handling medicolegal cases.
3	UG Curriculum equips to handle Negligence Issues	Yes (~52%)	No/Somewhat (~48%)	Just over half felt adequately trained; highlights curriculum gaps.
4	Is defensive medicine ethically justified	Yes (~60%)	No/Depends (~40%)	A considerable proportion justified defensive medicine.
5	Action if aware of colleague's negligence	Warn colleague (~55%)	Report to authority (~35%), Not sure (~10%)	Most preferred warning a colleague over formal reporting.

Table 4: Perceptions of Students on Training and Learning Approaches in Medical Law and Ethics.

S. NO.	QUESTION/CONCEPT TESTED	MOST COMMON RESPONSE (%)	OTHER RESPONSES (%)	INFERENCE
1	Received training on grievances/malpractice	Yes – 62.3%	Informal discussion – 27.6%, No – 10.1%	The majority had formal or informal exposure, but structured training is lacking.
2	Interest in medico-legal simulations/mock trials	Yes – 68.7%	Maybe – 20.1%, No – 11.2%	Strong student interest in practical learning methods like simulations.
3	Most confusing medico-legal topics	Documentation – 41.0%	Consent laws – 33.1%, Ethics in emergency – 17.3%, All topics – 8.6%	
4	Departments to train in laws and ethics	Forensic Medicine – 55.4%	General Medicine – 29.5%, Hospital Administration – 15.1%	Documentation and consent are major areas of difficulty, reflecting practical challenges.
5	Preferred mode for medico-legal learning	Simulations – 47.8%	Case-based discussions – 28.8%, Lectures – 13.7%, Online modules – 9.7%	Students recognize forensic medicine as the key specialty, but also value multidisciplinary input. Hands-on and interactive learning methods are most preferred over traditional lectures.

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Original Research Paper

A Cross-Sectional Study of Histopathological and Chemical Analysis of Various Organs in Cases of Poisoning at Tertiary Care Centre of South Gujarat Region

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ABSTRACT

Introduction: In most instances of fatalities resulting from poisoning, the determination of the cause of death is postponed until the viscera provide evidence of poisoning. Consequently, court proceedings, death certificates, and insurance claims filed by the relatives of the deceased remain unresolved. Death may occur immediately following the ingestion of poison or may be delayed for several days or even weeks, during which time the poisoning may not be the direct cause of death, as it could result from some distant intervening factor. In such situations, accurately identifying the cause of death can be challenging, as external appearances may not offer any clues, and internal examinations may only reveal visceral congestion. Therefore, histopathological findings are essential for studying organ pathology, where the poison has acted, been absorbed, and eliminated.

Aim: To conclude the cause of death, a pathological examination of the organs was conducted for individuals who died from poisoning or were suspected cases of poisoning.

Materials & Method: A total of 90 cases were collected for examination. In all 90 confirmed cases of poisoning, routine viscera were collected for chemical analysis and histopathological examination during post-mortem examination (PME). After receiving the reports, the results were analyzed.

Result: Among the cases examined, 71% (n=64) were male and 29% ((n=26) were female. Approximately 40% (n=33) of the cases had a history of insecticide ingestion, followed by corrosive substances and unknown materials. Of these, 90% (n=83) were classified as suicidal cases, while 10% (n=7) were deemed accidental. Notable histopathological findings were identified in various organs during the histopathological examination. In stomach mucosal necrosis, thrombosis in vessel, inflammatory infiltrate and sub-mucosal haemorrhage are observed in 50% (n=43) cases and mucosal ulceration with congestion was observed in 40% cases (n=42) while in lungs about 91.11% (n=82) cases shows pulmonary edema and pneumonia, inflammatory infiltrate in interstitial lung tissue while congestion was observed in 8.90% (n=8) cases. Fatty changes, hepatocyte degeneration and inflammatory infiltrate was observed in 46.7% (n=42) cases rest shows mix appearance of congestion, centrilobular necrosis and sinusoidal dilatation and in 7.0% (n=6) cases no abnormalities was observed. In kidneys, glomerulosclerosis and inflammatory infiltrate was observed in 30% (n=33) cases while rest showed congestion, in one case there was no changes observed.

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INTRODUCTION

The occurrence of poisoning that leads to death presents a considerable challenge in developing nations, where these events are mainly accidental. Incidents of poisoning are often featured in the daily news, with both print and visual media consistently showcasing different examples of such events.[1]

The World Health Organization estimated that 640 thousand people had died annually due to poisoning, of which, majority deaths occurred in low-income and

middle-income countries.[2] Significantly, 90% of these fatal incidents take place in developing nations, especially among those employed in agriculture. The increase in suicide rates can be attributed to the stress and anxiety that are common in contemporary life. Despite the considerable number of fatalities resulting from poisoning, the determination of the cause of death is postponed until the viscera provide proof of poisoning. This process requires a lengthy period for reporting, and as a result, court proceedings, death certificates, and insurance claims for the relatives of the deceased remain unresolved.

Death can happen immediately after ingesting poison, or it may be postponed for several days or even weeks, during which the poisoning might not be the direct cause of death; rather, it could result from some distant intervening factor. In such instances, identifying the precise cause of death can be challenging, as external appearances may not provide any indications, and internal examinations may sometimes fail to disclose anything significant upon gross inspection.

All organs appear congested during gross examination; however, histopathological analysis can uncover pathological changes in major organs such as the heart, lungs, stomach, liver, and kidneys, where the poison has acted, been absorbed, and subsequently eliminated. Therefore, this study involves the pathological examination of organs from individuals who died due to poisoning or in cases where poisoning is suspected.

MATERIALS AND METHOD

Following the approval from the IEC, a Cross Sectional Study has been conducted in the Departments of Forensic Medicine & Toxicology in partnership with the Department of Pathology at our institute. All deaths with a history of suspected or confirmed poisoning, either brought in dead on arrival in the casualty department or any treated poisoning case declared dead in the ward, have been referred for medico-legal post-mortem examination. In accordance with standard procedures, viscera collected for chemical analysis during the post-mortem were forwarded to the Forensic Science Laboratory in Surat. Concurrently, after noting significant changes in the Lung, Heart, Stomach, Liver, and Kidney, these viscera were preserved in a 10% formalin solution and sent for histo-pathological examination. Data was gathered over a period of one and a half years, from 2022 to 2023. A total of 90 cases (3.7%) were included in this study out of 2378 total cases, with 71% being male and 29% female. Deaths attributed to natural and undetermined causes, decomposed bodies, and cases of poisoning where the stomach had been removed were excluded from the study.

Observation: It has been observed that nearly 30% (n=27) of cases occur within the age group of 31 to 40 years, followed by 25% (n=22) in the 21 to 30 years age group. Additionally, a young child aged 1 year (1%) (n=1) and individuals over 60 years (4%) (n=4) were also noted in the study.

Among all cases, approximately 35% (n= 37) are attributed to HCL poisoning (corrosives), followed by agricultural poisonings. Within the various age groups, around 30% (n=10) of corrosive poisoning cases were identified in the 41 to 50 years age group, with about 23% (n=8) in the 31 to 40 years age group. In the 21 to 30 years age group, poison was not detected in 7 out of 20 cases (35%). A balanced distribution between males and females has been noted. Approximately 40% (n=31) of cases involved HCL poisoning, while another 40% (n=32) involved agricultural poison. Out of the total cases, poison was not detected in about 20% (n=21) of instances, with around 19% (n=12) in males. The FSL report for chemical analysis returned negative results. This may be attributed to the patient receiving treatment prior to death, a significant time lapse between poison ingestion and death, the poison being expelled through vomiting, or lavage being performed, resulting in no poison remaining in the stomach. Among cases of unknown poison ingestion, around 60% (n=54) show a positive chemical analysis report.

External Post-Mortem Examination: During external examination, froth from the mouth was observed in 30% (n=26) of cases, while a characteristic smell from the mouth was perceived in 20% (n=18) of cases. Staining of the teeth and lips was found in more than 60% (n=50) of cases. The lips were moist in over 60% (n=54) of cases and showed corrosion in 30% (n=31) Cyanosis of the nails was present in more than 90% (n=97) of cases.

Gross and Histopathological Examination of Organs

Stomach: Grossly, congestion was noted in all cases. Perforation was observed in approximately 35% (n=33) of cases (40% (n=26) in males and 22% (n=7) in females). Charred and corroded mucosa was noted in about 40% (n=35) of cases in both sexes. On histopathology, gastric congestion was observed in more than 55% (n=47) of cases. Mucosal ulceration, necrosis, inflammatory infiltrates, and thrombosed vessels were noted in around

40% (n=38) of cases. Submucosal hemorrhage was found in 5% of cases.(n=5)

Lungs: On gross examination, congestion was seen in all cases. Histopathologically, congestion and pulmonary edema were present in more than 50% (n=55) of cases of both sexes. Pneumonic changes and inflammatory infiltrates in the interstitial tissue were noted in about 30% (n=27) of cases.

Heart: Grossly, congestion was observed in all cases. On histopathology, congestion was noted in 45% (n=37) of cases. Advanced athromatous lesions were observed in 50% (n=32) of male cases, while intimal thickening was found in 10% (n=12) of cases across both sexes.

Liver: Gross examination revealed congestion in all cases. Histopathologically, congestion was observed in 30% (n=27) of cases. Hepatocyte degeneration and inflammatory infiltrates were seen in 30% (n=29), while sinusoidal dilatation was present in 15% (n=12) of cases.

Kidneys: Grossly, congestion was observed in all cases. Histopathologically, congestion was present in 70% (n=62) of cases. Inflammatory infiltrates were seen in 20% (n=20), and glomerulosclerosis was observed in 10% (n=7) of cases.

DISCUSSION

In the current study, the demographic distribution revealed that 71.1% of participants were male, while 28.9% were female, indicating a higher involvement of males compared to females. They are consistent with the research by KSN Reddy et al[3] which reported that 65.65% of cases were male. This elevated incidence among males is attributed to their greater exposure to stress and strain, as they often serve as the primary earners for their families.

The majority of victims in this study were within the age range of 31-40 years (30%), followed by those aged 21-30 years (24.4%) and 41-50 years (18.8%). These findings are little bit coincides with the research conducted by waghmare et al[4] and kukde et al[5] where 21-40 yrs age group is more involved. This particular age group is typically the most active in physical, mental, and social aspects, making them more susceptible to stress throughout their lives.

In the present study, Hydrochloric acid (31 cases,34.44%) and Organophosphurus compounds (32 cases, 35.56%) were the most commonly used substances among those who died by suicide. A study by Prajapati et al[6] found that Aluminum Phosphide (60.93%) was the most frequently consumed poison among suicide victims, followed by Hydrochloric acid (14.57%). In our research, Aluminum Phosphide was identified in 10 cases (31.25%), followed by Chlorpyrifos in 8 cases (25%) among all instances of organophosphurus poisoning.

Hydrochloric acid (HCl; pH1) is one of the most commonly abused acids. It is a widely used industrial product, primarily used as a cleaner for bathrooms and toilets. This substance is readily available in grocery stores and various pharmacies, with minimal regulations governing for its sale or purchase. Aluminum phosphide poisoning is prevalent due to its low cost and unrestricted availability in the market, where it is primarily utilized for grain preservation. In India, aluminum phosphide is sold under various brand names, including Alphos, Celphos, and Quickphos. In terms of mortality, hydrochloric acid can cause perforations in the oesophagus and stomach, leading to severe infections in the chest and abdominal cavities, which may ultimately result in death. Generally, the treatment for corrosive injuries is conservative in cases with minor complications, while life-threatening situations necessitate surgical intervention. The treatment approach requires the collaboration of multiple specialists, depending on the severity of the corrosive injuries, associated complications, and the patient's overall condition. Aluminum phosphide is more fatal, yet no specific antidote is available. In contrast, other poisons are less toxic and have corresponding antidotes. Upon ingestion, phosphine is released from aluminum phosphide, which is rapidly absorbed throughout the gastrointestinal tract and partially transported to the liver via the portal vein. It leads to cellular hypoxia.

Study conducted by Sutay et al[7] found that cyanosis found in the highest number of cases, followed by frothy discharge from the mouth and nostrils, which was observed in 19 cases (27.14%) due to significant accumulation of secretions in the respiratory passages. This finding aligns closely with the current study.

Research conducted by Waghmare et al[4] indicates that stomach congestion was observed in the highest proportion of cases, specifically 59.09%, which aligns with the findings of the current study. In this study, the alterations in the stomach were proportional to the effects of the causative agents. Most agents resulted in congestion and inflammation of the gastric mucosa, which explains the prevalence of these conditions. Submucosal hemorrhages were frequently noted in cases of insecticide poisoning, while corrosion, perforation, and wall softening

were also documented in instances of corrosive poisoning. In histopathological examination, study conducted by S. Waghmare et al.[4] which reported stomach congestion in 63.63% of cases, followed by submucosal hemorrhage in six cases. The stomach also exhibited a combination of corrosion, perforation, and wall softening, attributed to the corrosive effects and tissue destruction caused by acid exposure, leading to coagulative necrosis through protein precipitation. In our study, mucosal ulceration, necrosis, and inflammatory infiltrate were present in over 80% of corrosive poisoning cases. These results are comparable to those of S. Waghmare et al., who noted necrosis of the gastric mucosa in all corrosive poisoning cases. Our findings indicated that approximately 70% of poisoning cases exhibited congestion, which is similar to the 63.63%, reported by S. Waghmare et al. We observed sub mucosal hemorrhage in around 9% of cases, while it was noted in 27.27% of cases in the study by S. Waghmare et al.

A study by Siddhesh R.C. et al[8] demonstrated that organophosphurus poisoning resulted in congestion in all examined cases, with epithelial necrosis observed in 95% and pneumonic changes in 5%. In cases of corrosive poisoning, congestion and pulmonary edema were noted in 62.5% of instances, aligning closely with findings from S. Waghmare et al., who reported similar observations in 50% of cases. Furthermore, Siddhesh R.C. et al. conducted research on agricultural poisoning, revealing congestion in 100% of cases, with broncho-pneumonia present in 5%. In our study of insecticide poisoning, we noted congestion and pulmonary edema in 70% of cases, with pneumonia occurring in approximately 30%. The study by Kukde et al. reported intra-pulmonary hemorrhage in 40% and pulmonary edema in 60% of corrosive poisoning cases, which is consistent with our findings of congestion and pulmonary edema in 60% of cases, along with intra-alveolar hemorrhage in 15.63%. Kukde et al. also documented congestion (53%), intra-pulmonary hemorrhage (45.5%), and pneumonia (1.5%) in insecticide poisoning cases. Additionally, they observed pneumonia (11.8%), congestion (17.6%), intra-alveolar hemorrhage (41.2%), and pulmonary edema (17.6%) in cases where the poison was not identified. In our study, we found congestion with edema in 33.33% of cases, intra-alveolar hemorrhage in 14.28%, and pneumonia in 52.38% of cases where the poison was undetected. Such changes may be attributed to the inflammatory effects of toxins on the organs.

A study conducted by kanani et al[9] a cross sectional histopathological based study conducted on tissue samples of 52 cases of poisoning in which they found pulmonary edema (55.77 %), intra-alveolar hemorrhage (48.08 %), liver ballooning degeneration (48.08 %), acute tubular necrosis (51.92 %) in kidney. Stomach findings showed partial loss of rugosity (80.77 %), congestion (51.92 %), necrosis of the mucosa (30.77 %), congestion (71.15 %), denudation of the epithelium (48.08 %), and mucosal inflammation (48.08 %) as predominant findings. This is quite similar to our findings. In our study as pulmonary edema (61.11%), Inflammatory infiltrate in alveoli (30%), congested liver (30%), inflammatory infiltrate and hepatocyte degeneration (32.22%) and congested kidney (68.88%), combined inflammatory infiltrate and glomerulosclerosis (30%).

In histopathological examination of liver, S. Waghmare et al. documented fatty changes in 59.09% of cases, with ballooning degeneration noted in 13.63% in instances of insecticide poisoning. In our findings, fatty changes were observed in 17.39% of cases, alongside hepatocyte degeneration at 8.69%. Additionally, they reported fatty changes and sinusoidal dilation in 50% of cases related to corrosive poisoning, while our study found fatty changes in 12.5% and sinusoidal dilation in 21.88% of corrosive poisoning cases. S. Sutay et al. noted fatty changes at 34.88%, congestion at 46.51%, centrilobular necrosis at 9.3%, and sinusoidal dilation at 6.98% in cases of organophosphurus poisoning. Siddhesh RC et al observed congestion in 52.5% of cases, fatty changes in 20%, centrilobular necrosis in 10%, and sinusoidal dilation in 5% among organophosphurus poisoning cases. The breakdown of toxins results in by products that can inflict damage on the liver. Despite the liver's remarkable regenerative capacity, ongoing exposure to toxic substances can lead to significant and at times irreversible damage.

Kukde et al[5] founded heart congestion in all cases (100%), which aligns with the findings of our investigation. In cases of advanced athromatous lesions in the coronary artery, there is an accumulation of lipids, cells, and matrix components, including minerals, which are linked to structural disorganization, repair processes, and thickening of the intima, as well as deformities in the arterial wall. These findings are among the most common accidental histopathological observations noted in autopsies of other cases as well. Individuals with this condition often lead normal lives and are typically asymptomatic

In the current study, congestion of kidneys was identified as the most prevalent histopathological alteration, occurring in 68% of cases, followed by inflammatory infiltrate at 22% and glomerulosclerosis at 7%. Kukde et al find acute tubular necrosis (44.2%), cloudy degeneration (50%) and congestion (5.8%) in poisoning cases. This finding is contradictory to our study. We didn't find acute tubular necrosis of kidney in any cases. B B Jain et al[10] also found glomerular (74%) and intraparenchymal congestion (64%) as histopathological finding in majority of cases of insecticide poisoning.

A post-mortem study on histopathological finding of liver in poisoning cases was carried out by Kundal et al[11] in which they found 39% cases of Aluminium Phosphide poisoning and 32% cases of Chlorocompound group of insecticides and 27% cases of Organophosphurus poisoning cases among 100 cases (79 male, 21 female). They also observed congestion (66.6 %), degeneration (46%), mononuclear infiltration (51%), and fatty change (46%), and sinusidal dilation (10%) cases of poisoning. These observations are quite similar to present study.

Another study was carried out by R. S. Sibia[12] et al where they did retrospective analysis of poisoning cases at tertiary hospital. Over a period of 6 months data collection, they found total 71 cases (39 Male, 32 Female) of alleged poisoning cases. The major cases were found in the age group of 30-50 yrs. This is similar to our findings in which major cases fall into age group of 31-40 yrs (30%). In their study; out of total cases, aluminium phosphide (28.16%) is the major agent followed by organophosphurus (25.35%) and Organochlorines (11.26%) compound. This is similar to present study where we found around 40% cases of agricultural poison. Majority of pesticide exposure is seen more in middle and low income countries due to increased use of agrochemicals in agricultural sector.[13]

A 3 years retrospective study of histopathological examination in medicolegal autopsy done by Ajay Kumar et al[14] in which they have collected total 447 cases. Out of which, total cases of poisoning was 104 (21.01%). Based on Histopathological finding in various organs, they revealed that HPE is much effective in concluding final cause of death.

A prospective study on sociodemography profile of poisoning cases done by S K Dash et al[15] where over a period of 2 years; total 306 cases of poisoning were came for treatment at hospital out of which 62 cases were subjected to post-mortem examination. By revealing history from the near relatives as well as the police; they found that in a male to female ratio of 1.14:1, 53.3% cases belong to male with peak incidence of cases seen in age group of 21-30 yrs. This is quite similar to present study. They also found that on an average around 36.3% cases are of Agricultural, Insecticide Compound (Organophosphate, Organochlorines, Carbamates, Zinc Phosphide) and 11.5 % cases were of corrosive poisoning.

RESULT

Among the cases examined, 71% were male and 29% were female. Approximately 50% of the cases had a history of insecticide ingestion, followed by corrosive substances and unknown materials. Of these, 90% were classified as suicidal cases, while 10% were deemed accidental. Notable histopathological findings were identified in various organs during the histopathological examination.

In stomach mucosal necrosis, thrombosis in vessel, inflammatory infiltrate and sub-mucosal haemorrhage are observed in 60% cases and mucosal ulceration with congestion was observed in 40% cases while in lungs about 91.11 cases shows pulmonary edema and pneumonia, inflammatory infiltrate in interstitial lung tissue while congestion was observed in 8.90% cases.

Fatty changes, hepatocyte degeneration and inflammatory infiltrate was observed in 46.7% cases rest shows mix appearance of congestion, centrilobular necrosis and sinusoidal dilatation and in 7.0% cases no abnormalities was observed. In kidneys, glomerulosclerosis and inflammatory infiltrate was observed in 30% cases while rest showed congestion, in one case there was no changes observed.

Limitation: One potential limitation of the present research study is the lack of serum parameters that could have contributed to the assessment of functional organ involvement in poisoning cases. We have not included this in our part of study. Immunohistochemistry was also not studied in this study. Various toxins can lead to secondary metabolic disturbances that was also not studied.

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Table 1: Cases According to Age and Sex.

Age Group	Female (In%)	Male (In%)	Total
0-10	1 (4)	0 (Nil)	1
11-20	6 (23)	3 (5)	9
21-30	7 (27)	15 (23)	22
31-40	7 (27)	20 (30)	27
41-50	2 (8)	15 (23)	17
51-60	1 (4)	9 (16)	10
61-70	0 (Nil)	1 (2)	1
71>	2 (8)	1 (2)	3
TOTAL	**26**	**64**	**90**

Table 3: Histopathological Finding in Liver.

Liver-Histopathological Findings	Sex		Total
	Female (In %)	Male (In %)	
Centrilobular Necrosis	1 (4)	2 (3)	3
Congestion	11 (42)	16 (25)	27
Fatty Changes	3 (12)	10 (16)	13
Hepatocyte degeneration	2 (8)	7 (11)	9
Inflammatory Infiltrate	3 (12)	17 (27)	20
No Abnormality	2 (8)	4 (6)	6
Sinusoidal Dilation	4 (15)	8 (13)	12
TOTAL	**26**	**64**	**90**

Table 2: Histopathological Finding in Stomach and Lung.

	Stomach				Lung			
	Congestion	Mucosal Necrosis, Thrombosed Vessel, Inflammatory Infiltrate	Sub-mucosal Haemorr-hage	Total	Alveolar Congested Intraluminal Thrombi in Vessell	Congestion Pulmonary Edema	Pneumonia Inflammatory Infiltrate in Interstitial	Total
Female	13 (50)	10 (38.46)	3 (11.54)	26	3 (12)	13 (50)	10 (38)	26
Male	34 (53.13)	28 (43.75)	2 (3)	64	5 (8)	42 (65)	17 (27)	64
	47 (52)	38 (42)	5 (5.5)	90	8	55	27	90

Table 4: Histopathological Finding in Heart & Kidney.

	Heart				Kidney			
	AAL in Coronary	Changes of Healed MI	Congestion	Intimal Thickening	Congestion	Glomerul-osclerosis	inflamm-atory infiltrate	NAD
Female	6 (23)	0	16 (62)	4 (15)	20 (77)	0	5 (19)	1 (4)
Male	32 (50)	3 (5)	21 (33)	8 (13)	42 (66)	7 (11)	15 (23)	0
90 Cases	38	3	37	12	62	7	20	1

AAL: Advanced Athromatous Lesion. *NAD: No Any Abnormality.*
Note: Bracket in figure indicate 'n' number of cases.

Figure 1: Blackening and Thinning of Stomach Mucosa in Acid Poisoning.



Figure 2: Perforated Stomach Mucosa in Acid Poisoning.



Figure 3: Heart in Paraquat Poisoning.



Figure 4: Alveolar Harmorrhage.

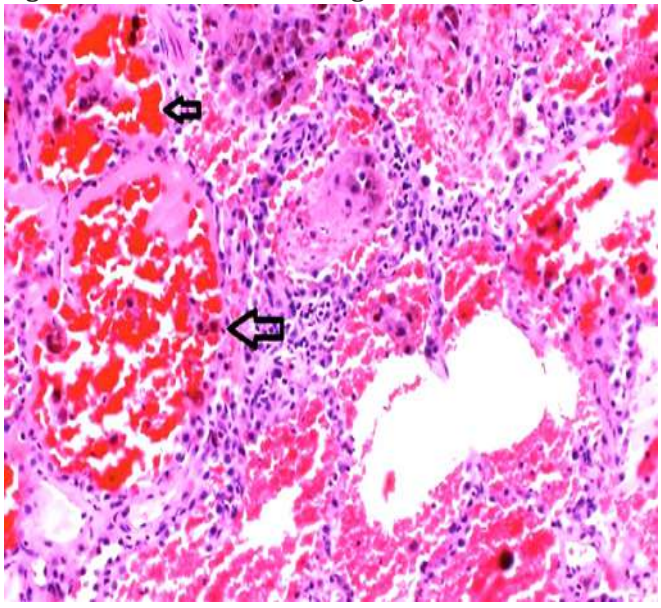


Figure 5: Inflammatory Infiltrate in Alveoli.

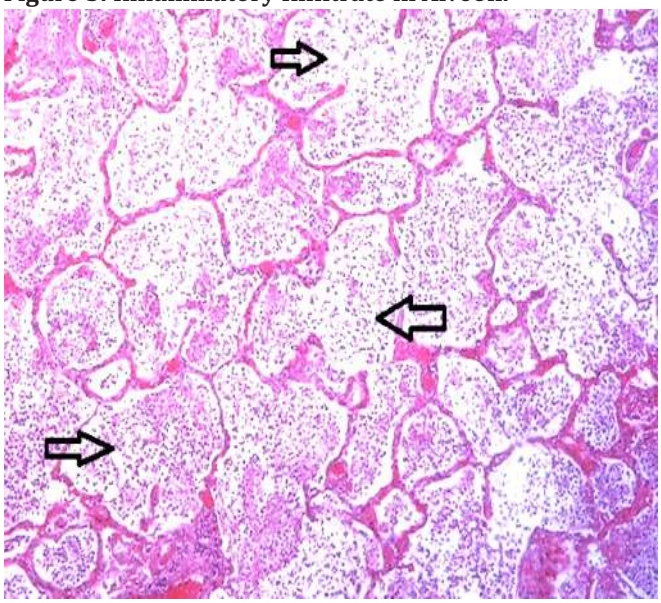


Figure 6: Hepatocyte Degeneration.

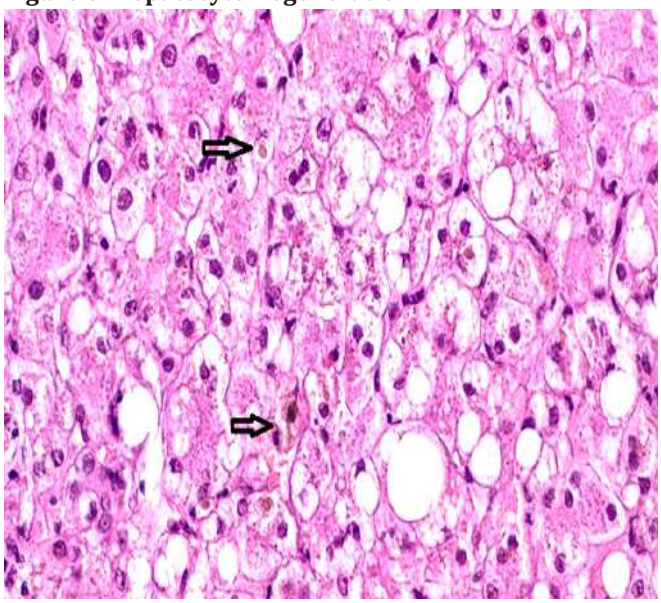


Figure 7: Glomerulosclerosis.

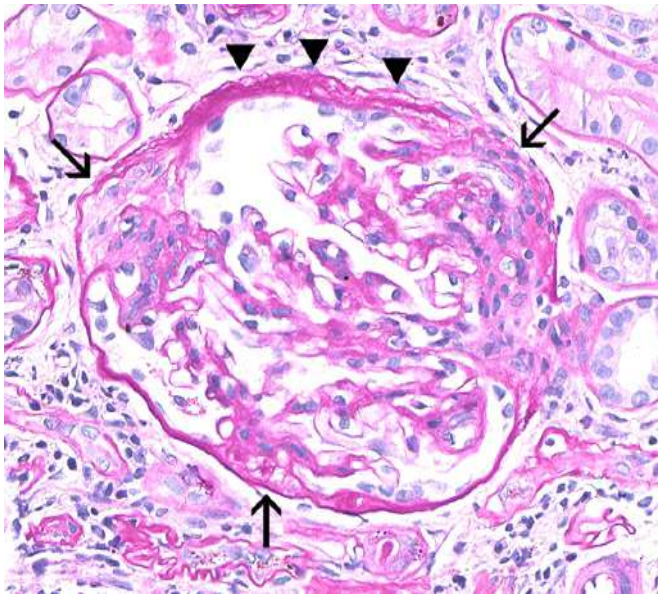
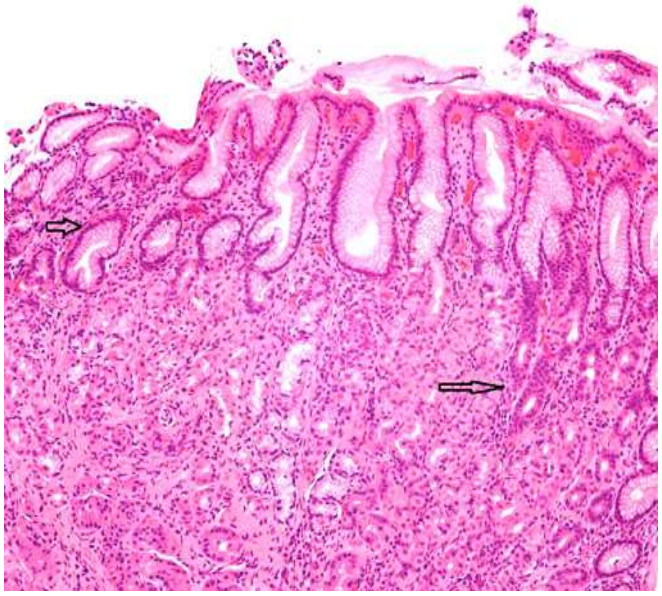


Figure 8: Inflammatory Infiltrate in Stomach Mucosa.



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Original Research Paper

An Autopsy-Based Comparative Study on Alcohol-Related Versus Non-Alcohol-Related Liver Pathologies

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ABSTRACT

Introduction: The liver is uniquely exposed to metabolic, toxic, infectious and circulatory insults. While chronic alcohol use remains a dominant cause of progressive liver disease, a substantial burden of non-alcohol-related and clinically silent liver pathology is encountered at autopsy.

Aim: To compare alcohol-related versus non-alcohol-related liver lesions detected at autopsy using six parameters: age, sex, histopathological lesions, gross morphology, cause of death correlation, and incidental/silent findings.

Materials and Method: The present study was undertaken in a leading tertiary care institute of North India to deepen the understanding of the distinct histopathological features and progression patterns of liver diseases influenced by alcohol consumption versus other etiologies, using autopsy specimens as a definitive diagnostic tool. Liver specimens were examined grossly and histologically (H&E, with special stains when indicated), and demographic/inquest data were reviewed to classify cases into alcohol-related and non-alcohol-related pathology groups.

Results: Alcohol-related pathology predominated in males (M:F ≈ 11.5:1) and in the third to fifth decades, with the classical histological spectrum of fatty change, hepatitis and cirrhosis being most frequent. The study observed fatty change in 34% of cases, hepatitis in 14% and cirrhosis in 8%. Grossly, greasy and nodular livers were common in alcohol-related cases. These lesions frequently contributed to natural deaths related to hepatic decompensation or multi-organ failure. Non-alcohol-related findings (congestion 6%, granulomas 2%, abscess 2%, incidental neoplastic or infiltrative lesions) were more heterogeneous and frequently incidental in trauma, poisoning, or cardiac deaths.

Conclusion: Autopsy histopathology distinguishes a predictable alcohol-related triad from a diverse spectrum of non-alcoholic liver pathology.

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INTRODUCTION

Autopsy examination remains a cornerstone to identifying disease processes, determining cause of death, and revealing silent lesions not diagnosed during life.[1] The liver, as the principal metabolic organ, is particularly vulnerable to injury from alcohol, viruses, toxins, drugs, metabolic syndrome, and circulatory disturbances.[2] Alcoholic liver disease (ALD) follows a well-defined pathological spectrum-fatty liver, alcoholic hepatitis, and cirrhosis-with significant clinical and Forensic implications.[3]

While alcohol is a major etiological factor, non-alcohol-related liver diseases also contribute significantly to morbidity and mortality. These include non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), chronic passive congestion secondary to cardiac disease, granulomatous lesions due to tuberculosis or sarcoidosis, and incidental neoplastic deposits.[4-7] Importantly, these conditions are often clinically silent and only discovered at autopsy.[2,8-15]

Multiple autopsy-based studies, both international and Indian, have highlighted the frequency of such silent liver lesions. Amarapurkar[11] reported a wide histological

spectrum in HIV-positive individuals, while Tuusov[12] in Estonia documented high prevalence of alcohol-related pathologies among premature deaths. Indian studies such as those by Behera,[13] Choudhury,[14] and Porwal[15] demonstrate similar findings, reinforcing that both alcohol-related and non-alcohol-related lesions are of great Forensic and epidemiological importance.

Histopathological evaluation in autopsy not only provides insight into hidden disease burdens but also refines cause-of-death determination in medicolegal settings.[16] This study evaluates and compares alcohol-related and non-alcohol-related liver lesions in autopsy cases using six well-defined parameters, with an emphasis on integrating published data with the Amritsar autopsy dataset.

MATERIALS AND METHODS

The present descriptive study was conducted on 100 consecutive autopsies performed at mortuary of Guru Nanak Dev Hospital attached with the Government Medical College, Amritsar. The Ethical clearance was obtained from the Institutional Ethics Committee prior to the conduct of study. Case selection: All cases were medicolegal autopsies. Decedents with advanced decomposition, extensively burned remains, drowning where hepatic autolysis precluded evaluation, and cases in which consent could not be obtained were excluded.

Specimen handling: After routine medicolegal autopsy, the liver was excised, weighed, and examined for surface changes and cut-surface morphology. Representative tissue samples from both lobes and any visible lesions were fixed in 10% buffered formalin, processed, embedded, and stained with hematoxylin and eosin. Special stains (e.g., Masson's trichrome, Ziehl–Neelsen) were applied when required. Data collection: Demographic information (age, sex, residence), case history (alcohol intake, comorbidities), and cause of death were obtained from hospital records, inquest papers, and family history when available.

Classification

- Alcohol-related pathology: documented history of alcohol abuse or classic alcoholic lesions (macrovesicular steatosis, ballooning degeneration, Mallory bodies, bridging necrosis, cirrhosis).
- Non-alcohol-related pathology: no alcohol history and/or lesions not directly linked to alcohol (congestion, granulomas, abscesses, hamartomas, metastasis).

RESULTS

In the Amritsar series (n=100), alcohol-related lesions were concentrated in males aged 21–50 years, accounting for nearly 78% of alcohol-positive cases. The male-to-female ratio was approximately 11.5:1 in alcohol-related pathology, consistent with other regional studies.[12,16,18] **Table 1** illustrates the age based predominant hepatic findings. Grossly, alcohol-related livers frequently displayed greasy cut surfaces (40%) and nodularity (25%) consistent with cirrhosis. Non-alcohol-related cases included nutmeg appearance (5%), cysts (2%), and otherwise normal cut surfaces (28%). Fatty change (34%), hepatitis (14%), and cirrhosis (8%) formed the alcohol-related triad. Non-alcohol-related lesions included chronic passive congestion (6%), abscess (2%), granulomas (2%), and incidental infiltrates.

Table 1. Age Distribution of Autopsy Cases (n=100).

Age (Years)	Cases (%)	Predominant Findings
<20	03 (03%)	Rare; non-alcoholic congestion/incidental
21–40	54 (54%)	Fatty change, hepatitis (alcohol-related)
41–50	24 (24%)	Cirrhosis, hepatitis (alcohol-related)
51–70	16 (16%)	Congestion/abscess (non-alcohol)
>70	03 (03%)	Degenerative/age-related changes

Table 2 demonstrates the histopathological observations in the present study. In 32% of cases, liver histology was unremarkable despite systemic disease or non-hepatic cause of death. Alcohol-related pathology was frequently contributory or primary to death. In contrast, non-alcohol-related lesions were more often incidental, with deaths due to trauma (35%), cardiac causes (30%), poisoning (7%), and sepsis (6%). Autopsies revealed numerous clinically unsuspected lesions. Alcohol-related cases sometimes showed early fatty changes in individuals dying of unrelated causes. Non-alcohol-related incidental findings included granulomas, leukemic infiltrates, bile duct hamartomas, and metastatic deposits.

Table 2. Histopathological Findings (n=100).

Finding	n (%)	Typical Association
Fatty Change	34	Alcohol-related/NAFLD
Hepatitis	14	Alcoholic/viral/inflammatory
Cirrhosis	8	End-stage alcoholic/chronic
Congestion	6	Cardiac/circulatory (non-alcohol)
Abscess	2	Infective (non-alcohol)
Granulomas	2	TB/sarcoidosis
Normal Histology	32	Mixed/incidental

DISCUSSION

This study reinforces the well-established triad characteristic of alcohol-related liver disease, which comprises fatty change, alcoholic hepatitis, and cirrhosis. These pathologies predominantly affect younger to middle-aged men, aligning with prior findings in multiple epidemiological studies.[3,12,15] Among these, fatty liver remains the most frequent and early histopathological manifestation, consistent with observations reported in diverse populations such as those from Greece, India, and Estonia.[8,12,15] This fatty infiltration of hepatocytes marks the initial response of the liver to excessive alcohol consumption and can be reversible with abstinence.

Although cirrhosis occurs less frequently than fatty changes in terms of case numbers, it presents a far graver prognosis and is associated with significantly higher mortality rates. This finding aligns with the data reported by Globa[16] and Bhagat[17] emphasizing the severe clinical consequences once irreversible fibrotic changes dominate the hepatic architecture. Cirrhosis represents the culmination of chronic alcohol-induced injury, often accompanied by complications such as portal hypertension and hepatic failure, highlighting its critical public health impact. Alcohol related pathology often leads to hepatic encephalopathy, variceal hemorrhage, and multi-organ failure secondary to cirrhosis or hepatitis. Comparable findings were reported by Globa[16] and Tuusov,[12] who emphasized the contribution of cirrhosis to premature mortality.

In contrast to alcohol-related liver lesions, non-alcohol-related liver pathologies exhibit a high degree of heterogeneity, influenced heavily by geographic, environmental, and demographic factors. The diversity of these lesions is reflected in their variable presentation and underlying etiology. Chronic passive congestion of the liver, frequently observed in individuals succumbing to cardiac-related deaths, was conspicuous in this group. Such congestion, as noted in studies by Rathod[18] and Simon,[19] occurs due to impaired venous outflow and contributes to secondary hepatic injury.

A noteworthy but relatively rare finding in this study is granulomatous hepatitis, which was similarly identified in Choudhury's work.[14] This condition is significant because it often reflects underlying infectious causes, such as tuberculosis, which remains endemic in certain regions. The detection of granulomatous inflammation in the liver through autopsy provides crucial insight into the burden of infectious diseases that may otherwise go undiagnosed during life. Alcohol related silent lesions have been highlighted in series from Burla, Imphal, and Ajmer.[13-15]

Additionally, incidental findings of neoplastic or infiltrative lesions during autopsy underscore the indispensable diagnostic value of postmortem examinations. Such lesions may remain clinically silent and undetected, yet their identification can reveal systemic diseases with significant implications for epidemiology and public health.[13-14] This highlights how autopsy studies serve not only forensic but also broader medical and research purposes by uncovering hidden disease burdens.

Moreover, autopsy data serve as a vital "public health mirror" that reflects the true prevalence of liver diseases within local populations. Several studies from regions like Ajmer,[20] Burla,[13] and Tehran[2] have demonstrated a high prevalence of silent non-alcoholic fatty liver disease (NAFLD) and alcoholic liver pathology, conditions that are often underreported or underestimated in clinical practice. These findings emphasize the importance of comprehensive postmortem evaluations to capture the full spectrum of liver pathology affecting communities.

The role of histopathology in Forensic Medicine cannot be overstated. As Pathak and Mangal[1] have emphasized, microscopic examination of liver tissue is essential for accurate determination of cause of death in medicolegal cases. Histopathological analysis provides definitive evidence to distinguish among various liver pathologies and helps identify potentially preventable disease processes. This knowledge can inform targeted community health interventions aimed at reducing liver disease burdens through strategies such as alcohol abuse prevention, tuberculosis control, and management of metabolic disorders.

CONCLUSION

In conclusion, this study affirms the well-established patterns of alcohol-related liver disease, notably characterized by a consistent triad that significantly contributes to mortality among young and middle-aged men. At the same time, it brings to light the wide variety of non-alcohol-related hepatic lesions, which tend to be more diverse and often incidental findings. The findings underscore the indispensable role of autopsy and histopathological evaluation-not only in enhancing forensic cause-of-death determinations but also as a powerful tool for monitoring liver disease prevalence at the population level. Ultimately, this approach supports

informed public health strategies aimed at preventing liver disease and reducing its burden across different communities.

Limitations of the Study

This study was based on medicolegal autopsies and therefore may not reflect the true prevalence of liver diseases in the general population. In many cases, detailed premortem clinical history and risk factor assessment were unavailable, limiting clinicopathological correlation. Being a single-center study with a limited sample size, the findings cannot be generalized. Advanced histopathological and molecular techniques were not routinely employed.

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Original Research Paper

Evaluation Of Oral Health Status In Children With Parental Usage Of Tobacco Products: An Observational Study

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ABSTRACT

Introduction: Tobacco use is very common practice in Indian household causing a whole series of oral health problems ranging from potentially cancerous changes to life threatening (oral cancer) and serious (periodontal disease) to social (bad breath) issues/problems. Parents act as role model for their children as children spend more time with them so they try to imitate them. Tobacco use in adult is a menace which we are continuously battling, when it comes to children our task is still worsened. Owing to the limited studies available, the study aims at exploring the potential impact of parental tobacco usage on oral and physical health of their children.

Aim: Evaluation of effects of parental use of tobacco on oral health of their children. This study will enable us to judge the burden of tobacco use among children which is rarely reported.

Materials and Method: Total of 750 students have been examined from 2 different schools of rural area in Palghar district of Maharashtra [351 male and 399 female]. After taking consent from the concerned authority for oral health examination, children were examined for BMI and oral findings, they were interviewed for nature of tobacco habits in their parents and the children underwent examination to identify any tobacco habits and related oral lesions. The data obtained was sent for statistical analysis.

Results: Out of 750 children, 33 children have tobacco habit which is 4.4%. Our study found that 234 out of 750 parents have tobacco habit which is 31.2%. Children who have tobacco habit with positive parental usage are 32 out of 234 parents using tobacco which is 13.67%. The statistical analysis revealed a significant association between dental caries in children and exposure to tobacco, with a p-value of < 0.038 (statistically significant association).

Statistically significant correlation ($p< 0.001$) was also observed between positive tobacco usage history in children and the incidence of teeth indicated for extraction. No statistically significant correlation ($p >0.05$) was observed between tobacco use with the prevalence of missing teeth and gingival health among children with a history of tobacco use as compared to children with no tobacco usage. The unpaired t-test analysis yielded no statistically significant difference ($p > 0.05$) in mean BMI values between the two groups, i.e., children without tobacco habits and children with tobacco habits.

Conclusion: This study highlights the growing concern over tobacco usage among children, as this dangerous habit is becoming increasingly prevalent, leading to addiction at a young age. It underscores the urgent need for tobacco cessation interventions for adults, recognizing the profound influence their habits have on their children.

Additionally, the study advocates for the creation and implementation of specialized tobacco cessation protocols tailored for children to address this escalating issue effectively.

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Keywords: Children; Oral Health Status; Parents; Tobacco; Dental Caries.

INTRODUCTION

Tobacco threatens oral health and health as well because it negatively affects our mental health, lifestyle, and body's function. Exposure to passive smoking may lead to various health issues in children, including asthma, allergies, nasal and sinus problems, tooth decay and increased risk of childhood cancer.

Oral health diseases are the most common non-communicable disease in the world and it is associated with negative impact on the quality of life. While existing research highlights the adverse effects of passive smoking on overall health, its specific impact on oral health remains understudied.[1] There is controversy regarding the relationship between parental usage of tobacco and its ill-effect on oral health of their children.[2]

Since second hand smoke exposure is a highly toxic threat to children's health, potentially triggering asthma, cough, wheezing, pneumonia, bronchitis and weight reduction, thus showing its effects on general health.[2] Children living with smoking parents are at higher risk of dental caries due to increased levels of cariogenic bacteria like S. mutans and Lactobacillus in their saliva.[3]

The development of caries is multifactorial, involving factors such as bacterial presence, poor oral hygiene, inadequate fluoride exposure, and reduced salivary flow. Exposure to passive smoke can lead to oral membrane inflammation, impaired salivary gland function, reduced serum vitamin C levels, and compromised immune function.[4] As deleterious effects of smoking on people's health and lifestyle are already proven, in addition to those it can also cause dental caries, discolouration of teeth and periodontal disease.

Longer duration of exposure to cigarette smoke can cause decrease oxygenation, reduce washing effect of saliva and intensify the decay process. Nicotine exposure can alter the balance between tooth demineralization and remineralization.[5] In addition to these aspects another important issue is, children using tobacco because of parental influence which can deteriorate their health. Thus, the study intends to assess the relationship between parental tobacco use and its effects on the oral health and body mass index (BMI) of their children, with a focus on the consequences of passive smoking exposure.

These observations are sparsely studied in the literature. So, our study aimed to evaluate of effects of parental use of tobacco on oral health of their children. This study will try to fill the knowledge gap, providing valuable insights for the development of preventive health programs in the community.

MATERIALS AND METHODS

An observational study in which total 750 students of either gender from class V to X were examined from 2 different schools of rural area in Palghar district of Maharashtra [351 male and 399 female]. The study protocol procedure was approved from the ethical committee, Government Dental College & Hospital, Mumbai vid e letter no 4090/2023 dt 12.10.23. After taking consent from the concerned authority of school for oral health examination, children were examined for oral findings such as dental caries, missing teeth, grossly decayed teeth/ teeth indicated for extraction, periodontal disease, potentially malignant lesions/conditions and BMI (Body Mass Index). They were interviewed for nature of tobacco habits in their parents and all the children were also asked and examined for type of tobacco habit and associated lesions in them. The information obtained was recorded in predesigned proforma. Oral cavity was examined using diagnostic instruments in artificial torch light. Teeth were dried and cleaned using cotton rolls as needed. The collected data was statistically analyzed using SPSS 26 software, with chi-square and unpaired t-tests performed to evaluate the results.

RESULTS

Total of 750 students from 2 different schools of rural area in Palghar district of Maharashtra [351 male and 399 female] were included in the study. Descriptive statistics of 750 children are presented in **Table 1**. Out of 750 children, 33 children have tobacco habit which is 4.4%. Our study found that 234 out of 750 parents have tobacco habit which is 31.2%.

Table 1: Descriptive Statistics of Tobacco use in Overall Population.

Tobacco Usage	Frequency (n)	Percentage (%)
Children	33/750	4.4%
Parents	234/750	31.2%
Children use, parents also use	32/234	13.67%
Children use but parent do not use	1/516	0.19%
Children do not use but	202/234	86%
parents used		

Children who have tobacco habit with positive parental usage are 32 (amongst 33 children using tobacco) out of

234 parents with tobacco habit which is 13.67%. When evaluated, statistical analysis revealed a significant association between dental caries in children and exposure to tobacco, with a p-value of < 0.038 (statistically significant association) as compared to children with no tobacco use **Table 2**.

Table 2: Comparison of Dental Caries status Among Children with Positive Parental use of Tobacco.

(N=234)	Frequency (n)	Percentage (%)
Children without tobacco habits (n= 202)	53 (26.2%)	149 (73.8%)
Children with tobacco habit (n=32)	2 (6.2%)	30 (93.8%)
	Chi square test value = 6.138, p< 0.038* (statistically significant association)	

The data demonstrate a highly statistically significant correlation (p< 0.001) between positive tobacco usage history in children and the incidence of teeth indicated for extraction, **Table 3**. The results indicate no statistically significant correlation (p >0.05) between tobacco use and the prevalence of missing teeth among children with a history of tobacco use, shown in **Table 4**. This study found no significant association between tobacco use and gingival health in children among tobacco users and non-tobacco users, **Table 5**.

Table 3: Comparison of Teeth Indicated for Extraction among Children with Positive Parental use of Tobacco.

(N=234)	Not Indicated For Extraction N (%)	Indicated For Extraction N (%)
Children without tobacco habits (n= 202)	171 (84.7%)	31 (15.3%)
Children with tobacco habit (n=32)	17 (53.1%)	15 (46.9%)
	Chi = 17.38, p< 0.001** (highly statistically significant difference)	

The unpaired t-test analysis yielded no statistically significant difference (p > 0.05) in mean BMI values between the two groups, i.e., children without tobacco habits and children with tobacco habits, shown in **Table 6**.

DISCUSSION

Passive smoking also known as secondhand smoke is the inhalation of tobacco smoke from other people. Secondhand smoke, comprising both sidestream smoke from burning cigarettes and exhaled smoke from smokers, poses health risks, and there is no safe level of exposure to it. Children are particularly susceptible to the harmful effects of secondhand smoke, which can impact both their oral and overall health.[2,5]

Table 4: Comparison of Missing Teeth among Children with Positive Parental use of Tobacco

(N=234)	Not Missing N (%)	Missing N (%)
Children without tobacco habits (n= 202)	192 (95%)	10 (5%)
Children with tobacco habit (n=32)	28 (87.5%)	4 (12.5%)
	Chi = 2.799 , p=0.094 (no statistically significant difference)	

Table 5: Comparison of Gingival Health among Children with Positive Parental use of Tobacco.

(N=234)	Healthy Gums N (%	Gingivitis N (%)
Children without tobacco habits (n= 202)	153 (75.7%)	49 (24.3%)
Children with tobacco habit (n=32)	28 (87.5%)	4 (12.5%)
	Unpaired t test = 2.180, p=0.140 (no statistically significant difference)	

Table 6: Comparison of Mean BMI among Children with Positive Parental use of Tobacco.

(N=234)	Mean BMI	SD
Children without tobacco habits (n= 202)	20.29	5.19
Children with tobacco habit (n=32)	21.11	5.66
	Unpaired t test = -0.818, p =0.414 (no statistically significant difference)	

Parental influence plays a significant role in shaping children's attitude and behavior, including those related to tobacco use and oral health. Exposure to parental tobacco use can increase the likelihood of children adopting similar habits and neglecting their oral health. Our study also revealed that out of 33 children who used tobacco, 32 had parents who also used tobacco, while only one child did not have a parental history of tobacco use. As we all know, kids

learn by observing, and when they see their parent's using tobacco, they may mimic the same habit. Unfortunately, this can lead to lack of emphasis on oral health, as children may not see it as a priority.

Our study revealed a concerning prevalence of tobacco habits among children, with 33 out of 750 participants (4.4%) exhibiting such habits. This sample represents only a small fraction of the population; the actual extent of the problem may be even more alarming. This topic has been largely neglected in discussions and research. A significant proportion of children reported tobacco habits in their parents i.e., 234 parents out of 750 which is nearly one-third.

As children spend a significant amount of time at home, parental influence plays a vital role in shaping their attitudes and habits. Children who grow up with parents who use tobacco are more likely to develop tobacco habits themselves. Our study found that 32 children (13.67%)

out of 234 tobacco using parents had adopted tobacco use themselves. It is worth noting that all the children who showed/had use of tobacco (32/33) has a positive history of tobacco usage in their parents. Only 1 child out of 33 acquired a tobacco habit independently, without any family members' influence. This indicates that guidance from parents is essential in helping children develop positive attitudes and behavior that will benefit them throughout their lives. Dental caries is a diet-induced infectious disease that is widely recognized to progress over time. A study conducted by Fatemeh Hamidifarid et al found that parental smoking significantly increased the incidence of caries in both primary and permanent teeth of children.[5] Data from another study conducted by Yuko Goto et al also suggest that exposure to tobacco smoke may have a dose-dependent effect on the development of dental caries.[6] In our study also the prevalence of dental caries among tobacco using children was 93.8% (30/32), indicating a strong association between tobacco use and dental caries. Hence a statistically significant correlation ($p< 0.038$) was observed between dental caries and tobacco use in children with positive parental use of tobacco as compared to children with no tobacco use. A significant difference ($p < 0.001$) was also found in the dental health of children with tobacco habits, with 46.9% (15/32) requiring tooth extraction.

In our study, no statistically significant difference was observed in the prevalence of missing teeth and gingivitis among children who use tobacco and have a positive parental history of tobacco use. In a study conducted by Raif ALAN et al also showed that there was no evidence for a causative role of parental tobacco smoking in periodontal disease.[7] Many people are unaware about the ill-effects of tobacco on health and the knowledge about the disease they cause was not considered to be serious or life threatening.[8] Research conducted by Gaurav Goyal et al stated that young population of North India lack Knowledge and Practice regarding consumption of areca nut, gutka and tobacco smoking.[9] As in addition to its oral health implications, tobacco use also has adverse effects on general health.

A study conducted by D. Gupta et al stated that if children are exposed to environmental tobacco smoke during childhood, it can be an important risk factor for asthma and respiratory symptoms in their adult life.[10] Therefore, we calculated the Body Mass Index (BMI) of children with a positive parental history of tobacco use. Notably, no statistically significant difference in BMI was observed between children with and without tobacco habits. A study conducted by Sajith Vellappally et al stated that Environmental tobacco smoke can cause dental caries in children, but findings are not conclusive for such studies in adults.[11] As we all know normal colour of gingiva ranges from pink to red, but exposure to passive smoking can deteriorates gingival health as it can lead to gingival pigmentation in children, resulting from increased melanin production.[12]

Parents' behaviour significantly influences their children's choices and there are studies which concluded that kids of tobacco users are more likely to use tobacco themselves which is similar to our study.[13-14] This kind of observation has never been discussed in some families. Tobacco cessation is a complex and challenging concept, with numerous barriers hindering successful quitting among adults. As we all know child learn what parents do, so the most influential method of educating children is through parental modelling, demonstrating good habits and a healthy lifestyle. Nida Talpur et al conducted a study which concluded that caries rate was high in smokers which can be attributed to stress in students and influence of tobacco use among siblings and parents who are smokers.[15]

In a study conducted by Aderonke A. Akinkugbe et al stated that as dental caries is a disease of multifactorial etiology, prenatal and partner smoking could be a possible risk factor for caries in offspring.[16] Another study conducted by Shahram Mosharrafian et al stated that children who are exposed to passive smoking have high rate of caries experience but this effect is primarily due to increase

plaque accumulation in children exposed to passive smoking.[17] Mei-Yen Chen et al conducted a cross-sectional study in rural area which showed that due to lack of knowledge about adverse effects of smoking on their surroundings and specially their child, they smoke around their children which was negatively associated with their health-promoting behaviours.[18] Today's children are tomorrow's citizens who hold the responsibility of building our nation. Tobacco menace is deep rooted, our study is highlighting the part which desire urgent and unified action.

LIMITATIONS

It is an observational study which relies on minors, as children are providing information about tobacco habit in their parents which can be limited and misleading, not appropriately reflecting true scenario. Future studies with more confirmatory objectives are required to evaluate tobacco usage in adults as well as in children as objective tests done for the same purpose would be more conclusive.

CONCLUSION

This study highlights the growing concern over tobacco usage among children, as this dangerous habit is becoming increasingly prevalent, leading to addiction at a young age. It underscores the urgent need for tobacco cessation interventions for adults, recognizing the profound influence their habits have on their children. Additionally, the study advocates for the creation and implementation of specialized tobacco cessation protocols tailored for children to address this escalating issue effectively.

CONFLICT OF INTEREST/FUNDING: Nill

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Original Research Paper

Seasons of Despair: A Medico-Legal Study of Suicidal Deaths and Their Socio-Demographic Correlates in Almora, Kumaon Hills of Uttarakhand

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ABSTRACT

Introduction: The Medico-legal autopsies plays a vital component in the present Day-to-day forensic practices that aids in determining the cause, manner and circumstances of death in all unnatural or suspicious cases. Patterns observed in autopsy populations often reflect broader social and environmental influences. A spring related rise in suicidal behaviour has been noted in literature recently, possibly linked to Seasonal Affective Disorder (SAD) and the "Broken Promise Effect." This current study evaluates the socio-demographic and medico-legal characteristics of autopsy cases in Almora, Uttarakhand.

Objective: To determine the demographic pattern, seasonal trends, and medico-legal features of autopsies conducted between July 2024 and June 2025 at SSJGIMS&R, Almora, with special emphasis on suicidal deaths.

Materials and Methods: This retrospective, descriptive study was conducted over a period of one year in the Department of Forensic Medicine & Toxicology, SSJGIMS&R, Almora, on a total of 108 medico-legal autopsies. Data from autopsy records, inquest papers, and police documentation were analyzed for demographic variables, season, manner and cause of death, and system involvement.

Result: Among 108 cases under study, 54 (50%) cases were suicides ie most common manner of death, predominantly seen in males (75%), with the highest incidence in the age group 31–40-year. Maximum number of suicidal deaths(39.8%) occurred during Spring season, suggesting a strong seasonal pattern possibly influenced by SAD and the "Broken Promise Effect." Hanging was the most common method of suicide.

Conclusion: Findings in our study revealed that middle-aged urban males forms a high-risk group, with pronounced seasonal peaks in spring seasons in the Kumaon region of India. Recognizing environmental and psychosocial contributors is essential for prevention of such deaths in the future

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INTRODUCTION

The Medico-legal autopsies are fundamental to forensic investigation, providing reliable evidence to interpret deaths that occur under suspicious, sudden, or unnatural circumstances. It acts as an essential tool in determining the cause, manner and circumstances of death in all cases of sudden, suspicious, or unnatural deaths. Beyond its legal significance, medico legal profiling provides valuable epidemiological insights into mortality trends, shows the prevailing socio-demographic, cultural, and environmental factors of a community. In regions such as Kumaon hills in Uttarakhand, where climate and geography vary markedly, mortality trends identified through autopsy holds significant value for public health and forensic interpretation. Understanding these patterns not only aids in the administration of justice but also helps the health authorities in developing preventive strategies.

Among the various manners of death, suicide remains a major contributor to unnatural deaths globally, driven by a complex interplay of psychological, biological, and socio-environmental factors Multiple studies conducted in countries other than India, demonstrate higher suicide counts during the spring months.[1-2]

The rise in suicides seen with the arrival of spring has been linked to two important concepts. One is Seasonal Affective Disorder (SAD), a mood disturbance influenced by changes in daylight. The other is the "Broken Promise Effect," which suggests that emotional distress can deepen when the improving weather and longer days of spring fail to match an individual's ongoing internal struggles. Together, these ideas offer a very clear explanation for why suicidal thoughts and actions may increase during spring, even as the external environment becomes more favorable.[5-6]

The present study was designed to examine the socio-demographic and medico-legal characteristics of all autopsy cases conducted over a period of one year at the Soban Singh Jeena Government Institute of Medical Sciences & Research (SSJGIMS&R), Almora, with a special focus on suicidal deaths and their seasonal variation. By assessing how suicide cases cluster during the spring months and considering their possible association with Seasonal Affective Disorder (SAD) and the "Broken Promise Effect," the study aims to underscore the role of seasonal psychological influences in shaping suicide patterns. These findings are aimed to serve as a foundational reference for future studies and to support forensic practitioners, law enforcement, and public health officials in planning focused preventive strategies and safety initiatives for the Kumaon region of Uttarakhand.

MATERIALS AND METHODS

This present retrospective descriptive study was conducted in the Department of Forensic Medicine & Toxicology at SSJGIMS&R, Almora. All medico-legal autopsies performed from 1st July 2024 and 30th June 2025 were included, a total 108 cases were considered as study population. All data were collected from postmortem registers, autopsy reports, police inquest papers, and hospital documents available at the medico legal records section of the department. The parameters such as age, sex, marital status, residence, seasonal pattern, time since death, manner and cause of death, asphyxial subtype, mechanism or weapon used, and major body system involved were taken for analysis in all cases

In order to fulfill the secondary objective emphasizing suicidal deaths, all suicide cases were further reviewed for demographic patterns, method adopted, and seasonal clustering, with special attention to cases occurring in the spring season to explore possible associations with Seasonal Affective Disorder (SAD) and the "Broken Promise Effect."

All data obtained were cross-verified and entered into a master table for further analysis. Descriptive statistical tools were employed, and results were expressed in frequencies and percentages. Ethical principles outlined by the Indian Council of Medical Research (ICMR) for biomedical research on human subjects were strictly followed. Institutional approval was obtained from the Head of Department and Principal, SSJGIMS&R, Almora, prior to initiation of the study. Complete confidentiality of personal identifiers and case-specific details was maintained throughout the research process.

RESULTS

Out of 108 medico legal autopsy cases, the age group 31–40 years accounted for the highest number of deaths (36 cases; 33.3%), followed by the age group 41–50 (24 cases; 22.2%) and the age group 21–30 years(15 cases; 13.9%). The age groups 0–10 years and 11–20 years each had 3 cases (2.8%) and no cases were reported above 80 years of age. Thus, the majority of deaths occurred among middle-aged adults (31–50 years).**(Figure 1)**

It was observed that, among the 108 cases, 81 (75%) were males and 27 (25%) females, showing a marked male predominance. **(Figure 3**). It was also observed that most of the individuals in this study were married (72; 66.7%), with unmarried persons accounting for only 36 cases (33.3%). A significant share of the deaths involved people were residence of urban areas (77; 71.3%), whereas rural residents constituted only 25 cases (23.1%). This urban predominance clearly suggests that, factors associated with city life such as psychosocial pressure, occupational demands, and lifestyle strain, may play a role in shaping the pattern of unnatural and suicidal deaths observed in this region.

The seasonal analysis during study period showed a clear trend that, spring seasons recorded the highest number of deaths (43 cases; 39.8%), followed by summer (38; 35.2%), while autumn (18; 16.7%) and winter (9; 8.3%) contributed comparatively fewer cases **(Figure 2)**. Analysis of death based on manner, reveals that suicidal deaths constituted 54 cases (50%), followed by accidental (37; 34.3%), natural (9; 8.3%), and homicidal (8; 7.4%).

Figure 1: Age wise Distribution of Medico Legal Autopsy Cases.

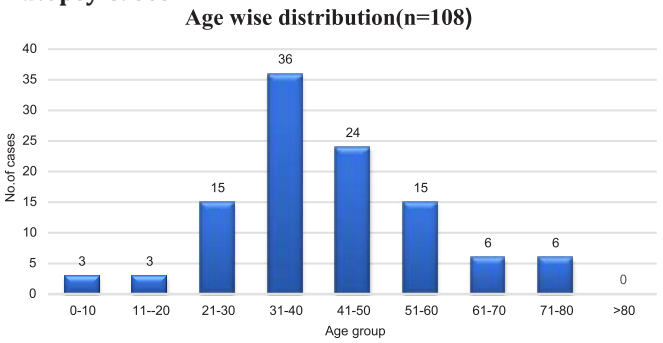


Figure 2: Seasonal Distribution of Medico Legal Autopsy Cases.

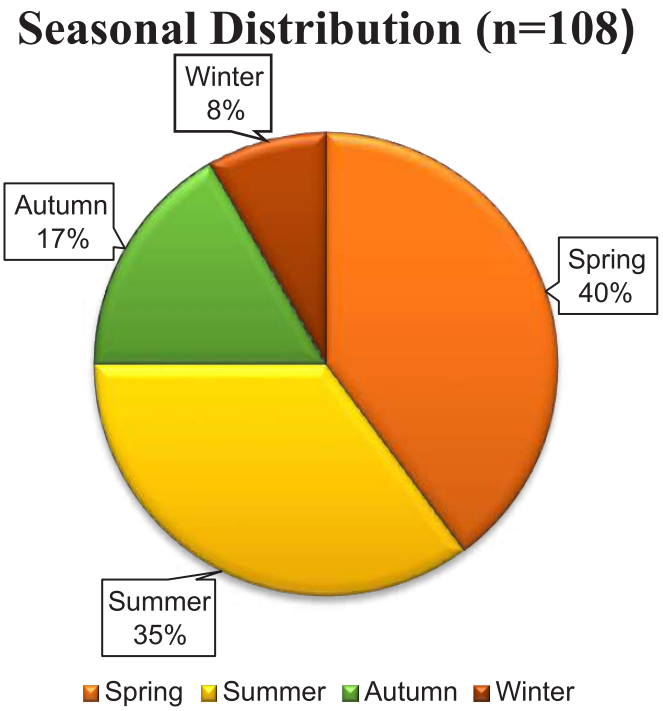


Figure 3: Distribution of Medico Legal Autopsy Cases Based on Manner of Death.

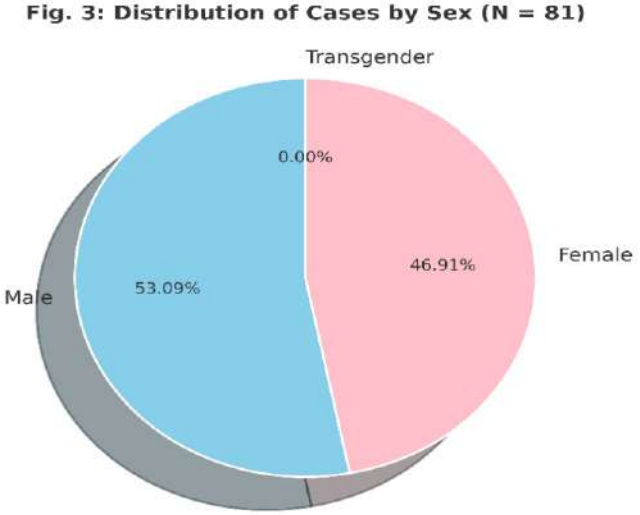


Figure 4: Distribution of Medico Legal Autopsy Cases Based on Manner of Death.

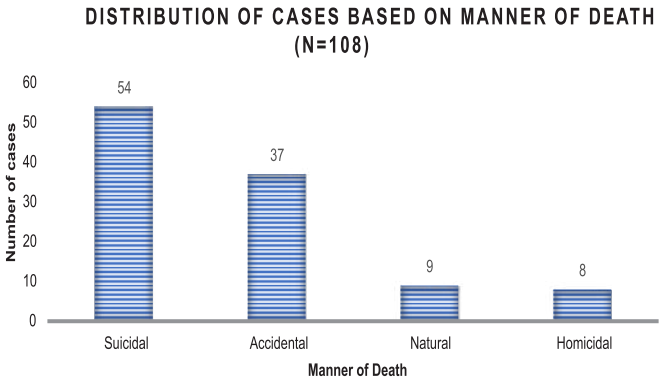
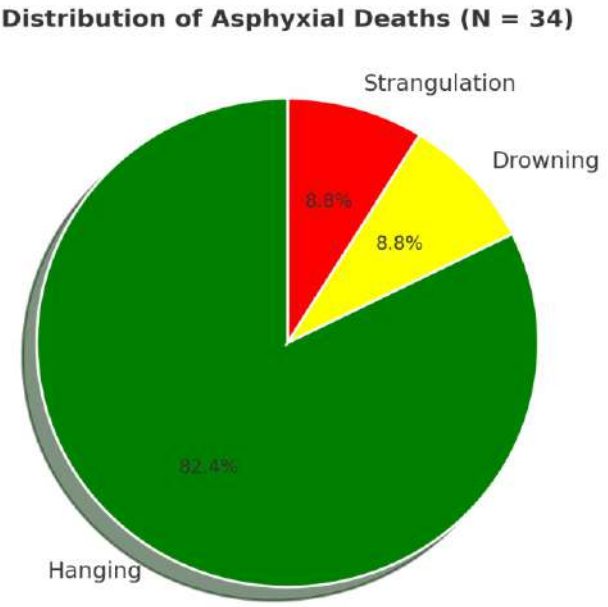


Figure 5: Distribution of Asphyxial Death Cases.



(Figure 4) The concentration of suicidal cases in spring points toward a notable seasonal influence, possibly linked to Seasonal Affective Disorder (SAD) and the "Broken Promise Effect." During this period, which typically represents renewal and positivity, individuals struggling with unresolved emotional distress may experience a sharper rise in psychological vulnerability.

It was also observed that, among all medico legal deaths, asphyxia (34 cases; 31.5%) was the leading cause, followed by vehicular accidents (20; 18.5%), poisoning (18; 16.7%), and natural causes (9; 8.3%). No railway or industrial accident was recorded. **(Table 1)**

Among the suicidal deaths, hanging was the most common method (28 cases; 51.8%), followed by poisoning (18; 33.3%). Most victims were brought in dead (84; 77.8%), with a majority found at home (45; 41.7%) or from roadside/public locations (39; 36.1%). The time since

death was estimated to be less than 12 hours in 57 cases (52.8%), 12–24 hours in 39 cases (36.1%), and more than 24 hours in 12 cases (11.1%).

The analysis of affected body systems revealed the respiratory system (42; 38.9%) as the most frequently involved, primarily due to asphyxial deaths, followed by the central nervous and skeletal systems (29; 26.9%), and the circulatory system (25; 23.1%). **(Table 3)**

Table 1:- Distribution of Cases based on Cause of Death.

Cause of Death	Number of Cases	Percentage (%)
Asphyxia	34	31.48%
Vehicular Accidents	20	18.52%
Poisoning	18	16.67%
Natural Cause	9	8.33%
Fall from Height	6	5.56%
Reserved Pending	6	5.56%
Electrocution	5	4.63%
Physical Assault	4	3.70%
Burns	3	2.78%
Snake Bite	2	1.85%
Firearm Ammunition	1	0.93%
Railway accidents	0	0.00%
Industrial accidents	0	0.00%
Total	**108**	**100.00%**

Table 2: Distribution of Cases based on Weapons Used.

Weapons Used	Number of Cases	Percentage (%)
Ligature Material	31	28.70%
Blunt force	27	25.00%
Poison	18	16.67%
Electric current	5	4.63%
Sharp Weapon	3	2.78%
Water/Liquid	3	2.78%
Thermal	3	2.78%
Animal Bite	2	1.85%
Firearm	1	0.93%
No specific Weapon	15	13.89%
Total	**108**	**100.00%**

Table 3: Distribution of Cases based on Type of Body System Involved.

Body System Involved	Number of Cases	Percentage (%)
Respiratory System	42	38.89%
Central Nervous System with Skeletal System	29	26.85%
Circulatory System	25	23.15%
Combined	10	9.26%
Gastro Intestinal System	2	1.85%
Total	**108**	**100.00%**

To put in a nutshell, our study clearly shows that suicides constituted the most common manner of death, with young and middle-aged men from urban areas being the most affected. Most of these incidents occurred during the spring months and frequently involved asphyxial methods, particularly hanging.

DISCUSSION

The present study revealed that among all medico legal autopsies conducted at SSJGIMS & R, Almora, Kumaon Hills of Uttarakhand, suicidal deaths constituted majority of the cases(50%)[1-2] with a significant springtime predominance (39.8%). This seasonal clustering points out that suicide as a major medico-social and forensic challenge in hilly northern regions of India. Similar findings have been noted in regional studies conducted by Singh PI et al, Kanchan et al, where suicide accounted for a major proportion of unnatural deaths.[1-2]

The basic parameters such as age and sex distribution analysis showed that maximum incidence of case were noted among 31 to 40 years age group and males, which is consistent with multiple Indian and global research studies reporting higher suicide rates among young to middle-aged men. This trend may be due to increased socio-economic responsibilities, occupational pressure, and greater access to lethal means. Similar basic observations have been noted in studies conducted by Singh PI et al, Kumar S et al, Khurana P et el and Mann Gr et al.[1,3,4,16,18] Married individuals accounted more, indicating interpersonal and psychosocial stress as key contributing factors in suicidal behaviour.[17]

A remarkable striking outcome of this research was that, the “springtime surge in suicides”, and this clear peak in spring parallels with multiple international observations of seasonal variation in suicide.[6-7] In an international multi-country analysis done by Yu et al. confirmed that spring peaks across continents irrespective of geography or culture.[6] This convergence strongly suggests that the seasonal influences transcend climatic boundaries, highlighting the interplay of biological, psychological, and social determinants.

One of the possible biological explanations for this rise in suicide cases during spring is the Seasonal Affective Disorder (SAD) model. In general, SAD is characterized by recurring depressive symptoms that fluctuate with seasonal changes, particularly variations in daylight exposure and photoperiod.[9-10] As winter shifts into spring,

the body usually experiences a swift adjustment in circadian rhythm along with changes in serotonin regulation and melatonin levels. These physiological changes can disrupt emotional stability and influence behaviour.[11,17] Due to this unusual "energy-before-mood" effect, where physical activity levels improve before emotional regulation is restored, it may heighten the risk of suicide tendency among indiviuals. As a result, individuals may be more likely to act on suicidal thoughts during early spring.[11-12] Several findings from meta-analyses suggests that suicide rates tend to rise noticeably during spring and early summer, showing an increase of roughly 11–23% compared to the winter months.[13]

Interestingly besides the biological explanations, the "Broken Promise Effect" provides an additional psychological perspective for the incidence of suicides more during spring. As per the original research done by Gabennesch, it points out that, when the outside world reflects renewal and positivity, individuals struggling with long-standing emotional difficulties may feel even more hopeless because the contrast between their inner distress and the improving environment becomes more pronounced.[11,13-14] This dissonance between societal positivity and personal pain, may precipitate to self-destructive acts, particularly among those facing unmet aspirations or emotional exhaustion.[15-16]

Recently a medico legal autopsy profiling study conducted by Mann Gr et al showed that, hanging was the leading cause of death in suicidal cases, in India and followed by Poisoning, this observation was in consistent with our study conducted in Kumaon hills of India ie. hanging (51.8%) and poisoning (33.3%).[4,18] The predominance of asphyxial mechanisms in our study explains the reason for high proportion of respiratory system involvement (38.9%).

Its very evident that, from both the forensic and public-health viewpoint, analysing medico-legal autopsy data is crucial for identifying mortality patterns as well as understanding how these vary across seasons. Incorporating seasonal indicators into routine medico legal autopsy documentation will help us in direct community-based prevention efforts and strengthen mental-health support services, particularly during spring and early summer when risk appears to be elevated. Although the retrospective nature of this study restricts firm conclusions about causation, the close agreement between our observations and prior regional as well as international research studies suggests that seasonal changes do have a notable impact on suicidal behaviour. [5-6,9-10]

CONCLUSION

Our research study revealed that suicidal deaths constituted the largest proportion of medico-legal autopsies conducted at SSJGIMS&R, Almora, Kumaon Hills of Uttarakhand, with a noticeable rise in cases during the spring months. Young and middle-aged urban males accounted for the most affected group, and hanging emerged out as the predominant method of self-harm. These observations highlight the significant influence of demographic and seasonal factors on suicide patterns in this hilly region of India.

The findings of our study strongly supports that Seasonal Affective Disorder (SAD) and the "Broken Promise Effect" underlie the springtime rise in suicidal deaths. Hence, addressing the requirement for cross-disciplinary collaboration between forensic experts, psychiatrists, and public health professionals.

From both medico-legal and preventive medicine perspective, systematic evaluation of autopsy data serves as an important indicator for identifying shifts in suicidal trends. By strengthening mental-health outreach, improving access to psychological support, and promoting awareness during high-risk periods might help to mitigate the self-inflicted deaths in upcoming years. Hence, with a beautiful and mindful quote "Spring brings light to nature but sometimes darkness to minds", we conclude our study.

LIMITATIONS

This retrospective study was completely based on previously recorded documents, the analysis depended on the quality and completeness of existing files, which may contain gaps or inconsistencies that could influence the findings and limiting the depth of analysis.

All medico legal autopsies cases under study were conducted at one government medical college in Almora, which may not accurately reflect the whole medico-legal patterns across the entire Kumaon region or other parts of Uttarakhand.

Key factors such as mental-health history, socioeconomic pressures such as substance abuse, prior self harm, specific environmental metrics like temperature changes, or humidity were not available in the records, limiting the ability to relate suicides to underlying psychosocial issues/ seasonal effects.

CONFLICTS OF INTERESTS/FUNDING: Nill

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Original Research Paper

Anthropometric Reconstruction of Stature from Percutaneous Long Bone and Digital Measurements in a North Indian Young Adult Population

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ABSTRACT

Introduction: Reconstructing stature from fragmented remains is an important part of the forensic identifying process. However, when non-native or skeletal-based regression equations applied to Indian populations, the errors are likely to be high in a view of ethnic differences in limb ratios. The objective of this study was to establish population-specific regression models for the estimation of stature using percutaneous measurements of long bones, arm span and digital segments in a North Indian young adult sample.

Materials and Method: A cross-sectional study was conducted on 150 healthy North Indian students (77 males, 73 females) aged between 19 and 21 years. Stature, arm span and percutaneous lengths of the humeri, radii, ulnae, femora and tibiae as well as hand/ finger dimensions were taken. The formulas were calculated by regression analysis according to sex, based on Pearson's correlation.

Results: Sexual dimorphism was seen for all p< 0.001). The arm span correlated best with stature in men (r=0.68) and women (r=0.81), with the lowest SEE. Among the long bones, tibia and ulna were the most consistent predictors. Interestingly, digital dimensions such as index finger and hand length were found to be robust proxies when excluding principal long bones. The resulting percutaneous models showed superior performance compared with existing skeletal standards.

Conclusion: This research emphasizes that it is important to apply regional, sex-specific regression formulas in forensic practice. Arm span and hand measurements can be used in place of conventional long bone measures among North Indians.

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INTRODUCTION

Identification of the human remains is an important issue in forensic science especially in cases of dismemberment, decomposition and mutilation where direct measurement of height is not possible. Height, as well as age, sex and ancestry are included in this biological profile that can help reduce the number of possible identities for a victim.[1]

Anatomical methods that add up skeletal units have high accuracy but they decide on full skeletal recovery, something not always accomplished in mass disasters or cases of high-impact trauma. As a result, regression models based on long bone lengths are now the established mathematical tool in stature estimation.[2]

Nevertheless, anthropometric studies have shown that the relationship between limb lengths and height is population-specific as it is under genetic, environmental and dietary control factors that determine body composition.[3] The formulae derived either from the western world underestimate or overestimate our population and hence, regression models are needed to be developed for India.[2,4]

In addition, dry bone standards are still the basis for most of available historical standards and do not take into account the thickness of soft tissues present in fresh body

or living bodies measurements. This difference highlights the importance of "percutaneous" criteria having direct application to body donors and living subjects.[5,6]

Recent research has even widened the scope from long bones to investigate the effectiveness of "digital morphometry"- that is, hand and finger dimensions—as trustworthy estimates for stature when primary limb bone remains are missing.[7]

However, for the forensic purpose population from North India only limited data on long bone and digital percutaneous measurements are available. The present study tries to fill this lacunae by obtaining sex-specific regression equations for the estimation of stature taking into account multiple parameters in a young adult North Indian population.

MATERIALS AND METHODS

This cross-sectional study was performed on 150 healthy undergraduates (77 men and 73 women) aged 19 to 21 years from North Indian population. Those with a history of skeletal deformities, fractures, or nutritional disturbances influencing growth were not included in the study. All subjects provided written informed consent before participation.

Anthropometric measurements were recorded from the right side of the body to ensure uniformity. Height measurements were obtained to the nearest 0.1 cm in a barefoot standing position according to the Frankfort horizontal plane, using a standard stadiometer. Body weight were measured by digital weighing balance calibrated.

Percutaneous lengths of the long bones (femur, tibia, humerus, radius and ulna) and arm span were taken with a flexible steel tape and an anthropometer rod, identifying standard bony landmarks.[5] Short segments such as those of hands and fingers were measured in centimetres using a sliding calliper 0.01 cm precision. Statistical analysis was carried out, employing Pearson's correlation and linear regression to form sex-based models for stature estimation.

RESULTS

The total number of subjects (77 males and 73 females) involved in the study was 150, between age group ranges from 19–21 years. Significant sexual dimorphism ($p < 0.001$) was noted in all measured dimensions. Males were taller, heavier, and had greater skeletal dimensions compared to females; the mean stature for males was 172.40 ± 7.00 cm while that for females was 161.43 ± 7.61 cm.

Correlation Analysis

We calculated Pearson's correlation coefficients (r) to determine the relationship between stature and somatometric parameters. In the whole population, Arm Span showed the highest positive correlation with stature ($r = 0.783$, $p < 0.001$), closely followed by Thumb Length ($r = 0.599$) and Index Finger Length ($r = 0.586$).

Sex-specific analyses revealed specific nuances. The correlation between Arm Span and stature was especially high in females ($r = 0.812$), this variable being sensibly superior to all the rest. It is worth noting that the bone lengths of the upper limb (Humerus, Radius, Ulna) demonstrated moderate correlations in females [$0.38 \leq r \leq 0.41$], while Foot Length ($r = 0.099$; $p = 0.405$) and trunk measurements at the umbilicus did not reach significant levels of correlation with height.

In comparison, males had moderate-to-strong correlations over a broader set of variables. In males, Arm Span ($r = 0.684$) stood out once more as the best predictive variable, while digital measurements such as Index Finger ($r = 0.602$) and Ring Finger ($r = 0.595$) also had better predictive power than the Femur ($r= 0.352$) and Tibia ($r= 0.360$).

Regression Models and Stature Estimation

Linear Regression formulas for stature reconstruction were proposed. The performance of the models was assessed by the Standard Error of Estimate (SEE) and Coefficient of Determination (R2).

Single Parameter Models: Among all the models, the regression equation of Arm Span showed the best estimation (lowest SEE values), with SEE = 4.48 cm for females and 5.14 cm for males. Among the hand dimensions, the Index Finger was found to be the best single digit for stature estimation in males (SEE = 5.63 cm), while Hand Length was the best predictor in females (SEE = 7.08 cm).

Multifactorial Models: Stepwise multiple regression analyses were used to test whether combining parameters improved predictive accuracy. The composite of all five long bones (Femur + Tibia + Humerus + Radius + Ulna) achieved an R2 = 0.330 for males and 0.327 for females. Although this multi-factorial approach was superior to single long bone models (e.g., Femur only yielded an R2 = 0.124 in males), it still fell short of the simple Arm Span

model (R2 = 0.468 in males).

Comparative Accuracy

The analysis showed that proximal limb segments (Humerus, Femur) were generally less reliable predictors than distal ones (Hand, Fingers) or composite measurements (Arm Span) in this percutaneous population. Trunk (Umbilicus to Suprapubic) measurements had weak to no association ($r < 0.25$) and high SEE values (>9.0 cm), thus not being suitable for forensic stature estimation in this population.

DISCUSSION

Estimation of stature from incomplete human remains is one of the key factors in forensic anthropology. The present study on a North Indian young adult population has expounded the somatometric relationship of stature with percutaneous dimensions of long bones, arm span, and digital segments.

The findings confirm the biological sexual dimorphism, wherein all recorded parameters have male means that are significantly larger ($p < 0.001$) than female means. This dimorphism, accounted for by the longer duration of pubertal growth and a different hormonal profile in males, requires sex-specific equations to be computed as pooling data would yield extremely large standard errors.[1]

Stature Surrogate of Arm Span

The most important aspect of this study was the remarkable reliability of arm span in estimation of stature compared to all long bones individually. This is consistent with results obtained in a North Indian population in which males showed arm span to be 2–3 cm greater than height.[8] Our findings support the classic "Rule of Squares" (which states that arm span equals height, an approximation that underestimates stature slightly in this population).

It is reported elsewhere in the world that arm span was greater than height in both White and Black populations; however, the North Indian proportions seem closer to their Caucasian samples.[9] Thus, the resultant regression for arm span should be the preferred method of stature estimation in forensic and clinical cases where the trunk is damaged but upper limbs remain intact.

Comparison with Long Bone Standards

It was particularly important in this study that the percutaneous models derived could be compared against well-established historical standards. When the classical equations of Trotter and Gleser[2] were applied to our male data, the femur MAE was 10.29 cm-almost twice that of our population-specific model (MAE = 5.26 cm).

This difference is primarily methodological since the Trotter and Gleser equations were based on dry skeletal remains, whereas our measurements included soft tissue (skin, muscle, and fat).[2] The "soft tissue offset" results in a different regression slope, which explains why the East African percutaneous tibia formula performed significantly better (MAE = 6.34 cm) than skeletal standards.[5]

Additionally, there was a notable difference when compared to regional standards for Punjabi males.[10] Although their models fit better than Western formulae, they yielded larger error margins (MAE ≈ 7.24 cm for tibia) than our study (MAE ≈ 5.08 cm). The results likely reflect a "secular trend" in growth-the biological phenomenon in which modern populations show increased stature and altered limb proportions due to improved nutrition and healthcare.[11]

Moreover, formulae developed for Central Indian populations gave higher errors for distal limb segments (Radius MAE > 10 cm) in our North Indian sample.[3] This confirms the anthropological principle that regression equations are highly population-specific and cannot be applied across regions without reducing accuracy.[12]

Digital Morphometry and Distal Segments

The comparison of hand and finger dimensions is an emerging trend in forensic science, especially when long bones are missing.[13] Our research shows that hand length is a strong predictor of stature (SEE = 5.9 cm for males), consistent with international data from Australia[14] and Turkey,[6] where SEEs range between 4.5–5.5 cm. A notable finding in our male group was the significant predictive value of the Index and Ring fingers, which correlated similarly to the tibia.

This challenges the traditional reliance on long bones and indicates that, in mass disasters involving limb fragmentation, intact hand segments can be effectively used for stature estimation.[7] Furthermore, our findings align with prior research showing foot length to be a stronger stature predictor in males than females due to earlier foot growth cessation in females.[4]

Multifactorial Analysis

The multifactorial approach revealed that combining bone lengths (Femur + Tibia + Humerus) increased the R^2 value compared to single-bone models. However, consistent

with other studies,[15] the improvement was minimal relative to the added complexity.

Interestingly, in our percutaneous dataset, the "upper limb" combination (Humerus + Radius + Ulna) performed better than the "lower limb" combination, which contrasts with skeletal research where the femur is often the gold standard. We attribute this to the difficulty of locating the percutaneous femoral landmarks in living individuals due to gluteal muscle mass, whereas arm and forearm landmarks are more superficial, allowing greater measurement precision.

Limitations of the Study

The major limitation of this study is the small sample size (n = 150), so that some lengthy tails of the North Indians which may suffer from over representativeness. Also, the research was limited to 19-21-year-olds. Although epiphyseal fusion is complete at this age in large part, some late-maturing males could have remaining growth left in their skeletons and slightly biasing the regression compared to an entirely adult mature sample.

Thirdly, measurements were only made from the right (as in life): it is known that human limbs exhibit directional asymmetry and these formulae may not equally well apply to left lateralised individuals. Lastly, being a percutaneous study, the range of error accounts for variations in muscle tone and subcutaneous fat which can differ in decomposed forensic osseous remains where soft tissue is not as present.

Suggestions for Future Research

In future studies, it may be necessary to increase the sample size for a larger age range (18–60 years) to represent aging loss of stature and verify whether such proportions exist among older subjects. We suggest that other investigators should estimate the 'soft tissue correction factor' by performing a comparison study with imaging (e.g. CT, MRI) to examine the precise difference in percutaneous and osteometric length within subjects.

Furthermore, validation of the prediction models with independent samples in the same region is warranted to assess the generalization capacity of the derived regression equations. Last, studies which include foot breadth and hand breadth in addition to lengths may assist in development of multivariate models that could decrease the Standard Error of Estimate and determine appropriate thresholds for forensic identification.

CONCLUSION

The present investigation suggests that percutaneous limb and digital segment measurements are good predictors of height in "North Indian" young adult subjects. Arm Span as the best single predictor, confirming its role as a principal proxy where standing height is not measurable. The findings unambiguously show that the population-specific regression equations perform much better than those based on historical skeletal standards and/or regional derived formulae in modern forensic context applications. Additionally, the high predictive value of Hand and Finger dimensions provides an important alternative for the estimation of fragmentary bones when the large, long bones have been destroyed.

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Table 1: Summary of Linear Regression Analysis and Correlation of Anthropometric Parameters (Long Bones) With Stature.

Parameter	Sex	Mean ± SD (cm)	r	R^2	SEE (cm)	Regression Equation	p
Humerus	Female	29.92 ± 3.47	0.340	0.116	7.211	H = 0.75(humerus) + 139.14	0.0033
	Male	32.35 ± 3.09	0.485	0.235	6.166	H = 1.10(humerus) + 136.85	<0.001
	Mixed	31.16 ± 3.49	0.515	0.265	7.851	H = 1.35(humerus) + 125.11	<0.001
Radius	Female	24.95 ± 2.64	0.383	0.147	7.082	H = 1.11(radius) + 133.82	0.0008
	Male	27.55 ± 3.26	0.474	0.225	6.209	H = 1.02(radius) + 144.36	<0.001
	Mixed	26.28 ± 3.24	0.555	0.308	7.617	H = 1.57(radius) + 125.88	<0.001
Ulna	Female	25.30 ± 2.57	0.413	0.171	6.982	H = 1.23(ulna) + 130.41	0.0003
	Male	26.88 ± 3.09	0.416	0.173	6.413	H = 0.94(ulna) + 147.04	0.0002
	Mixed	26.11 ± 2.94	0.479	0.229	8.042	H = 1.48(ulna) + 128.28	<0.001
Femur	Female	41.60 ± 4.00	0.337	0.114	7.218	H = 0.64(femur) + 134.73	0.0035
	Male	43.43 ± 4.53	0.352	0.124	6.600	H = 0.54(femur) + 148.77	0.0018
	Mixed	42.54 ± 4.36	0.394	0.155	8.417	H = 0.82(femur) + 131.95	<0.001
Tibia	Female	36.59 ± 3.19	0.424	0.180	6.943	H = 1.01(tibia) + 124.42	0.0002
	Male	39.39 ± 4.05	0.360	0.130	6.578	H = 0.62(tibia) + 147.85	0.0014
	Mixed	38.02 ± 3.90	0.503	0.253	7.916	H = 1.18(tibia) + 122.31	<0.001
Humerus + Radius + Ulna	Female	80.17 ± 6.34	0.519	0.270	6.646	H = 0.45(Hu) + 0.67(Ra) + 0.79(Ul) + 111.05	<0.001
	Male	86.79 ± 7.75	0.574	0.330	5.853	H = 0.74(Hu) + 0.68(Ra) + 0.12(Ul) + 126.65	<0.001
	Mixed	83.55 ± 7.81	0.647	0.419	7.031	H = 0.76(Hu) + 1.01(Ra) + 0.46(Ul) + 104.93	<0.001
Femur + Tibia	Female	78.19 ± 5.68	0.489	0.239	6.736	H = 0.48(Fe) + 0.87(Ti) + 109.83	<0.001
	Male	82.82 ± 7.62	0.401	0.161	6.503	H = 0.33(Fe) + 0.41(Ti) + 141.83	0.0017
	Mixed	80.55 ± 7.11	0.533	0.284	7.778	H = 0.42(Fe) + 0.95(Ti) + 113.02	<0.001
All Long Bones	Female	158.36 ± 10.80	0.572	0.327	6.476	H = 0.24(Fe) + 0.57(Ti) + 0.56(Ra) + 0.53(Ul) + 0.22(Hu) + 96.55	<0.001
	Male	169.61 ± 14.27	0.574	0.330	5.936	H = -0.00(Fe) + 0.01(Ti) + 0.67(Ra) + 0.12(Ul) + 0.74(Hu) + 126.57	<0.001
	Mixed	164.10 ± 13.84	0.660	0.435	6.979	H = -0.03(Fe) + 0.39(Ti) + 0.83(Ra) + 0.37(Ul) + 0.69(Hu) + 100.45	<0.001

Table 2: Summary of Linear Regression Analysis and Correlation of Anthropometric Parameters (Digits) with Stature.

Parameter	Sex	Mean ± SD (cm)	r	R^2	SEE (cm)	Regression Equation	p
Thumb	Female	6.16 ± 0.65	0.370	0.137	7.123	H = 4.32(thumb) + 134.83	0.0013
	Male	6.89 ± 0.71	0.523	0.273	6.011	H = 5.18(thumb) + 136.70	<0.001
	Mixed	6.53 ± 0.77	0.599	0.359	7.334	H = 7.10(thumb) + 120.65	<0.001
Index Finger	Female	7.02 ± 0.84	0.320	0.102	7.264	H = 2.88(index finger) + 141.20	0.0058
	Male	7.89 ± 1.12	0.602	0.362	5.632	H = 3.75(index finger) + 142.80	<0.001
	Mixed	7.46 ± 1.08	0.586	0.343	7.423	H = 4.93(index finger) + 130.25	<0.001
Middle Finger	Female	7.89 ± 0.89	0.331	0.110	7.234	H = 2.83(middle finger) + 139.14	0.0042
	Male	8.74 ± 1.36	0.591	0.350	5.686	H = 3.05(middle finger) + 145.73	<0.001
	Mixed	8.32 ± 1.23	0.562	0.316	7.574	H = 4.19(middle finger) + 132.20	<0.001
Ring Finger	Female	7.23 ± 0.95	0.363	0.132	7.143	H = 2.92(ring finger) + 140.34	0.0016
	Male	8.07 ± 1.24	0.595	0.354	5.668	H = 3.35(ring finger) + 145.40	<0.001
	Mixed	7.65 ± 1.18	0.576	0.332	7.487	H = 4.45(ring finger) + 133.00	<0.001
Little Finger	Female	6.05 ± 0.77	0.327	0.107	7.244	H = 3.26(little finger) + 141.72	0.0047
	Male	6.85 ± 1.21	0.576	0.331	5.765	H = 3.33(little finger) + 149.60	<0.001
	Mixed	6.46 ± 1.09	0.563	0.317	7.571	H = 4.71(little finger) + 136.59	<0.001

Table 3: Summary of Linear Regression Analysis and Correlation of Anthropometric Parameters (Distal Segments) with Stature.

Parameter	Sex	Mean ± SD (cm)	r	R^2	SEE (cm)	Regression Equation	p
Hand Length	Female	17.48 ± 1.19	0.383	0.147	7.083	H = 2.46(hand length) + 118.45	0.0008
	Male	18.78 ± 1.64	0.485	0.235	6.166	H = 2.07(hand length) + 133.61	<0.001
	Mixed	18.14 ± 1.57	0.563	0.317	7.567	H = 3.27(hand length) + 107.76	<0.001
Foot Length	Female	23.12 ± 1.74	0.099	0.010	7.629	H = 0.43(foot length) + 151.40	0.4052
	Male	25.00 ± 1.97	0.540	0.291	5.936	H = 1.92(foot length) + 124.50	<0.001
	Mixed	24.08 ± 2.08	0.505	0.255	7.904	H = 2.22(foot length) + 113.62	<0.001
Arm Span	Female	163.09 ± 10.02	0.812	0.659	4.480	H = 0.62(arm span) + 60.91	<0.001
	Male	176.62 ± 15.45	0.684	0.468	5.144	H = 0.31(arm span) + 117.64	<0.001
	Mixed	169.99 ± 14.69	0.783	0.612	5.701	H = 0.49(arm span) + 84.38	<0.001
Supra Sternal to Supra Pubic	Female	48.11 ± 5.06	0.267	0.071	7.390	H = 0.40(SS to SP) + 142.15	0.0226
	Male	51.36 ± 4.86	0.318	0.101	6.684	H = 0.46(SS to SP) + 148.82	0.0051
	Mixed	49.77 ± 5.20	0.409	0.168	8.356	H = 0.72(SS to SP) + 131.29	<0.001
Umbilicus to Xiphisternum	Female	17.22 ± 3.71	0.026	0.001	7.664	H = 0.05(U to X) + 160.52	0.8287
	Male	17.73 ± 2.14	0.252	0.063	6.824	H = 0.82(U to X) + 157.81	0.0283
	Mixed	17.48 ± 3.01	0.133	0.018	9.077	H = 0.40(U to X) + 159.96	0.1046
Umbilicus to Suprapubic	Female	15.89 ± 3.42	0.008	0.000	7.667	H = 0.02(U to S) + 161.15	0.9472
	Male	16.11 ± 2.87	0.224	0.050	6.872	H = 0.55(U to S) + 163.59	0.0517
	Mixed	16.00 ± 3.14	0.104	0.011	9.109	H = 0.30(U to S) + 162.18	0.2054

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Original Research Paper

Visual Forensics of Deepfake Images: Manual Detection and Classification of Visual Artifacts

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ABSTRACT

Introduction: The present work investigates the natural human ability to recognize and counter the negative impact of deepfake technology. Artificial Intelligence (AI) and machine learning play a crucial role in creating highly realistic synthetic media, known as deepfakes. Deepfakes have become a powerful tool for spreading misinformation and creating inappropriate content. Social media platforms are flooded with such synthetic media, raising serious concerns about the authenticity of digital information directly available to users. Moreover, there is a lack of knowledge and awareness among layman about the authenticity of such media.

Objective: To address this issue, manual analysis of hundreds of deepfake images, focusing on identifying visual anomalies without the aid of specialized software or tools, is performed.

Materials and Methods: Through this process, various visual markers are discovered, ranging from common to rare, that can help distinguish deepfake images.

Results: These indicators are intended to contribute to public awareness and enhance digital media literacy, helping social media users recognize and question the authenticity of deepfake media.

Conclusion: This study highlights the importance of identifying visual anomalies and empowering the users to identify and safeguard themselves from deepfakes and its harmful impact.

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INTRODUCTION

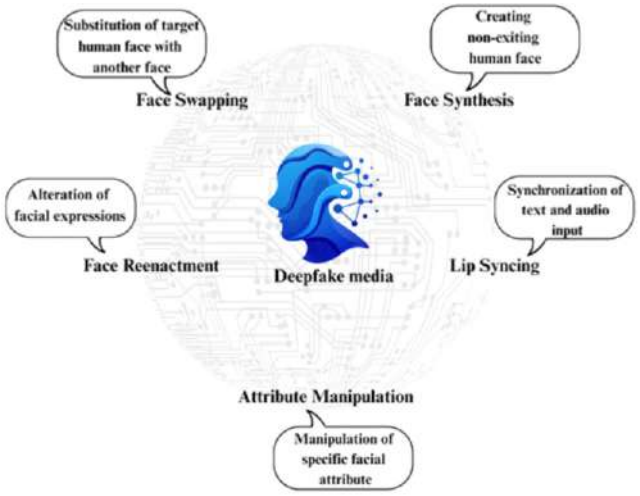
The rapid evolution of artificially generated content, such as deepfakes, has made it increasingly difficult for human beings to distinguish between fiction and reality.[1] Deepfake media, such as images and videos, are generated using deep learning tools, including Generative Adversarial Networks (GANs) and Autoencoders. GANs consist of two competing neural networks: the generator network and the discriminator network. The generator creates synthetic images, and then it is analysed by the discriminator alongside real images.

The generator network learns to produce images that deceive the discriminator, while the discriminator network learns to differentiate between real and fake images. In the process of iterative training, GANs can produce highly realistic deepfakes. On the other hand, Autoencoders could be used as feature extractors that encode and decode facial features.

During the training phase, the autoencoder learns to compress a facial image into a low-dimensional latent representation while preserving the most critical features. Further, this latent space representation is used to reconstruct the original image with minimum loss of details. Now, for deepfake creation, two autoencoders are utilized, one trained on the source's face and the other on the target.[2] This enables the system to map the facial features of the source onto the target, facilitating realistic face swaps.

Among all the existing deepfake approaches, as shown in **Figure 1**, face swapping is the most popular one. In this technique, the existing face is replaced with the target face in the original image or video. Further, such deepfake media can be used to blackmail the target person or to defame, or for any other illegitimate purpose.[3]

Figure 1: Deepfake Media Generation Approaches.



The advanced professional multimedia editing tools, including deep learning tools, already play a legitimate role in the film and entertainment industry and the video games industry. However, with the application of such tools, hyper-realistic media can be created with minimal effort or without having artistic skills in multimedia editing, which leads to the exploitation of such technology.[3]

Such illegitimate media can influence public opinion, and it can give rise to various crimes like extortion, cyberbullying, fabrication of evidence, etc. The analysis of such forged content is essential for controlling and minimizing the adverse effects of deepfake media.[1] The study focuses on developing a preliminary forensic analysis framework, appropriate for image authentication, which means that software or tool assessment, is not required at this level. This evaluation involves manual assessment and data entry, which will further assist the forensic examiner and it, will also help the common man in the identification of synthetic media.

MATERIALS AND METHODS

This study explores the natural human ability to recognize deepfake content without relying on advanced forensic tools or automated systems. The primary focus is on conducting a structured, manual analysis of visual inconsistencies present in manipulated media.

a) Dataset and Sample Selection

The images analyzed in this study were sourced from the Kaggle test dataset (https://www.kaggle.com/datasets/manjilkarki/deepfake-and-real-images? resource=download), made publicly available on Kaggle. It is one of the most comprehensive open-access repositories of deepfake content. For this study, only deepfake images were considered, with the objective of identifying common visual flaws or inconsistencies within the manipulated media itself. A total of 400 still image samples were extracted from the deepfake dataset, generated using advanced generative adversarial networks (GANs). Each sample is a 256 × 256 jpg image, consisting of a frontal or near-frontal view of a subject's face, ensuring that visual analysis could be carried out effectively.

To ensure a balanced sample and proper analytical validity, image selection was conducted using inclusion and exclusion criteria as outlined below.

b) Inclusion and Exclusion Criteria

Inclusion Criteria

- Images have frontal or near-frontal human face views.
- Images with sufficient lighting and resolution for identifying facial details.
- Variety of subjects: diverse in age, gender, and ethnicity.
- Deepfake samples were generated using known synthesis techniques.

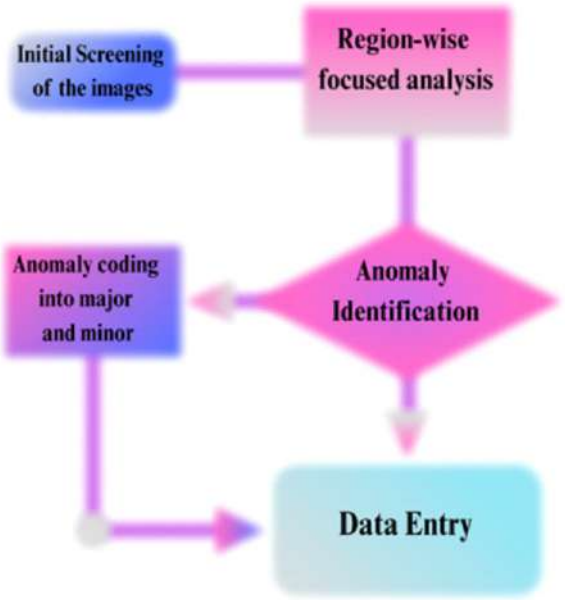
Exclusion Criteria

- Images with extreme motion blur or poor lighting.
- Images with a partial or side view of the subject.
- Images with non-human subjects, digital avatars, or cartoons.
- Samples generated with unknown techniques.

c) Manual Evaluation of Data

A structured, multi-step manual evaluation framework **(Figure 2)** was developed. This framework ensures systematic and consistent inspection of all samples. It targets specific facial regions known to exhibit anomalies related to deepfakes and facilitates the stepwise identification, classification, and documentation of inconsistencies. An initial assessment was conducted on all 400 images to capture spontaneous perceptual cues. Each image was examined for disrupted facial harmony, lack of coherence in presentation, and visual discomfort that might indicate manipulation artifacts. After the broad-

Figure 2: Stepwise Framework for the Manual Visual Detection of Deepfake Images.



level review, a detailed region-specific inspection followed. Images were systematically examined across predefined regions of interest: Hair and hairline, forehead and skin surface, eyes and eyebrows, mouth, lips, and oral cavity, jawline and facial contour. This detailed inspection linked our earlier perceptual assessment with the later forensic-level analysis.

Detected irregularities were documented and classified into anomaly type, including unnatural skin texture, blurred boundaries, lighting inconsistencies, facial asymmetry, and localized distortions. This categorization enabled consistent characterization of manipulation artifacts. Each anomaly was further classified as major or minor based on its frequency in the dataset. Frequently observed inconsistencies were categorized as major, while rare occurrences were labelled as minor. The classified anomalies were then recorded and consolidated into a structured dataset for analysis.

DISCUSSION

The findings of this study provide compelling evidence that, despite the growing sophistication of deepfake generation techniques, synthetic media continues to exhibit discernible visual anomalies. Through rigorous manual analysis of 400 deepfake samples, the study identified recurrent abnormalities, particularly in the rendering of hair, skin, and facial outlines that exposes the underlying manipulation and limitations of current generative models.

Table 1 and **Table 2** illustrates the distribution of major and minor anomalies of 400 deepfake images. A significant portion of the dataset, 43% of the samples, exhibited hair-related irregularities, including unnatural texture blending, incomplete strand definition, and directional inconsistencies. Literature has emphasized about the deepfake models frequently exhibit difficulty in accurately synthesizing hair, largely due to its fine structural complexity and dynamic variability. Irregularities in this hair region often arise from the inability of generative models to reproduce high-detail textures consistently, resulting in unnatural or incoherent visual output.[2,4-7] Similarly, edge-related anomalies were identified in 37% of the samples, most often manifesting as blurred or poorly defined facial contours. Such issues commonly occur due to post-processing operations employed to blend synthetic faces with real backgrounds. Literature has focused on the blending techniques that improve overall visual coherence. However, they often reduce edge sharpness, which is an important sign of authenticity in high-resolution images.[4,8] Previous literature has shown that blending operations improve overall visual coherence but often reduce edge sharpness. Edge sharpness is an important signal for authenticity in high-resolution images. Furthermore, edge inconsistency has been identified as a key marker in deepfake detection. This highlights the importance of the edge-related artifacts seen in this study.[4,9] Previous research has shown that generative models often oversimplify skin rendering. This leads to overly smooth surfaces and uniform colors that miss realistic textural details. In line with these findings, skin-related artifacts appeared in 36% of the samples. These artifacts usually showed up as a lack of pores, wrinkles, blemishes, and other fine details. The absence of natural lighting gradients and small textural variations highlights the limits of synthetic algorithms in reproducing subtle but crucial signs of facial authenticity.[10,11] Major anomalies such as hair distortions, edge blurring, and unnatural skin textures, as shown in **Table 1**, are especially promising as robust detection features for both real-time and forensic applications. Minor anomalies, including unnatural teeth rendering, inconsistent skin tone, and misaligned eye direction, though less prevalent, as shown in **Table 2**, remain diagnostically significant. Previous literature has shown that even sophisticated generative models still have difficulty reproducing subtle features, especially in small oral and ocular areas. Although synthesis quality has improved, low-level artifacts continue to appear. These issues may go unnoticed by the

Table 1: Overview of Various Frequencies of Major Visual Inconsistencies.

ANOMALIES	NO. OF SAMPLES
Hair	172
Edges	148
Skin	144
Eyes	91
Background	63
Wrinkles	52
Marks	47
Teeth	37
Lips	24
Mouth	20
Specs	17
Eyebrows	11
Alignment	11

Table 2: Overview of Minor Anomalies Having A Lower Frequency.

ANOMALIES	NO. OF SAMPLES
Blurred Edges	75
Abnormal/unnatural Skin Texture	64
Blurred Background	59
Unnatural Wrinkles	52
Unnatural Marks	45
Discrepancies In Eye Direction.	42
Difference In Skin Tone	40
Smudging Of Skin	38
Unnatural Teeth	35
Smoothening Of Skin	32
Unrealistic And Different Dimensions Of Eyes.	32
Abnormal Specs	23
Missing Details Of Fine Hairs	24
Unusual Lines	21
Abnormal Teeth And Lips.	17
Unrealistic Hair	19
New Face Is Placed On The Body	13
Unusual Spots	12
Unnatural Alignment	11
Misplaced Eyes	10
The Eyes Are Misaligned	9
Extra Hair Strands	5
Merging Of Body And Background	4
Facial Glitches	3
Blurred Eyes And Eyebrows	3

casual observer, but experts can identify them through careful inspection or automated detection methods. These anomalies are especially relevant in forensics, as they reveal both the developing abilities and the ongoing weaknesses of next-generation deepfake generation techniques.[12] Furthermore, this study underscores the crucial role of human awareness and perceptual training in combating deepfakes. While automated tools are essential, equipping individuals, particularly in fields like journalism, security, and forensic science, with the knowledge to recognize frequent visual markers can significantly enhance media literacy and detection accuracy. Literature has highlighted the need to combine human perceptual skills with automated detection systems. While algorithms perform well in processing large amounts of data, they often miss subtle irregularities that human observers can notice.[2] This study supports that view and emphasizes the value of a hybrid detection method. It shows that some anomalies, especially small inconsistencies in texture and edge definition, are better identified through manual examination.[13] These results strengthen the argument for merging algorithmic efficiency with human expertise to ensure effective and reliable deepfake detection.[14]

Limitation of the Study

Despite providing the valuable insights into the visual anomalies in 400 still deepfake images, the study is constrained by certain limitations like exclusion of video-based deepfake samples and subjective manual interpretation.

CONCLUSION

This study showed that even with new techniques, deepfake images still have noticeable visual issues, especially in hair patterns (43%), facial edges (37%), and skin textures (36%). The proposed evaluation framework captured common problems like blurred edges and unnatural textures, as well as rare but significant flaws that show the limits of current deepfake models. These findings offer useful indicators for forensic analysis and emphasize the importance of thorough manual checks to support automated detection systems. Overall, the results advocate for a mixed detection approach that combines software tools with human knowledge for more trustworthy and effective deepfake identification.

Conflicts of Interest/Funding: Nil

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Original Research Paper

Profile of Drowning Deaths at a Tertiary Care Centre in Ambala District of Haryana, India: An Autopsy-Based Retrospective Study

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ABSTRACT

Introduction: Drowning remains a significant yet preventable cause of unintentional mortality worldwide. This study explores the epidemiological and medico-legal aspects of drowning-related fatalities based on autopsy records in a tertiary care setting in Ambala, Haryana. The study was carried out to identify patterns in age, gender, seasonality, place, and manner of drowning among victims subjected to postmortem examination, and to determine the most vulnerable groups.

Materials and Method: A retrospective descriptive analysis was conducted over 12 years (2013–2024) using autopsy and police inquest records. The study comprises 62 cases that met the inclusion criteria and were studied for demographic and circumstantial variables. The data was tabulated, and descriptive data analysis was performed using Microsoft Excel 2021.

Results: Drowning accounted for 3.3% of all medicolegal autopsies. Males constituted the majority (88.7%), and the highest incidence was among individuals aged 11-20 years (29%). The majority of the victims were from rural areas (75.8%) and were mostly daily-wage laborers (35.5%), followed by students (24.2%). The peak drowning season was monsoon, constituting 43.5% of cases, with canals being the most frequent site (61.3%) of these drownings. Accidental drowning (64.5%) was the predominant manner of death. Froth from the mouth or nostrils was observed in 64.5% cases. The decomposition changes were present in 53.2% of the autopsied bodies.

Conclusion: This study highlights critical patterns in drowning-related deaths in Northern Haryana. The findings underline the need for targeted community awareness, improved water safety infrastructure, and region-specific prevention strategies.

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INTRODUCTION

The World Health Organization (WHO) defines "drowning as the process of experiencing respiratory impairment from submersion or immersion in liquid."[1] According to the WHO, data from the Global Health Estimates 2019 revealed that an estimated 2,35,642 people died from drowning globally, making it one of the predominant causes of accidental deaths.[2] The National Crime Records Bureau (NCRB) report of 2022 shows that among the causes of mortality due to accidents in India, drowning is responsible for 9.1% of the deaths and is attributed as the third major cause of accidental deaths. Drowning is also the third major cause of suicidal deaths in India, constituting 5% of such deaths in India.[3]

Haryana is an agrarian state of India with rivers, canals, ponds, and seasonal water bodies that cause unintentional and intentional drowning. As per the NCRB 2022 data, 743 people died of drowning in Haryana. Among them, 360 people died due to accidental falls into water bodies. Continuing the 2022 NCRB data, 125 people implemented drowning as the means of suicide.[3] Still, there has been no region-specific forensic data concerning the Ambala

district of Haryana to inform drowning prevention strategies. Autopsy studies can provide useful demographics and describe the various manners of death, such as accidental, suicidal, or homicidal, seasonality, and other attributes.

The study's primary objective was to evaluate the patterns and trends of drowning deaths reported for postmortem examination at a tertiary care setting in Ambala, Haryana, India, and to find out the vulnerable groups of the victims in these drowning deaths.. Drowning presents itself as a critical challenge in public health contexts, law enforcement investigations, developing targeted drowning prevention strategies, and necessitating research efforts for these preventable fatalities.

MATERIALS AND METHODS

This retrospective descriptive study was conducted at the Department of Forensic Medicine & Toxicology of a tertiary care hospital in Ambala, Haryana, which carries out around 150-160 medicolegal autopsies every year. The study was conducted with prior institutional ethical committee approval (IEC-3433). All cases with a clear history of drowning and subjected to autopsy during the period of 12 years (January 2013 to December 2024) were included. All the cases without a history of drowning but were subsequently found to be drowning-related deaths were also included.

The unknown cases and cases with a history of drowning, but subsequent postmortem examination ruling out drowning, were excluded. Satisfying the inclusion and exclusion criteria, 62 drowning-related fatalities were included in the study. An anonymised proforma was created using data gathered from police inquests and postmortem examination reports. The data was tabulated, and descriptive data analysis was performed using Microsoft Excel 2021.

RESULTS

Drowning Deaths Among Medicolegal Autopsies

During the study period from January 2013 to December 2024, 1863 medicolegal postmortem examinations were conducted. The drowning-related fatalities satisfying the study's inclusion criteria constituted 62 cases, i.e., 3.3% of all the medicolegal autopsies conducted.

Demography

Among the 62 victims, 55(88.7%) were males and seven (11.3%) were females. The majority of them were Hindus, 47(75.8%), followed by Sikhs, 10(16.1%), and the remaining five (8.1%) were Muslims.

Residence, Occupation, and Marital Status

Most of the cases were from rural areas, 47 (75.8%), and 15 (24.2%) were from urban locality. The majority of the victims were daily wage laborers, 22 (35.5%), followed by 15 (24.2%) students, six (9.7%) were farmers, five (8.1%) were business owners, three (4.8%) were employed in private jobs, and one (1.6%) case each of a government employee and a home maker. The occupations of nine deceased were not available in the records. As per records, half of the deceased, 31 (50%), were married, and 29 (46.8%) were unmarried. The marital status was not known in two cases.

Age group-wise Distribution

The age of the drowning victims ranged from 2 to 76 years. The maximum drowning deaths were observed in the 11-20 years age group, 18 (29%), followed by 11 (17.7%) in 21-30 years, 10 (16.1%) in 31-40 years, 7 (11.3%) in 41-50 years, 6 (9.7%) in 51-60 years, 4 (6.5%) in less than 10 years age group and three (4.8%) each in 61-70 and 71-80 years age group. Almost half of the victims, 30 (48.4%), were in the 2nd to 4th decade of life. Male victims were more in all age groups except the 1st decade, where female drowning fatalities were more. Most of the male drownings, 15 (24.2%), were in the 11-20 years age group, followed by 11 (17.7%) in the 21-30 years. No female drowning-related deaths were noted in the 3rd to 7th decades of life. Male victims were found in all the age groups **(Table 1)**.

Table 1: Shows Drownings According to Age Group and Gender.

Age Group (in years) (n=62)	Gender	
	Male (n=55)	Female (n=7)
0-10 (n=4)	1	3
11-20 (n=18)	15	3
21-30 (n=11)	11	0
31-40 (n=10)	10	0
41-50 (n=7)	7	0
51-60 (n=6)	6	0
61-70 (n=3)	3	0
71-80 (n=3)	2	1

Season, Month, and Year-wise Distribution

Most of the drownings were in the monsoon or rainy season (June to August), constituting 27 (43.5%) of the cases, followed by 17 (27.4%) in winter (December to February), 10 (16.1%) in Autumn (September to

November), and least in summer (March to May), with 8 (12.9%).

The maximum number of drowning were recorded in June (10, 16.1%), followed by 9 (14.5%) in August. No drowning death was recorded in November **(Figure 1)**. The highest number, 10 (16.1%) of drowning fatalities, occurred during the year 2014, followed by 7 (11.3%) in 2019, and the least 3 (4.8%) in 2016 **(Figure 2)**.

Figure 1: Shows Stack Lines Depicting the Month-wise Distribution of Drowning Deaths.

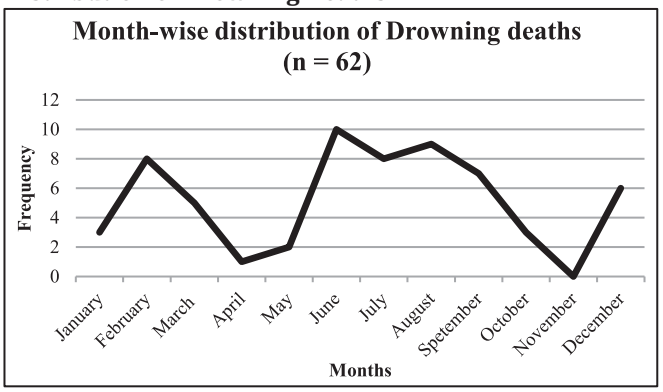
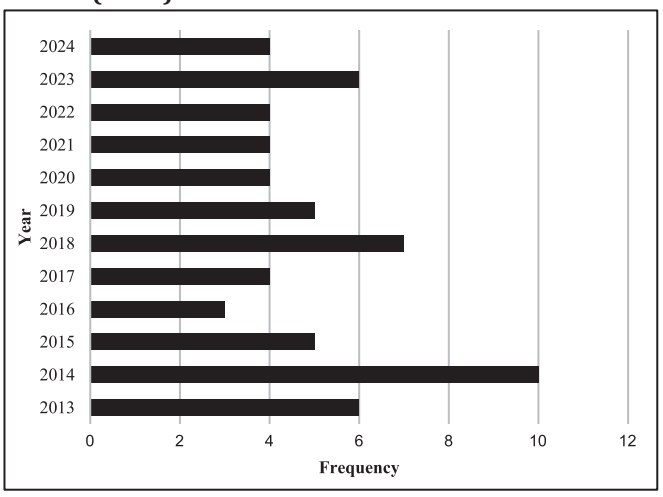


Figure 2: Shows Year-wise Incidence of Drowning Deaths (n=62).



Place of Incidence, Manner of Drowning, and Survival Duration of Victims

The majority of the victims drowned in canals, 38 (61.3%), followed by pond and stagnant water, with nine (14.5%) cases each, five (8.1%) in a river, and a single case of drowning in a water tub. Most of the cases were of accidental drowning, with 40 (64.5%) cases, and 10 (16.1%) were suicidal drowning. In 12 (19.4%) cases, the manner of drowning was undetermined. No homicidal drowning-related fatality was observed in our study. All the victims were brought dead to the hospital.

Most of the accidental and suicidal drownings occurred in canals, with 19 and seven cases, respectively. Accidental drownings were noted in all the places of incidence in this study. Whereas no suicidal drownings were observed in the river and the water tub **(Table 2)**.

Table 2: Shows Drowning Deaths with their Place of Incidence and Manner.

Place of Incidence (n=62)	Manner of Drowning		
	Accidental (n=40)	Suicidal (n=10)	Undetermined (n=12)
Canals (n=38)	19	7	12
Pond (n=9)	7	2	0
Stagnant Water (n=9)	8	1	0
River (n=5)	5	0	0
Water Tub (n=1)	1	0	0

Froth and Decomposition Changes

Most of the drowning victims, 40 (64.5%), were noted with froth from the mouth or nostrils. The decomposition changes over the body were present in 33 (53.2%) cases.

DISCUSSION

The drowning fatalities comprised 3.3% of all postmortem examinations conducted during the study period. A similar observation was noted in studies by Shubhendu et al. and Raut et al., where the drowning deaths constituted 3.3% and 3.7% respectively, of all the postmortem examinations conducted at their respective centers. [4-5] Dhillon et al. and Venkatesulu et al. observed a higher frequency of drownings, with 8.8% and 12.4% cases among the medicolegal autopsies, respectively, conducted at their postmortem examination centers.[6-7] Whereas, studies by Dhakar et al. and Mohanty et al. had a lower percentage of drowning cases among all the autopsies conducted, with 1.3% and 2.7% respectively.[8-9]

The study showed a male preponderance among the drowning victims, comprising 88.7% of the deaths. A similar result was observed in most of the studies.4,5,7-15 Most of the victims were of the Hindu faith. This observation is similar to a study by Phad et al., conducted at Nagpur, Maharashtra, India.[10] The majority of the victims were from rural domiciles, and the rest were from urban areas. Studies by Shubhendu et al., Raut et al., and Phad et al. have also observed a higher frequency of drowning victims from rural areas.[4-5,10]

In the present study, more than one-third of the drowning victims were daily-wage laborers, followed by students and then farmers. Venkatesulu et al. also observed a maximum number of victims among the laborers.[7] In the study by Mohanty et al., most of the victims were

unemployed.[9] Whereas Phad et al. had the majority of students in the fatal drownings, and observed that laborers were the third most common occupation behind farmers.[10] Half of the deceased in the present study were married; in two cases, the victim's marital status was not known, and the rest were unmarried. The studies by Shubhendu et al., Raut et al., and Phad et al. also found that drowning is more common among married individuals.[4-5,10] Whereas, in the studies by Dhakar et al., Mohanty et al., and Kumar N et al. higher percentage of unmarried individuals were involved in drowning.[8-9,13]

The majority of the drowning deaths belonged to the 11-20 years age group, followed by the age groups of 21-30 and 31-40 years. The studies by Raut et al., Kumar P et al., and Palimar et al. also observed 11-20 years as the most common age group of drowning victims.[5,11,14] Whereas, the study by Kanchan et al. had most of the victims in the 0-10 years followed by the 11-20 years age group.[15] In the studies by Venkatesulu et al. and Kumar N et al, the 21-30 years age group was most commonly involved.[7,13] In contrast, the studies by Shubhendu et al., Dhakar et al., and Kumar N et al. observed the majority of the drownings in the 31-40 years age group.[4,8,12]

Most of the drownings were in the rainy season. This observation aligns with studies by Dhakar et al., Phad et al., and Palimar et al., where the drowning incidents were common during the rainy season.[7,9,13] In contrast, the studies by Raut et al., Kumar N et al., and Kumar N et al. were conducted in the Beed district of Maharashtra, Pauri Garhwal District of Uttarakhand, and South-Western Maharashtra, respectively, observed more drowning in the summer.[5,12-13] In our study maximum number of drownings was recorded in June, followed by August. Similarly, June was observed by Kumar N et al. when most of the drowning happened.[12] Whereas, studies by Raut et al. and Venkatesulu et al. had maximum drowning incidents in April and August, respectively.[5,7]

Most of the drownings happened in canals in the present study, followed by ponds and stagnant water. No other study was found in the literature with higher frequencies of drowning in the canals. The studies by Shubhendu et al. and Phad et al. had the maximum drowning in the lakes.[4,10] Venkatesulu et al. and Kumar N et al. had the maximum cases of drownings in the rivers.[7,12] Kumar P et al. had a higher frequency of drownings in lakes and rivers combined.[11] Raut et al. and Dhakar et al observed that the majority of the drownings occurred in the wells or ponds.[5,8] The study by Kanchan et al. was conducted at a center near the coastal region of Karnataka and had the majority of drownings at the sea, followed by in wells and ponds.[15]

Most of the drownings were accidental, followed by undetermined manner, then suicidal. This finding is similar to most of the other studies, where among the determined manner of drowning, accidental drownings were more common, followed by intentional drownings.[4,7-15] No homicidal drownings were observed in our study. Similarly, Dhakar et al. also had no homicidal drownings in their study.8 In contrast, other studies have reported incidences of homicidal drownings.[4,7,9,11-15]

Frothing from the mouth and nostrils was noted in 64.5% of victims in this study. The studies by Venkatesulu et al., Mohanty et al., and Kumar P et al showed frothing from the mouth and nostrils in 56.5%, 54.6%, and 44% cases, respectively.[7,9,11] Singh et al. observed frothing from the mouth in 80.4% cases of drowning.16 The decomposition changes in the bodies retrieved were observed in 53.2% of cases. The study by Mohanty et al. had similarly observed decomposition changes in 52% of the drownings.[9] The studies by Venkatesulu et al. and Kumar P et al. had a lower percentage of decomposition changes present over the body, with 12.3% and 16% cases, respectively.[7,11]

CONCLUSIONS

This autopsy-based retrospective analysis provides valuable insights into the profile of drowning deaths in this region of Haryana. Males in the second and third decades of life, particularly from rural settings, emerged as the most affected group. The data further emphasize a seasonal trend, with a sharp rise in cases during the monsoon months and a higher prevalence in canal-associated drownings. Accidental drownings were the most frequent, followed by suicides, with no homicidal incidents reported. The findings advocate for proactive water safety interventions, particularly in high-risk areas such as canals. Community-level education campaigns, fencing around open water bodies, and timely rescue measures can help mitigate such preventable fatalities. Future studies with broader datasets and the inclusion of psychosocial factors could offer a deeper understanding and enhance drowning prevention strategies.

Limitations of the Study

The activities the deceased were involved in just before the incident of drowning were not available uniformly in the records. The stressors for suicidal drownings were not available in the records. The data regarding the swimming

abilities of the deceased were not available.

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Original Research Paper

Comparative Evaluation of Modified Demirjian and Nolla's Method for Dental Age Estimation in Young Football Players

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ABSTRACT

Introduction: Dental age estimation is a fundamental aspect of forensic science, paediatric dentistry, and orthodontics, often employed in both clinical and legal contexts. It is mainly important when birth records are unavailable or unreliable, such as in cases involving refugees, adoptees, or judicial age disputes. Teeth, unlike many other physiological markers, show a predictable and sequential pattern of development and mineralization, making them reliable indicators of biological maturity. Among the widely used methods, the Modified Demirjian Method and Nolla's Method are prominent for assessing dental maturity.

Aims : The designated group was taken to compare these methods and assess their accuracy in estimating the actual age of the players.

Materials and Method: This study was conducted in the department of Forensic Medicine and Toxicology, GMCH Chandigarh. A total of 180 players among the age group of 9 to 19 years, came to our department in November, 2024, for Age estimation for their Football Championship. Out of which 68 players required OPG for their age estimation.

Results: In our study we found that, the mean of actual age was approximately 15.73 years (±2.13 years). When their dental age was estimated using the Modified Demirjian Method, the average estimated age was found to be 17.40 years (±1.34 years), which tended to overestimate the actual chronological age in most cases. In contrast, the Nolla's Method calculated a mean estimated age of 15.40 years (±1.70 years), which showed a tendency to underestimate the actual age.

Conclusions: When their dental age was estimated using the Modified Demirjian Method, the average estimated age was found to be 17.40 years (±1.34 years), which tended to overestimate the actual chronological age in most cases. In contrast, the Nolla's Method calculated a mean estimated age of 15.40 years (±1.70 years), which showed a tendency to

Keywords:

Dental age, OPG, Modified Demirjian Method, Nolla's Method, third molar, forensic odontology, adolescent age assessment, panoramic radiographs.

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INTRODUCTION

Age estimation based on dental development is a useful method in forensic medicine because teeth develop in a regular pattern and are not significantly influenced by environmental factors. This makes dental examination valuable in forensic medicine, anthropology, and pediatric assessment, especially when age information is not available.[1] Among the available radiographic methods, the Demirjian and Nolla methods are widely used. These have been adjusted for different groups to improve the accuracy

of age estimation.

The Demirjian method, developed in 1973, evaluates the development of the seven left permanent mandibular teeth. It assigns a maturity score to each stage, which is then used to estimate dental age.[2] The Nolla's method 1960, divides tooth mineralization into eleven stages, from the start of mineral formation to the closure of the tooth's root apex. This method works well for younger age groups, but may underestimate the age of older adolescents. Research shows better results when the third molars are fully developed.[3]

Thorakkal Shamim et al. highlighted the importance of dental methods in medicolegal age assessment, especially when documentary proof of age is unavailable or doubtful.[4] Their work emphasizes how dental evidence can support legal and forensic decision-making in a scientific manner. AlQahtani et al. introduced the London Atlas, which provides detailed visual charts of tooth formation and eruption stages across growing individuals.[5] This atlas has improved the accuracy and consistency of dental age estimation by offering population-based reference data.[5]

Jayaram J et al. simplifies the scoring system and its reliability have made it popular worldwide.[6] However, there have been reports of age overestimation in some populations, leading to the creation of region-specific standards.[7-8] They also emphasized the importance of considering population-specific growth patterns and recommended resetting the existing methods for different populations.[8]

To address the limitations of each method, Schmeling A et al. suggested using both methods together for more accurate age assessment.[9] For adolescents around 16 years old, we assessed the reliability of both the Modified Demirjian and Nolla methods. This age is significant in legal contexts, as it is often the cutoff for decisions regarding criminal responsibility or asylum status.[9] However, this issue becomes more pronounced in late adolescence, when dental maturation slows and third molar development becomes more variable. Due to this variation, some authors advise against using third molars for age estimation.[10]

To evaluate the applicability of these methods in a South Asian population, we compared the estimated dental ages with the actual chronological ages.

Aim and Objective of the Study: To estimate and compare the dental age using the Modified Demirjian's method and Nolla's method in adolescents/ players and evaluate which method closely correlates with their actual chronological age.

Sample Size: A total of 68 players were included in the study. Each entry consisted of demographic data, actual age, number of permanent teeth, third molar space, and scores as per the Modified Demirjian and Nolla's methods.

MATERIALS AND METHOD

This study was conducted in the department of Forensic Medicine and Toxicology, GMCH 32, Chandigarh after the clearance from Institutional Ethics Committee with vide reference No. GMCH/IEC/2024/1162, dated 15.04.2024. A total of 180 players among the age group of 9 to 19 years, came to our department in November, 2024, for age estimation for their National Football Championship. Out of all, 68 players required OPG for their age estimation. The designed group was taken to compare these methods (Nolla and Demirjian) and assess their accuracy in estimating the actual age of the players when compared with their chronological ages.

RESULTS

Comparison between Chronological and Estimated Dental Age : The chronological ages of the 69 adolescents/players in the study ranged from 9 to 16.8 years, with a mean age of 15.73 ± 1.03 years. When dental age was assessed using the Modified Demirjian method, the average dental age was 17.40 ± 1.31 years, showing a consistent increase in age when compared to the actual age. In comparison, Nolla's method showed a mean age of 15.44 ± 1.75 years and generally underestimated the participants ages. The difference between the methods highlights their typical biases - Demirjian tends to overestimate age, while Nolla tends to underestimate it.

Evaluation of Permanent Dentition and Third Molar Space: The number of erupted permanent teeth ranged from 27 to 32, with a mean of 28.39 ± 1.03, indicating that most participants had almost complete permanent dentition. Regarding third molar space, 26 individuals had no space for third molars on OPG, while the others showed better-developed spaces in the maxilla, mandible, or both. Although third molar development can help estimate age in late adolescence, the variation in these cases suggests that it should be used as a supplementary indicator, not as a primary measure.

Modified Demirjian Scoring Method: Scores from the Modified Demirjian method ranged from 93.35 to 100, with a mean of 97.63 ± 2.05, indicating advanced dental

development in the study group. When broken down by jaw, the average scores for the maxilla and mandible were 74.60 ± 4.89 and 75.04 ± 5.28, respectively. These similar scores for upper and lower teeth suggest a consistent pattern of maturation, though the overall higher scores also reflect the overestimation of age seen with this method in this population.

Nolla's Scoring Method: Nolla's scores varied between 119 and 159, with the mean of 149.33 ± 9.23. The minimum and maximum dental ages were 10 and 17 years, using these scores. This technique was generally more accurate than the Demirjian approach for age estimation. The greater standard deviation (±1.75 years) for Nolla's estimates reflects more variation at the individual level, but also better differentiation of small differences in coming times.

Figure 1 and **Figure 2** shows the comparison of Scores and the calculated age using both Demirjian and Nolla's Methods. This analysis supports the conclusion that while the Demirjian method is more consistent, Nolla's method may offer greater accuracy.

DISCUSSION

The present study demonstrates that the Modified Demirjian Method provides a reasonably close approximation to chronological age in adolescents, although a consistent upward change was noted. The mean dental age obtained through this system was about 17.6 years, slightly higher than the mean chronological age of 16.25 years.

This trend is similar the original observations reported by Demirjian and colleagues, who also described a tendency for their scoring system to advance dental age estimation in older age groups.[2] Similar patterns of overestimation-typically falling within a range of 0.5 to 1.5 years, have been reported across several independent populations, reinforcing that this bias is inherent to the method rather than a demographic change.[2,7]

In contrast, the Nolla's Method produced age estimation, that was substantially lower than the participants actual ages, averaging around 11.8 years. This pronounced underestimation aligns with long-standing concerns regarding its application in adolescents, where dental development has largely stabilized and only the third molars remain as differentiating structures.[3-4]

Because Nolla's approach relies on incremental staging of calcification, its precision decreases once most permanent teeth have reached apex closure, limiting its usefulness beyond the early teenage years.[3] AlQahtani et al. similarly noted that the method retains its diagnostic value primarily in younger children, with accuracy declining sharply in individuals older than approximately 14 years.[5] Our findings reassure this limitation, suggesting that the method should be used cautiously when assessing older

Figure 1: Comparison Graph of Score for Dmerjian and Nollas Method.

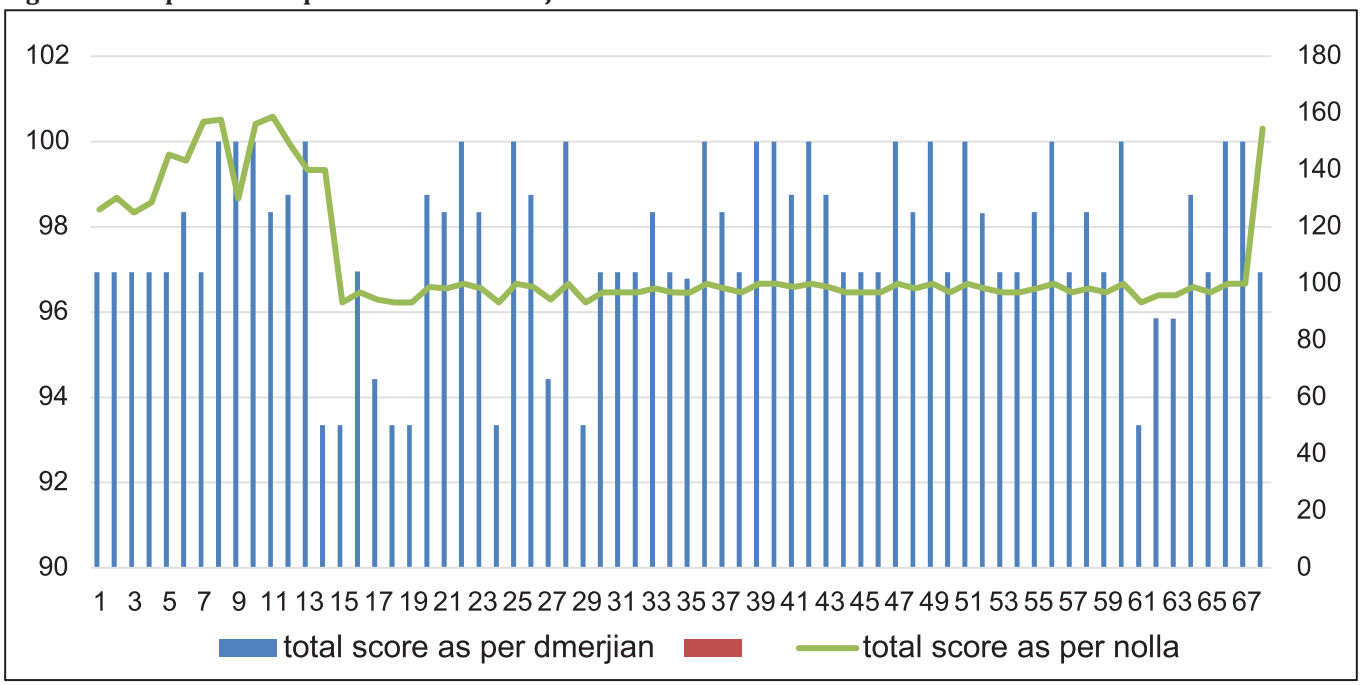
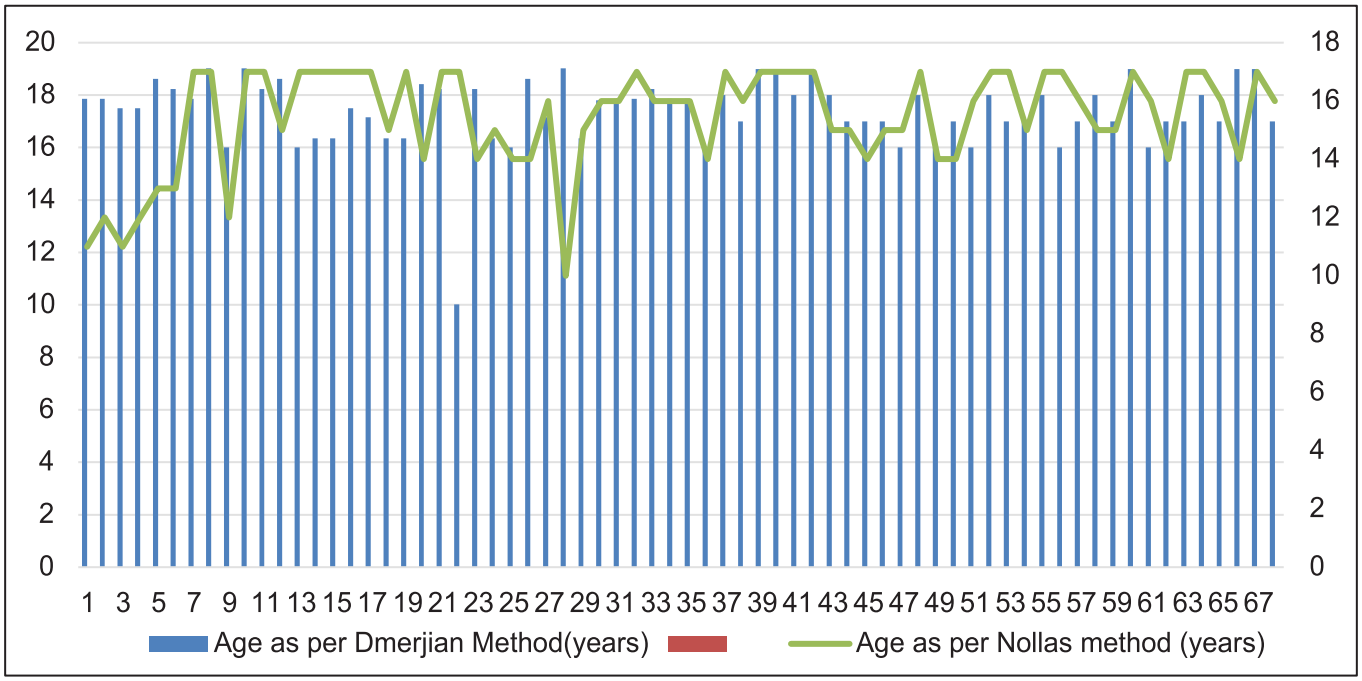


Figure 2: Comparison of Age with Demirjian and Nolla's Method.



individuals.

The differences we noticed in how third molars develop in our study make it solely rely on the maturity of wisdom teeth. Variations in the spacing and growth of third molars, along with differences between dental arches, show how inconsistent this could be. Past research by Mincer, Liversidge et al, has pointed out that third molar development is influenced by various biological, genetic, and environmental factors.[7,10]

When looking at all these observations together, it seems like what Schmeling et al., found makes sense. They pointed out that no single dental method works perfectly for estimating age in all stages of development. They suggested that using a combination of methods or adjusting them for different populations could give more accurate results.[9] These matches with the work of Jayaraman et al., who demonstrated that modifying Demirjian's scoring system for South Indian populations significantly improved age-estimation accuracy.[6]

In general, our results shows that the Modified Demirjian Method is still quite useful for estimating the age of teenagers around 16 years old, especially when combined with specific targets for a particular population. On the other hand, the Nolla Method tends to underestimate ages significantly in this age group, so we should not use it without adjustments. To improve the accuracy and reliability of dental age estimation, future studies should involve larger and more diverse groups of people and consider other indicators of skeletal maturity.

CONCLUSION

Our study found that the Modified Demirjian method often estimates people to be older than they are, suggesting a bias towards overestimation, with estimated age of 17.40 years compared with an actual mean of 15.73 years. On the other hand, Nolla's approach provides age estimates that are closer to the participants chronological ages, providing a mean of 15.40 years, although slightly underestimating in general. This indicates that each method has its own strengths.

Limitations:

1. Sample Size: The sample size in this present study was small, that is, 69 adolescents/players from only one region, thus making it difficult to generalize the results to larger or more diverse populations.

2. Small Age Range: Participants were mainly in their mid-teen years by which time dental development is largely completed. This limits the overall general result to such an accuracy in younger or older age groups.

3. Subjective Scoring Errors: In both methods as described by Demirjian and Nolla, the way in which we see and interpret OPG is totally dependent on us, so small errors of perspective or scoring of the teeth could make a difference.

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Original Research Paper

Anthropometric Correlation between Percutaneous Length of Long Bones of Lower Limb and Stature in Adult Males - A Cross-Sectional Study from the North Indian Population

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ABSTRACT

Introduction: Stature estimation from skeletal remains is crucial in forensic anthropology and medical identification. Long lower limb bones, notably the femur and tibia, predict height, but their correlation differs by population, requiring population-specific models. Bilateral right-left limb measurement discrepancies may further reduce accuracy. This study aimed to assess the correlation between stature and percutaneous femur and tibia lengths, evaluate bilateral asymmetry, and develop regression equations specific to adult North Indian males.

Materials and Method: A cross-sectional study was carried out on 250 healthy adult males, belonging to the age groups of 18-40 years, in North India. Stature, right and left femur length, and right and left tibia length were measured with the help of standard anthropometric techniques. Pearson's correlation was utilized for determining the association between the bone lengths and stature. Bilateral asymmetry was tested using paired t-tests. Formulation of stature estimation equations was done through simple and multiple regression analysis. The statistical significance was defined as $p < 0.05$.

Result: All long-bone measures correlated positively with stature ($p < 0.001$). The right femur had the strongest correlation ($r = 0.924$), followed by the right tibia (0.861), left tibia (0.837), and left femur (0.825). Significant bilateral asymmetry was found in femur and tibia lengths ($p < 0.001$). According to simple linear regression models, the right femur is the most trustworthy predictor ($R^2 = 0.854$; Standard Error of Estimate, SEE = ±4.156 cm). The multiple regression model encompassing all four bone lengths demonstrated strong predictive power ($R^2 = 0.855$, $p < 0.001$); only the right femur was significant because of multicollinearity.

Conclusion: The lengths of the femur and tibia are significant predictors of height in adult North Indian males, with the right femur exhibiting the greatest accuracy. Pronounced bilateral asymmetry points to the need for side-specific regression models, in particular, in the forensic cases of incomplete remains. The population-specific equations obtained provide solid tools for forensic and anthropological applications.

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INTRODUCTION

One of the most taxing and crucial tasks in criminal investigation is identifying unknown body. In instances of severely decayed corpses, burnt or dismembered remains, skeletal evidence is the most effective means of identification. Stature is a critical biological metric used in physical anthropology, forensic investigations, and clinical anatomy and its estimation from skeleton remains has long been documented as an essential tool in medicolegal investigations.[1]

It also plays a part in osteoarchaeological research to find out sexual dimorphism and changes throughout time. It is influenced by several genetic and epigenetic variables, including diet, sex, age, physical activity, and

environmental circumstances.[2] There are two ways to estimate someone's stature: the anatomical method, which typically necessitates the complete skeleton, and the mathematical method, which employs various regression equations. There are numerous studies which have illustrated the correlation between various body measurements and stature.[3]

It is essentially based on the fact that the individual's height is found to have a specific and linear correlation with distinct human bones and anatomical structures.[4] In situations where direct height measurement is impractical, like in incapacitated individuals, those on bed rest, or cases of spinal or limb disorders, long bones like the femur and tibia because of their strength, size, and high survival rate in skeletal remains serve as dependable alternatives for stature estimation.[5,6]

Historically, several skeletal parts, from the femur to tiny bones like metacarpals, have been utilized for stature measurement. Although smaller elements can provide reasonably accurate outcomes, long bones consistently exhibit greater predictive value.[7] Bone pieces, whether proximal or distal, have been utilized; nonetheless, whole long bones are favoured as the standard due to their superior precision. All these investigations uniformly concluded that regression equations and correlation coefficients are distinctive to populations.

Consequently, these metrics must be tailored for distinct populations and revised periodically to account for secular and temporal variations.[8] Another important anthropological aspect is bilateral asymmetry, which refers to differences between right and left side bones. Although slight asymmetry is normal, significant differences can affect accuracy in forensic applications if only one side bone is available.[9]

The present study aims to determine the relationship between stature and femur/tibia lengths in adult males, to derive regression equations for stature estimation and to evaluate bilateral asymmetry between right and left femur and tibia.

MATERIAL & METHOD

Study Design and Participants: This cross-sectional study was conducted in the Department of Anatomy, Faculty of Medicine and Health Sciences, SGT (Shree Guru Gobind Singh Tricentenary) University, Gurgaon, Haryana. All participants were given detailed information about the purpose of the study, procedures, and potential advantages. Informed consent was written and guaranteed confidentiality and the right to withdraw at any given time without any repercussions. Before starting the study, the institutional ethical committee approval was obtained.

Sample size and Participant Selection: A predetermined sample size was calculated for regression analysis, ensuring sufficient precision to detect moderate correlations between stature and long-bone dimensions at 80% power and 5% significance. A total of 250 healthy adult male volunteers aged 18 to 40 years were recruited by convenient voluntary sampling. Eligibility criteria included North Indian origin male subjects, healthy, and free from musculoskeletal deformities, past fractures, neurodegenerative conditions or lower-limb surgeries. Individuals with conditions preventing accurate anthropometric measurement (e.g., severe obesity, joint swelling) and declined to provide written informed consent were excluded.

Measurement Protocol and Reliability: All measures were taken into account at one time of day in the anthropology lab of the Department of Anatomy, to minimise diurnal variation. A single, qualified investigator did all the measurements to reduce the observer bias. All the devices were calibrated in centimetres up to the nearest 0.1 cm, and the accuracy of calibration was verified prior to each test.

To assess intra-observer reliability, measurements were done three times under the same conditions, and the average outcome was obtained to be analysed. Bilateral measures were averaged from the left and right data. Measurement error or biological improbability was the only reason to exclude outliers based on standardised residuals (>±3). All anthropometric procedures followed the international recommendations.

Anthropometric Measurement: All instruments (stadiometer and sliding callipers) underwent daily calibration. Measurements were conducted by a sole trained investigator to reduce intra-observer variability. Measurements were obtained on the right and left sides separately.

Stature: A conventional stadiometer with an accuracy of 0.1 cm was used to assess stature. The subjects positioned their heads upright, heels together, and stood barefoot in the Frankfurt horizontal plane. After taking three consecutive measurements, the average was noted.

Femur Length (Right and Left): This measurement was taken from the most prominent palpable point of the greater trochanter to the most prominent palpable point of

the lateral epicondyle of the femur. The individual positioned themselves with ankles together atop the box. The lengths of the right and left femoral bones were subsequently measured percutaneously. Three repeated readings were documented on each side and averaged.

Tibia Length (Right and Left): Tibia length was measured by the linear distance between the medial condyle and the medial malleolus with the assistance of a standard anthropometry calliper. Knee flexion was performed, and the subjects were in a position to enable accurate palpation. Three consecutive measurements per side took place and were averaged.

Data Management & Statistically Analysis: Data were entered into Microsoft Excel and cross-verified for accuracy. Outliers were screened using standardized residual analysis and retained only if biologically plausible. Data were analyzed using SPSS (version 20). Descriptive statistics (mean ± SD) were computed for stature and all long-bone measurements. Pearson's correlation assessed the relationship between stature and bilateral femur and tibia lengths. Paired t-tests evaluated right–left differences. Simple linear regression was applied separately for each bone, and a multiple linear regression model was constructed using all four predictors (right/left femur and tibia) to estimate stature. Model fit was evaluated using R, R^2, adjusted R^2, standard error of estimate (SEE), and significance level $p < 0.05$.

RESULTS

Descriptive statistics for the stature, femur, and tibia length for both sides, left and right, are presented in **Table 1**. Pearson's correlation analysis was utilized to analyze the strength of the relationship between stature and each measured long-bone (femur & tibia) dimension, evaluated individually for the left and right sides **(Table 2)**. All measurements presented statistically significant positive correlation coefficients with stature ($p < 0.01$). The correlation coefficients of the right femur (0.924) and tibia measurement (0.861) was greater than left side femur (0.825) and tibia measurements (0.837).

To determine the potential differences between contralateral measures, paired-sample t-tests were used on each dimension **(Table 3)**. The purpose of this analysis was to determine the presence of statistically significant bilateral asymmetry, which implied that side-specific regression models should be created for stature estimation. These findings revealed that the two differences were statistically significant. It is a

Table 1: Descriptive Statistics for Stature and Lower Limb Measurements (Right& Left) (N=250).

Parameters	Minimum	Maximum	Mean±SD
Stature	117.60	194.50	172.99±10.8
Right Femur	31.75	52.51	46.80±2.9
Right Tibia	25.87	42.79	37.99±2.5
Left Femur	31.00	52.50	46.74±3.0
Left Tibia	25.86	42.79	37.98±2.5

Table 2: Correlation Between Stature and Lower Limb Measurements in Both Sides (Right & Left) (N=250).

Parameters	r	P value (2 tailed)
Right Femur	0.924***	0.000
Right Tibia	0.861***	0.000
Left Femur	0.825***	0.000
Left Tibia	0.837***	0.000

Table 3: Comparing Left and Right Measured Parameter.

Parameters	Difference in mean (Right-Left)	Standard Error (Difference)	t-value	p-value	95%CI of Difference
Femur Length	8.80	0.07	122.37	0.000	8.66, 8.95
Tibia Length	8.76	0.11	75.80	0.000	8.53, 8.98

Table 4: Simple Linear Regression Analysis Predicting Height from Long Bone Lengths.

Predictor	Regression Equation	R	R2	Std Error of Estimate	P value
Right Femur (RF)	11.960+3.441xRF	0.924	0.854	4.156	0.000
Right Tibia (RT)	32.172 + 3.706 × RT	0.861	0.742	5.526	0.000
Left Femur (LF)	36.687 + 2.916 × LF	0.825	0.681	6.141	0.000
Left Tibia (LT)	39.848 + 3.505 × LT	0.837	0.700	5.954	0.000

Table 5: Multiple Regression Equations for Estimation of Stature (cm) from Lower Limb Measurements.

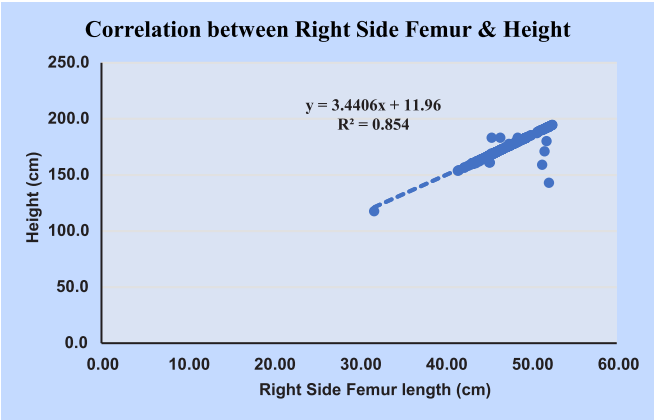
Regression Equation	R	R2	Std Error of Estimate	P value
Height (cm) = 11.781 + 3.224(Right Femur) + 0.205 (Right Tibia) + 0.001(Left Femur) + 0.065(Left Tibia)	0.924	0.855	4.173	<0.001

methodological rigour approach that will enable practitioners to implement the most precise predictive model possible. In this regard, the formulae obtained in this study should be applied by first defining the side of the body part where possible with the help of relevant anatomical landmarks and then using the corresponding regression equation. Equations of linear regression were formulated concerning each of the measured dimensions **(Table 4)**. The predictive significance of each variable was assessed, and model fit was measured by the standard error of estimate (SEE), which is a measure of the residual variation about the regression line: Y = B + MX, where Y represents stature, M represents the regression coefficient, X represents measured dimensions, B represents constant in the equation. A low value of SEE shows the higher accuracy in the estimated stature. All the derived equations had statistically significant regression coefficients. Right Femur length produced the best model (SEE = ±4.156 cm; R^2 = 85%), whereas by contrast left femur was poor with the highest SEEs (±6.141) and the lowest explanatory power (R 2 = 68%).

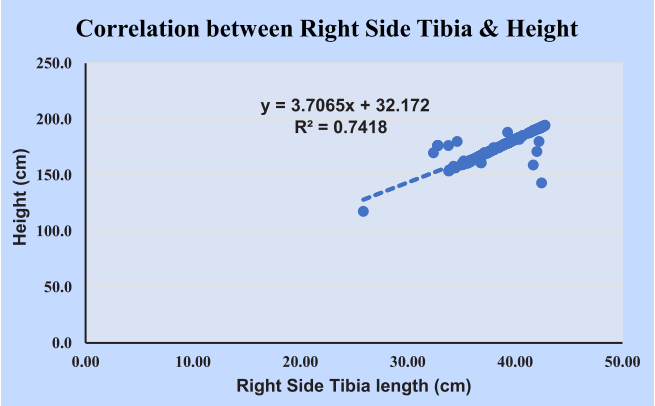
For determining stature, the lengths of the right femur, right tibia, left femur, and left tibia were utilized in a multiple linear regression analysis **(Table 5)**. Total model was statistically significant ($F(4,245) = 359.87$, $p < 0.001$), demonstrating that height is significantly predicted by the combined limb data.

It was found that the model fitted excellently with R = 0.924 and R 2 = 0.855, that is, 85.5 % of the variation in stature could be attributed to the four bone lengths. The adjusted R 2 was 0.852, indicating minimal shrinkage and ensuring the model is stable. Durbin-Watson statistic (2.004) indicated that there was no autocorrelation of the residuals. The only predictor which significantly helped in estimating the stature was the right femur length (β = 0.866, $p < 0.001$). When all four variables were included, only the right tibia (p = 0.569), left femur (p = 0.997), and left tibia (p = 0.831) did not independently predict height, which might possibly be attributed to multicollinearity (VIF values 4.9 to 11.8).

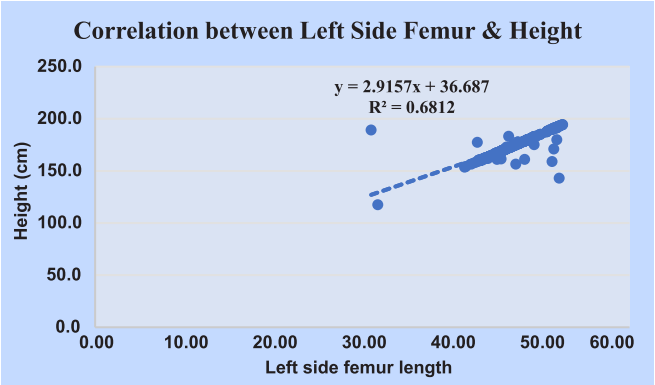
Graph 1: Scatter Diagram Showing Correlation Between Right Side Femur and Height Length in Males.



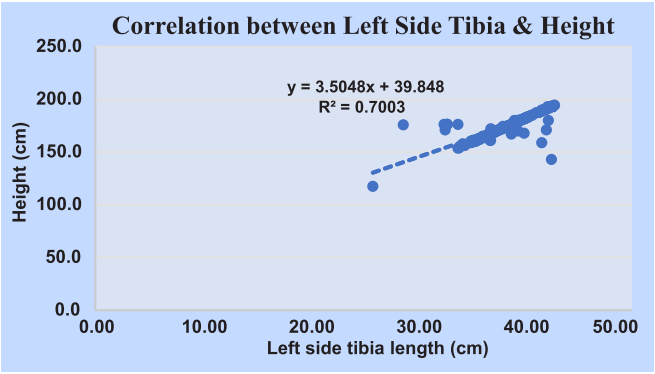
Graph 2: Scatter Diagram Showing Correlation Between Right Side Tibia and Height Length in Males.



Graph 3: Scatter Diagram Showing Correlation between Left Side Femur and Height Length in Males.



Graph 4: Scatter Diagram Showing Correlation between Left Side Tibia and Height Length in Males.



DISCUSSION

One of the most important aspects of postmortem examination is identifying the identity of deceased, particularly after a natural or man-induced disaster, when specific identification is often required. Stature is a critical

characteristic of the reconstruction of the individual's biological profile among the significant biological parameters that can be obtained from skeletal remains. The regression models are important in terms of forensic and medico-legal perspectives. Stature estimation helps to optimize the biological profile of unknown persons and enhances the accuracy and defensibility of identifications in disaster victim recovery, criminal cases, and judicial proceedings. The current study found a robust and statistically significant positive association between long-bone length and stature, confirming that femoral and tibial dimensions are valid predictors of height in adult males. Among all examined variables, the right femur had the highest predictive capacity.

The correlation between stature and right and left femur length (r = 0.924, r=0.861) observed in this study is consistent with previous research by Hauser R (2005) and Chansa Mulenga (2019), who also reported that the femur is the most accurate long bone for reconstructing stature across populations.[10-12] Similarly, study conducted on Indian populations by Kumar P (2014) confirmed that the femur demonstrates the highest predictive value among weight-bearing bones. It was also clear from this study that prediction of stature is also influenced by sex and racial factor.[13] The tibial correlations in the present sample (right tibia r = 0.861; left tibia r = 0.837) also closely align with findings from Saknure MB et al.,[14] who emphasized the tibia as the second-strongest long bone for stature estimation, while the study of Salawu AA et al. reported the strongest associations of tibial measurements with stature across sexes, while intercondylar width consistently displayed the weakest.[15] Another prior research conducted in both African[16] and non-African populations,[17-18] underscoring the tibia's effectiveness as a reliable predictor of height, irrespective of the methods employed, be they percutaneous or osteological. Harrington KI et al. , for example, highlight the consistent correlation between tibia length and height across diverse ethnic populations, underscoring the bone's reliability in stature estimation.[19] The strength of the correlation observed in this study falls within the confidence ranges reported in the wider literature, supporting the notion that femoral and tibia measurements may serve as universal indicators for estimating human height.

Bilateral asymmetry was found to be statistically significant in both femur and tibia ($p < 0.001$). The current study demonstrated marginally elevated correlation coefficients for the right-sided bones in comparison to the left. The trend has been reported in several previous studies suggesting that there is possible functional dominance or more mechanical loading on the right in predominantly right-handed populations. Study of Shrestha I et al. in 2019 observed Bilateral asymmetry in the tibial bone length by 44% and reported Males were more likely than females to exhibit asymmetry in length in the right tibia.[20] Present study is in line with another research which showed bilateral asymmetries in the long bones in relation to several components.[21-22] Our findings also align with those of Krishan K et al. (2010) where notable asymmetry has been documented in lower limb length.[23] The anatomical basis of this asymmetry is still debated, but it may be enhanced by population-specific functions and development. Minor but consequential discrepancies in contralateral bone lengths may affect the reconstruction precision when formulas that are side-specific are not applied. The lateral variation in this work is identified, which justifies the creation of separate regression equations on both sides, thereby increasing the predictive precision in forensic and anthropological applications.

In the simple regression models, the equation involving the right femur gave the best result with a lower standard error of estimate (SEE = ±4.156cm) and a maximum coefficient of determination ($R2 = 0.854$). The left femur, however, had the lowest predictive value ($R 2 = 0.681$; SEE = +6.141), which is probably because there is more biological variability or a measurement asymmetry. Equivalent regression-based models have been utilised in other studies, including that Brits DM et al.,[24] which also found different values of the coefficient of variation based on the population characteristics and the applied analysis methods. The multiple regression model that included all four long-bone lengths showed a very excellent predictive ability ($R2 = 0.855$), which implied that a combination of the length of the femur and tibia explained nearly 85 percent of the variance in the stature. Nevertheless, the multiple regression model was found to be statistically significant; however, only right femur contributed independently as a significant predictor in the presence of other bones. The nonsignificant coefficients of right tibia, left femur, and left tibia indicate multicollinearity, which is a well-known problem when several long bones (growing in correlated patterns) are placed in same model. This was supported by the high VIF values (ranging from 4.9 to 11.8). This demonstrates that multiple regression equations provide more accurate stature estimates than

single-dimension models, a finding consistent with earlier studies.[25-26]

The multiple regression equation is nevertheless a powerful model for real-world applications where there are numerous bones available, even though it is susceptible to multicollinearity. When just partial remains are found, forensically speaking, it may be more reasonable to use simple regression equations generated from individual bones. Overall, the findings support the notion that femur and tibia lengths, particularly the femur, are reliable indicators of adult male stature and are in line with international research. The reliability of these bones in recreating biological profiles in forensic cases involving North Indian populations is demonstrated by the strong predictive values found in this study.

CONCLUSION

The current study demonstrates that adult North Indian male's femur and tibia lengths are extremely accurate measures of stature. The right femur was the best predictor of all the long bones examined; it had the lowest SEE and the highest correlation. Significant bilateral asymmetry was observed, which justifies the use of side-specific regression equations to achieve precision. The independent predictor, which was the only variable that showed multicollinearity between the measured variables, was the right femur, despite the multiple regression model having a strong predictive fit. The regression equations developed in this research apply to specific populations and are invaluable tools in forensic anthropology, medico-legal inquiries, and archaeological research in Northern India. It is recommended that future research should employ larger and more diverse samples, that they should include female subjects, and employ advanced imaging modalities. Such steps will contribute to further refining stature prediction models and enhancing their use in different demographic groups.

LIMITATIONS:

This study only included adult males between the ages of 18 and 40, hence the developed regression equations could not be applicable to adolescents, older adults, or individuals with age-related skeletal abnormalities. Additionally, the use of percutaneous measurements and the restriction to a male population in North India limit greater generalizability.

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Original Research Paper

Forensic Investigation on Blue Stamp Pad Inks using UV - Visible Spectroscopy

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ABSTRACT

Introduction: Stamp-pad inks are frequently used in numerous authorized documents particularly in contracts, wills, declarations, cheques and postal cards etc. These inks and stamps used to provide authenticity and credibility to document. Any alteration in stamp inks can dispute the document or question its authenticity. To establish the authenticity of a document, examination of stamp pad inks can play a pivotal role in forensic investigation for the purpose of legal procedures. In the present study, a total of 13 samples (B1 to B13) of blue stamp pad inks of different brands were collected from Punjab region of North-West India. Four different substrates were utilized including A4 sheet, bond paper, ivory paper, and postcard envelop to analyse the effect of substate on stamp pad inks. The stamp pad ink samples were analysed using UV-Visible Spectroscopy which is rapid,cost effective method. On the basis of visual peak asesement three samples of inks were selected for substrate study (B3, B11 & B12). The result revealed that blue stamp-pad ink samples were distinguished from each other with a discriminating power of 83.33% using visual examination and UV/Visible spectra. The findings provide insightful information about blue stamp pad inks differentiation which further help in forensic document examinations.

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INTRODUCTION

Modern development and digitalization of technology has made analysis of ink tremendously stimulating.[1] Ink analysis plays a pivotal role in questioned document cases, such as fogery or where authentication of document is unidentified or questioned. With the innovation, counterfeiters are also using contemporary methods for falsifying the documents and hence making the examination process more tedious.[2]

Over a decades numerous types of inks are frequently used among which stamp pad inks are extensively accepted to acknowledge authorship to the agreements, legal binding, personal or professiona documents, cheques, artwork, funding, or other official records. Across Asian region, manual signatures along with stamps are frequently employed to validate personal and official documents.[3]

Any tampering or modifications made in any document that can question its authenticity can be validated by comparing the stamp seal inks on various sections of the document. Therefore, stamp pad inks distinction and identification play a essential role in judicial competence, as they may be important in determining the legitimacy of papers under scrutiny.[4]

In instances where allegations are made that in some cases, the seal ink stamped on the development contract and the document of the developmental right application are the same, examination of stamp inks is beneficial in such cases. In document analysis: **(I)** chemical profiling of ink, **(ii)** sequence analysis of intersecting ink strokes and **(iii)** ink age estimation in questioned documents are significant for solving disputes.[5] Chemical composition of modern inks are significantly more complex due to increased use of synthetic dyes, additives, organic solvents etc as compared of ancient inks.[6]

Modern inks are composed of lubricants, pigments, solvents, deformers, emulsifiers, surfactants, humectants, resins, modifiers, pH-adujusting substances, biocides/ baceteriostatic agents etc.[7] Various analytical techniques, such as TLC and HPTLC, were deployed to correlate the values of retention factor values of blue stamp pad inks.[8] Consequently, the analysis's findings can be utilized to confirm or refute the truth that the seal ink employed in the

two aforementioned documents is the same or not.[7] Bacteriostatic and biocides are also added to the ink to prevent bacteria and fungi.[9-10]

The first case included the chemical analysis of the ink on a disputed document, was reported in the court of New York County in 1889, suggesting that the ink testing is not a recent development.[11] Various destructive as well as nonde-structive techniques such as chromatographic techniques including thin-layer chromatography (TLC),[8] high-performance liquid chromatography (HPLC),[12] and gas chromatography-mass spectroscopy (GC-MS)[13] can be utilized for the identification of various components and their concentration in the inks.

These chromato-graphic techniques are destructive in nature as the sample is consumed during analysis. Whereas, certain non-destructive practices such as, attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy,[12] UV- visible spectroscopy,[14] and Raman spectroscopy[15] can be utilized for the identification and differentiation of different inks based on their chemical compositions.

The objective of the present study is to use UV-Visible spectroscopy to analyze the chemical consituents of blue stamp pad inks to differentiate between them using their spectral profile to inspect forgeries in document analysis. The study showed that blue stamp pad ink samples were differentiaed from each other.

MATERIALS AND METHOD

Study Design: The research was conducted as analytical and comparative analysis of blue stamp pad inks.

Place of Study: The study was carried out in the Punjabi University, Patiala.

Time Period: The study was conducted for the period of six months.

Ethics: No ethical permission was required for this research.

Exclusion Criteria: Stamp pad inks other than blue were excluded.

Inclusion Criteria: The study included only fresh and unused blue stamp pad ink samples.

Sample Size: The study included thirteen blue stamp pad ink samples in triplate purchased from local markets.

MATERIAL

A total of 13 blue stamp pad inks of different brands were collected from the local shops of various districts of Punjab, India. All the samples were collected in triplicates and details of these samples are given in **Table 1**. Samples for the analysis were prepared by diluting stamp pad ink in methanol. As substrate study was also conducted to analyse the substrate variabiity. Four different substrates were utilized including A4 sheet, Bond paper, Ivory paper and Postcard envelop. Three samples of inks were selected for substrate study (B3, B11 and B12). Stamp pad ink was imprinted on the different surfaces and let it rest for 24 hours. Samples were extracted from surfaces using methanol.

Table 1: Sample Description of Blue Stamp Pad Inks.

S.No.	Sample Code	Brand	Sample No.
1	B1	Ashoka	3
2	B2	Golden (Ashoka)	3
3	B3	Camlin	3
4	B4	Faber Castell	3
5	B5	Office Mate	3
6	B6	Supreme	3
7	B7	Kanchan	3
8	B8	Gold Star	3
9	B9	Shiny	3
10	B10	Colop	3
11	B11	Saket	3
12	B12	Select	3
13	B13	Tro Ink	3

METHOD

Pristine samples for UV-VIS spectrometry were prepared by diluting 0.2 µl stamp pad ink with 4 ml methanol and for substrate study samples were prepared. SHIMADZU UV-1700 Pharma Spec instrument was used for the analysis. The parameters were kept constant during the analysis which are illustrated in **Table 2**. In both the cuvettes methanol was filled and analyzed under UV-Visible light to record the background. Each sample and three substrate samples were analyzed thrice in the range of 190-800 nm. The spectrum of each ink was recorded and saved. After each analysis, the cuvette was cleaned with methanol to avoid contamination.

Table 2: Instrumental Parameters.

Wave Lenghth Range	190-800 nm
Scan Speed	Medium
Sample Interval	Automatic Interval
Measuring Mode	Absorbance
Slit	1 nm

RESULTS

A total of thirteen blue stamp pad ink samples in triplate exhibited absorption within the 190–800 nm range. The maxima and minima absorbance points for all blue stamp pad ink samples were observed for each sample as given in **Table 1.3.** In sample B1 has seven maxima (777, 732, 634, 476, 358, 316, and 236 nm) and seven minima (760, 650, 582, 364, 341, 306, and 193 nm) points.

Sample B2 has five maxima (581, 358, 303, 251, and 207 nm) and four minima (429, 341, 282, and 233 nm) points. Sample B3 has four maxima (577, 301, 248, and 204 nm) and three minima (430, 277, and 237 nm) points. Sample B4 has five maxima (578, 301, 248, 221, and 201 nm) and five minima (664, 442, 283, 243, and 206 nm) points.

Sample B5 h a s six maxima (621, 582, 407, 303, 249, and 204 nm) and five minima (722, 614, 442, 276, and 240 nm) points **[Figure 1]**. Sample B6 has four maxima (578, 302, 248, and 208 nm) and three minima (458, 272, and 237 nm) points **[Figure 2]**. Sample B7 has seven maxima (756, 573, 366, 349, 302, 249, and 207 nm) and seven minima (752, 659, 456, 364, 325, 273, and 233 nm) points **[Figure 3]**. Sample B8 has five maxima (579, 348, 302, 248, and 207 nm) and five minima (694, 436, 430, 278, and 235 nm) points **[Figure 4]**.

Figure 1: UV-visible Spectrum of Sample B5.

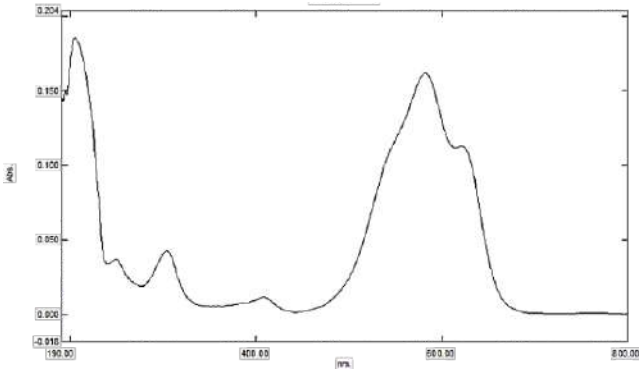


Figure 2: UV-Visible Spectrum of Sample B6

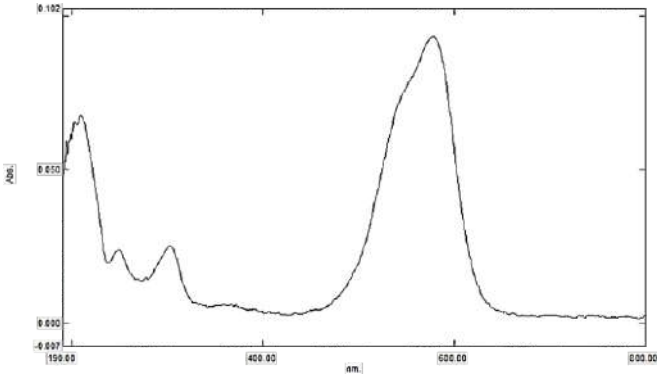


Figure 3: UV-Visible Spectrum of Sample B7

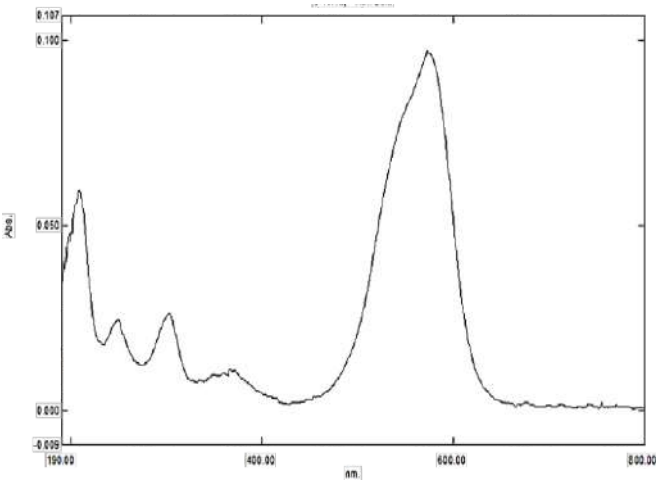
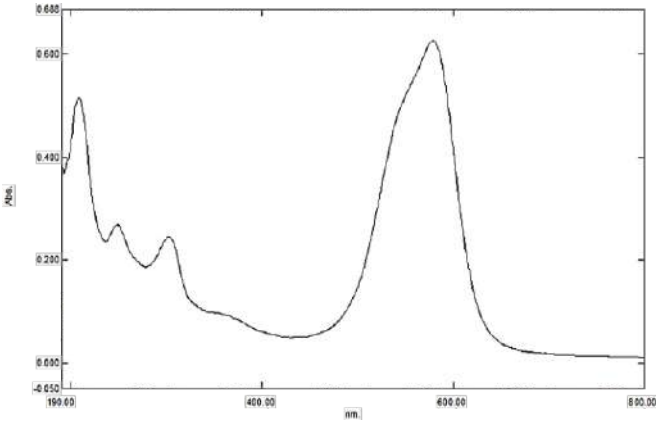


Figure 4: UV-Visible Spectrum of Sample B8



Sample B9 has eight maxima (770, 626, 561, 406, 352, 308, 256, and 204 nm) and seven minima (709, 579, 457, 394, 332, 280, and 245 nm) points. Sample B10 has seven maxima (718, 623, 370, 356, 308, 251, and 205 nm) and five minima (682, 475, 336, 282, and 242 nm) points. Sample B11 has five maxima (683, 581, 302, 252, and 207 nm) and five minima (679, 422, 342, 285, and 232 nm) points **[Figure 5]**.

Sample B12 has seven maxima (740, 685, 584, 351, 301, 252, and 206 nm) and five minima (734, 464, 336, 293, and 235 nm) points [Figure 6]. Sample B13 h a s six maxima (693, 604, 586, 332, 261, and 216 nm) and five minima (663, 485, 293, 255, and 202 nm) points.

Discriminant Analysis:

Based on descriptive and visual examination of **Table 3**, the discriminating power (DP) is assesed. Primarily it is a pair-based evaluation approach which inspected the resemblances and variations among the samples. Smalldon and Moffat described this and later, it is used by

Table 3: Maxima and Minima Absorbance Points for All Blue Stamp Pad Ink Samples.

Sample	Maxima (nm)	Minima (nm)
B1	777, 732, 634, 476, 358, 316, 236	760, 650, 582, 364, 341, 306, 193
B2	581, 358, 303, 251,207	429, 341, 282, 233
B3	577, 301, 248, 204	430, 277, 237
B4	578, 301, 248, 221, 201	664, 442, 283, 243, 206
B5	621, 582, 407, 303, 249, 204	722, 614, 442, 276, 240
B6	578, 302, 248, 208	458, 272, 237
B7	756, 573, 366, 349, 302, 249, 207	752, 659, 456, 364, 325, 273, 233
B8	579, 348, 302, 248, 207	694, 436, 430, 278, 235
B9	770, 626, 561, 406, 352, 308, 256, 204	709, 579, 457, 394, 332, 280, 245
B10	718, 623, 370, 356, 308, 251, 205	682, 475, 336, 282, 242
B11	683, 581, 302, 252, 207	679, 422, 342, 285, 232
B12	740, 685, 584, 351, 301, 252, 206	734, 464, 336, 293, 235
B13	693, 604, 586, 332, 261, 216	663, 485, 293, 255, 202

Figure 5: UV-Visible Spectrum of Sample B11

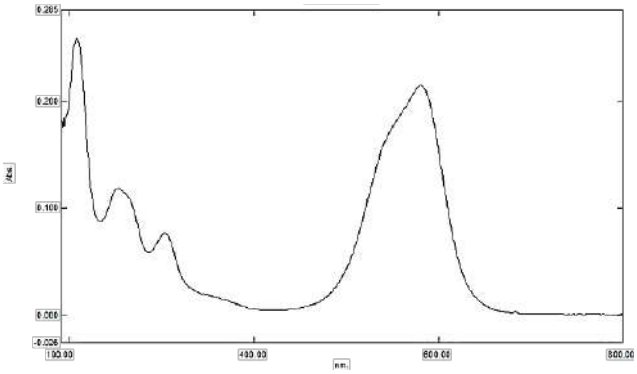
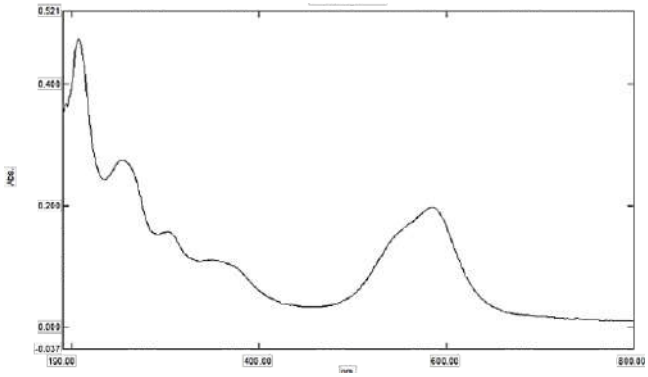


Figure 6: UV-Visible Spectrum of Sample B12



many researchers for discrimination purposes. According to Smalldon and Moffat, DP is calculated[17]:

$$DP = \frac{\text{Total number if discriminating pairs of samples}}{\text{Total no. of possible pairs of samples}} \times 100$$ [17]

To calculate the total number of possible pairs[18]:

$$\text{Total Number of Possible Pairs} = \frac{n\,(n-1)}{2}$$

$$\text{Total Number of Possible Pairs} = \frac{13\,(13-1)}{2}$$

In the conducted study, as the total of 13 samples of blue stamp pad inks, thus the total number of possible pairwise combination are calculated as 78. After an in-depth inspection of all the samples, it is found that set of thirteen samples (B1B2, B1B3, B1B4, B1B5, B1B6, B1B7, B1B8, B1B9, B1B10, B1B11, B1B12, B1B13, B5B7) could not distinguish by descriptive analysis. Thirteen paired of ink samples from blue stamp-pad revealed resembling peaks through UV–Visible spectroscopy which show no distinction. Consequently, after visual analysis, the DP value is:

$$DP = \frac{78 - 13}{78} \times 100$$

Substrate Study:

Three stamp pad ink samples (B3, B11, and B12) were randomly selected to study the effect of substrate on stamp pad inks. The substrates such as A4 sheet (A), bond paper (B), ivory sheet (I), and postcard envelopes (PC) were included in this study. UV-visible spectra of stamp pad inks extracted from the various substrates were compared with those of pristine (S) stamp pad ink samples.

Figure 7: Spectral Comparison of Sample B3 in Pristine form and on Substrates

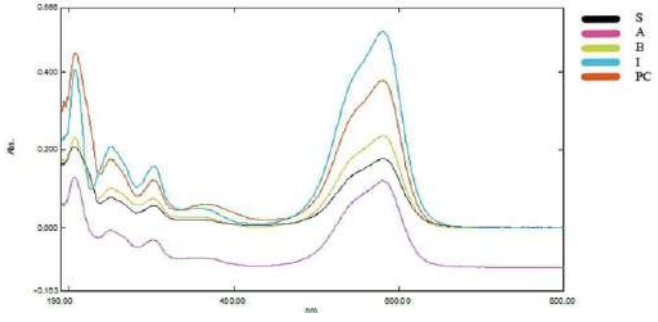


Figure 8: Spectral Comparison of Sample B11 in Pristine form and on Substrates

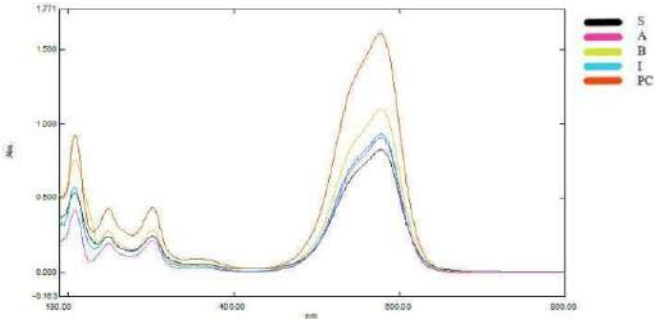
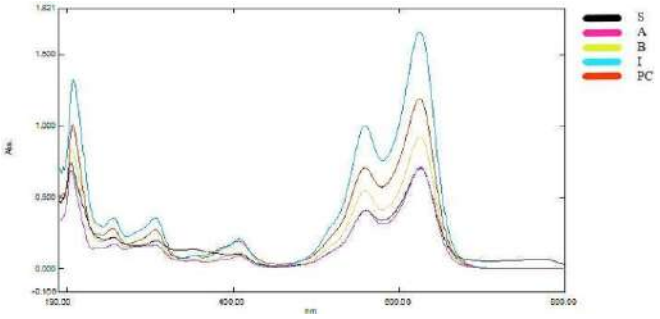


Figure 9: Spectral Comparison of Sample B12 in Pristine form and on Substrates



The substare study unveiled that UV-visible spectra of stamp pad inks extracted from the various substrates were compared with those of pristine (S) stamp pad ink samples. Visual examination of spectra demonstrated that pristine spectra of the selected stamp pad ink samples (B3, B11, and B12) were similar to the spectra of stamp pad inks extracted from the various substrates **[Figure 7, 8, 9]**.

DISCUSSION

The study demonstrated that peaks in the UV-visible spectra ranges from 190-800 of samples were compared with the previously published data. A peak at 200-225 nm was observed in all the samples except for sample B1 which might be due to the presence of solvent glycerol. The peak at 250-275 nm was present in samples B2, B3, B5, B8, B9, B10, B11, B12, and B13 which may be due to the presence of water.

The peak at 300-325 nm was observed in all samples except for samples B1, B4 and B13 which may be due to the presence of some additives including waxes, plasticizers, soaps, and detergents. A peak was observed at 575-600 nm in all the samples except for samples B1, B4, B7, B9, B10, and B13 which may be due to the presence of methyl violet dye (Adam, 2009; Lee et al., 2015).[14,16]

UV-visible spectral comparison of blue stamp pad ink pristine samples: Visually inspected samples were further discriminated by comparing the presence and absence of peaks in each sample's corresponding UV-visible spectrum (190-800 nm). **Table 1.4** presents a complete overview of the wavelengths at which peaks are present in blue stamp pad ink samples.

CONCLUSION

The comparative evaluation of blue stamp pad inks demonstrated that despite their visual appearce their spectral profile is differenent from each other. The variation between their maxima and minima absorbance

Table 4: Comparative Table of Peaks Observed at Various Wavelengths in Blue Stamp Pad Ink Spectra.

Samples	wavelength (nm)																						
	200-225	225-250	250-275	275-300	300-325	325-350	350-375	375-400	400-425	425-450	450-475	475-500	500-525	525-550	550-575	575-600	600-625	625-650	650-675	675-700	700-725	725-750	750-775
B1	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-
B2	+	-	+	-	+	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
B3	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
B4	++	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
B5	+	+	+	-	+	-	-	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-	-
B6	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
B7	+	-	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
B8	+	-	+	-	+	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
B9	+	-	+	-	+	-	+	-	+	-	-	-	-	-	+	-	-	+	-	-	-	-	+
B10	+	-	+	-	+	-	+	-	+	-	-	-	-	-	-	-	+	-	-	-	+	-	-
B11	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
B12	+	-	+	-	+	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
B13	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-

points, peak intensities and their overall spectral profiles helps to discriminate the samples with each oher, confirming that UV-visible spectroscopy is the most reliable, cost effective and non-destructive technique that can be utilized for both qualitative and quantitative analysis.

The results revealed that discrimination power (DP) of pristine blue stamp pad ink samples are 83.33% which was calculated by visual inspection of presence and absence of peaks at a particular wavelength. The findings of this study could help forensic document examiners to analyze stamp and stamp pad ink samples efficiently and accurately. However sample size may be considered as the limitation of this study. Despite such limitation, this research offers a low-cost and effective method for forensic examination and can potentially contribute to the broader domain of forensic identification. Further researches with more sample size and environmental factors may contribute further in forensic science.

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Original Research Paper

Rugoscopy as a Supplement Tool for Profile Generation in Barwala Region, Panchkula (Haryana)

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ABSTRACT

Introduction: Accurate age and sex estimation is critical in forensic investigations. Dentition and palatal rugae patterns are two anatomical structures offering potential markers for personal identification. This study aimed to estimate age based on the eruption of permanent teeth and assess gender differences using rugoscopy in individuals from the Barwala region of Haryana.

Materials and Method: A cross-sectional study was conducted on 320 healthy dentulous individuals aged 10–24 years with known age proof (Adhaar card) attending the outpatient department at Swami Devi Dyal Dental Hospital and College, Barwala, Panchkula (Haryana). Clinical evaluation of tooth eruption followed the FDI notation system. Age estimation was based on Wheeler's eruption chronology. Maxillary impressions were used to create study casts, and palatal rugae were analyzed for number, length, and shape. Data was analyzed using Microsoft Excel and SPSS v1.0.0.1406.

Results: The estimated age matched actual age in 78 participants, while 94 showed an overestimation of 1 year, and 144 showed underestimation of 1–4 years. Females exhibited earlier eruption of third molars compared to males. Rugae counts were higher in males (1450) than in females (1376), with males exhibiting more secondary and fragmentary rugae. Males showed longer primary rugae, while females had slightly longer secondary rugae. Common rugae shapes observed were curved and straight in both sexes, with wavy forms more common in females.

Conclusion: Permanent tooth eruption is a reliable method for age estimation in individuals under 25 years, though slight overestimation was observed. Rugoscopy demonstrated significant sex-based differences and may serve as a supplementary tool in forensic sex identification.

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INTRODUCTION

Forensic odontology plays an essential role in human identification, particularly when conventional methods such as fingerprinting or DNA profiling are not feasible because of decomposition or availability. Its applications extend across various domains, including criminal investigations, disaster victim identification, and age or sex estimation in legal and humanitarian contexts.

Age estimation is especially critical in cases involving unidentified remains, juvenile justice, and asylum determinations. Among the various indicators, tooth eruption stands out as a genetically programmed yet environmentally influenced developmental marker, making it a reliable parameter for age estimation in children and adolescents.[1]

In addition to dental development, anatomical structures like palatal rugae-irregular mucosal ridges located on the anterior third of the hard palate-are gaining prominence in forensic identification. These rugae patterns are highly individualistic, remain relatively stable throughout life, and demonstrate resistance to trauma, decomposition, and

extreme environmental conditions.[2-3]Traditionally utilized for personal identification, palatal rugae are now being explored for their potential in sex determination as well.[2]

Moreover, in rare forensic scenarios involving bite marks on soft materials, palatal rugae impressions may be inadvertently recorded, allowing for supplementary identification through rugoscopy.[4] Despite extensive literature on tooth eruption and palatal rugae morphology as separate forensic tools, limited studies have attempted to correlate both parameters simultaneously for combined age and sex estimation. Such integrative approaches may enhance forensic accuracy, particularly when dental or skeletal remains are partially preserved.

Furthermore, region-specific anthropological variations necessitate localized data for better forensic applicability. To address this gap, the present study aimed to evaluate the correlation between tooth eruption and palatal rugae morphology for age and sex estimation among young individuals in Barwala (Haryana), thereby contributing to the establishment of region-specific forensic standards.

MATERIALS AND METHODS

Study Design and Participants

This was a clinical, cross-sectional study conducted at Swami Devi Dyal Dental Hospital and College, Barwala, Panchkula, Haryana. Ethical clearance was obtained from the Institutional Ethical Review Board. A total of 320 participants aged 10–24 years were selected based on inclusion and exclusion criteria.

Participants were Divided into Four Age Groups:

Group 1: 10–13 years (n = 80)

Group 2: 13.1–16 years (n = 80)

Group 3: 16.1–18 years (n = 80)

Group 4: 18.1–24 years (n = 80)

Each group had an equal number of males and females (n = 40).

This study will include Mixed dentition (Primary and secondary dentition), and permanent dentition patients as per there chronological eruption pattern. Participants will be excluded if they have any known allergies to dental impression materials, as this may pose a health risk. Individuals with severe malocclusion, cleft palate, or other congenital or acquired palatal anomalies will also be excluded to maintain uniformity in the study sample. Additionally, those currently using dentures or braces, or with a history of orthognathic surgery, will not be eligible to participate. Patients with a history of facial trauma or scarring that could affect oral structures, as well as those who are unwilling or unable to provide informed consent or participate in the study, will also be excluded

Age Estimation Procedure

Clinical examination was conducted using diagnostic kits. Tooth eruption patterns were recorded using FDI notation. The last erupted tooth was noted, and estimated age was calculated based on Wheeler's eruption chronology.[5] Actual age was recorded from government issued ID (Aadhaar Card) and compared.

Gender Estimation Procedure via Rugoscopy

Alginate impressions were taken, and casts were prepared using Type 2 dental stone. A midline was marked along the mid-palatal raphe. Rugae were analyzed for:

Number: Count on both sides of the midline.

Length: Measured using a digital vernier caliper. As per Thomas and Kotze classification.[6-7] Primary: >5 mm, Secondary: 3–5 mm, Fragmentary: <3 mm

Shape: Classified as straight, curved, wavy, circular, and unification (converging or diverging arms).[8] Three independent examiners analyzed the casts to avoid inter-observer bias, and mean values were recorded.

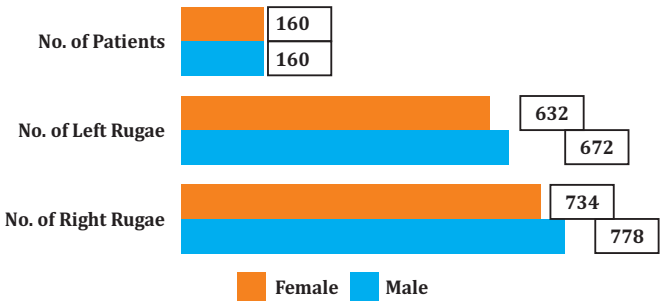
Statistical Analysis

Data were analyzed using Microsoft Excel (2013) and SPSS (v1.0.0.1406). Confidence intervals were set at 95%, with $p < 0.05$ considered statistically significant.

RESULTS

There are 4 groups in total, each with 80 participants, leading to a grand total of 320 patients included in the study.

Table 1: Comparison of Right & Left Rugae.



When the number of palatal rugae in male and female subjects, with 160 participants in each group were compared, Males showed a higher count of both left (672

vs. 632) and right rugae (778 vs. 734) compared to females. This suggests a slight gender-based difference in rugae patterns, more prominent on the right side. The mean and standard deviation of the sum of right and left palatal rugae were assessed in 160 male and 160 female subjects.

For the right palatal rugae, males demonstrated a higher mean count (4.86 ± 0.85) compared to females (4.59 ± 0.94). Similarly, for the left palatal rugae, males also showed a higher mean (4.20 ± 0.75) than females (3.95 ± 0.79). The total rugae counts further supported this trend, with males exhibiting higher numbers on both sides-778 right and 672 left, compared to females, who showed 734 right and 632 left rugae. Overall, these findings indicate that males tend to have a slightly greater number of palatal rugae on both sides than females, with relatively low variability within each group.

Inferential analysis revealed that the difference in right palatal rugae counts between males and females was not statistically significant ($p = 0.0585$). In contrast, a statistically significant gender difference was observed for the left palatal rugae ($p = 0.0417$), suggesting a meaningful side-specific variation.

Analysis of palatal rugae shape patterns showed that straight and curved rugae were the most commonly observed forms in both genders. Males exhibited higher frequencies of straight (n = 580) and curved (n = 758) rugae compared to females (straight: n = 414; curved: n = 462). Wavy rugae were more frequently observed in females (n = 378) than in males (n = 274).

Circular rugae represented the least common pattern in both groups, while unification patterns were almost equally distributed between males (n = 186) and females (n = 184). Statistical analysis demonstrated a significant gender difference in the distribution of wavy rugae patterns ($p < 0.05$), whereas no statistically significant differences were observed for straight, curved, circular, or unification rugae patterns ($p > 0.05$).

Table 2: Age Estimation Findings.

Age Difference	No. of Subject
+1 year	94
0 year (Exact)	78
−1 year	40
−2 years	38
−3 years	66
−4 years	4

Eruption was found earlier in females, particularly for third molars (32/40 females vs. 26/40 males in Group 4). Greatest variability in eruption pattern was seen in 10–13 year old, consistent with mixed dentition.

Table 3: Rugae Count and Distribution.

Rugae Type	Male (n)	Female (n)
Primary	1242	1240
Secondary	128	58
Fragmentary	80	68
Total	**1450**	**1376**

Males had more total rugae and secondary/fragmentary types. Rugae were more numerous on the right side in both genders.

Table 4: Rugae Length (Mean).

Type	Male (n)	Female (n)
Primary	9.98	9.07
Secondary	3.99	4.01

Straight and curved types were dominant in both genders. Wavy rugae were more common in females. Chi-square analysis revealed a statistically significant gender difference in the distribution of wavy rugae patterns ($p < 0.05$). Straight, curved, circular, and unification patterns did not show statistically significant differences between males and females ($p > 0.05$).

Table 5: Rugae Shape Patterns.

Shape	Male	Female
Straight	580	414
Curved	758	462
Wavy	274	378
Circular	30	24
Unification	186	184

DISCUSSION

Accurate age and gender estimation plays a pivotal role in forensic science, especially in contexts such as disaster victim identification, criminal investigations, and civil litigations involving age disputes. The present study utilized two established forensic tools, permanent teeth eruption patterns and palatal rugae morphology (rugoscopy) to estimate age and gender in a sample population from the Barwala region, Panchkula Haryana. The significance of this dual-method approach lies in its ability to provide cross-validation and greater reliability, particularly when other identification modalities are unavailable.

Palatal rugae develop during the third month of intrauterine life and are primarily under genetic and embryological control, which determines their number, shape, and orientation.[9] Although the basic rugae pattern remains stable throughout life, minor changes may occur due to growth, orthodontic treatment, trauma, or oral habits without altering their fundamental configuration.[9-10] Previous studies have concluded that length of rugae is increased significantly till 10 years of age thereby remaining stable througho6ut life suggestive of underlying palatal growth.[11] Genetic and molecular influences, including bone morphogenetic protein signaling pathways, have also been implicated in rugae morphology, supporting their individuality and forensic relevance.[12]

Age Estimation Using Dental Eruption Patterns

The chronology of tooth eruption has long been recognized as a reliable biological indicator for age estimation, especially in children and adolescents. Our study found a general trend where the estimated dental age was often higher than the actual chronological age. This may reflect regional variations in eruption timing, influenced by factors such as genetics, nutrition, socioeconomic status, and environmental exposures.

The results are consistent with previous studies, such as those by Kaul and Tewari,[13] Aggarwal KK, Kaur A, Kumar R[14] which reported region-specific differences in eruption timelines among North Indian populations. In our Barwala cohort, we observed greater variation in eruption patterns within the 10–13 years age group, reflecting the transitional phase of mixed dentition. In contrast, the 15–18 and 21–24 years age groups showed more homogenous patterns, likely due to the full eruption of permanent teeth.

Additionally, a Sex-based difference in eruption timing was evident: female subjects generally exhibited earlier eruption of teeth compared to males. This supports findings from studies such as those by Lakshmappa, et al[15] and Elamin & Liversidge,[16]

which noted earlier dental development in females. The early eruption in females is thought to be linked to earlier overall physiological maturation.

Rugoscopy and Gender Estimation

The palatal rugae patterns in this study provided complementary data for sex identification. Consistent with literature to Nayak et al,[17] Paliwal et al,[18] we found sexual dimorphism in the number, length, and shape of rugae. While the total number of rugae was higher in males, females exhibited greater mean length in secondary rugae, which aligned with the findings of Kapali et al.[19]

A particularly interesting observation was the predominance of certain rugae shapes-such as wavy and curved patterns in both genders, but with a higher frequency of straight rugae in males and more balanced distribution in females. This suggests that although certain patterns are common across sexes, specific morphometric differences may enhance gender differentiation.

Unification patterns, especially diverging and converging types, were almost equally distributed among males and females in our study. However, directional differences (right vs. left rugae) revealed that the right side had a higher rugae count in both genders, a pattern that could have developmental or functional implications that warrant further investigation.

Comparative Utility in Forensic Settings

The combination of dental and palatal rugae analysis enhances the accuracy of forensic identification. In mass disaster scenarios, where fingerprints and DNA may be compromised, rugoscopy offers a non-invasive, stable, and easily recordable method of identification. The resilience of rugae patterns to post-mortem changes and environmental stress supports their utility, as noted by Mutalik et al[20] and Trobo Hermosa.[21]

While dental eruption patterns are best suited for age estimation, especially in individuals below 25 years, rugoscopy may be particularly valuable for gender identification and supplementary personal profiling.

Challenges and Limitations

Despite the positive outcomes, several limitations must be acknowledged:

Inter-observer variation in measuring and classifying rugae patterns, although minimized by using multiple examiners, could introduce bias.

The cross-sectional design of the study prevents longitudinal tracking of eruption patterns or rugae changes over time.

The sample was limited to a specific geographic region (Barwala), which may affect the generalizability of the findings to other ethnic or regional populations.

Future studies with larger, multi-regional samples, and the integration of digital image analysis tools for rugae assessment could provide more objective and scalable

applications of these forensic methods.

CONCLUSION

Dental age estimation is an accepted and reliable method, particularly in children and adolescents, due to predictable eruption sequences.[22-23] This study confirms earlier eruption in females, consistent with prior studies in Indian populations.[13] Slight overestimation of age was observed, possibly due to environmental, nutritional, and genetic factors.[24]

Palatal rugae patterns showed significant gender-based variations. Males had more rugae overall, with longer primary rugae and more secondary and fragmentary types. Wavy rugae were more prevalent in females, aligning with other Indian studies on rugoscopy.[25-26] These findings support rugoscopy as a supplemental tool for gender estimation, especially in forensic contexts where conventional methods are unavailable. Further research with larger samples and integration of radio-graphic data is recommended.

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Original Research Paper

Ethical Doctor-Patient Relationship: Do We Actually Practice It ? An Observational Survey at a North Indian Tertiary Care Centre

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ABSTRACT

Introduction: Ethical practice in the medical field has gained prominence as an essential component of professional conduct. However, existing research largely focuses on stakeholders' attitudes rather than on actual bedside behaviour of physicians or variations across clinical settings such as outpatient departments (OPDs), wards, and emergencies.

Objectives: This observational study aims to assess the standards of patient care and ethical practice among physicians, with a focus on the influence of physician experience and work setting. It also seeks to identify potential causes for observed differences in clinical behaviour.

Materials and Methods: A total of 75 physicians from five high-patient-load departments were observed in OPDs, wards, and emergency settings. A corroborated questionnaire was used to evaluate physician behaviour, with emphasis on patient-centredness, communication, and adherence to ethical norms. Data were analysed to compare performance across gender, years of experience, and clinical setting.

Results: A higher degree of patient-centric care was associated with male physicians and with increased years of experience, although some variations were noted. The outpatient setting recorded the highest scores, followed by emergency settings, with wards showing the lowest performance. Observable disparities in ethical and patient-centred behaviour were evident across settings and experience levels.

Conclusion: The findings highlight significant variations in ethical practice and patient-centred care across clinical settings and levels of experience. These disparities underscore the need for targeted training, institutional support, and systemic reforms to strengthen ethical standards and preserve the integrity of the doctor–patient relationship.

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INTRODUCTION

Ethical medical practice is the cornerstone of present-day clinical practice. The four pillars of ethics – autonomy, beneficence, non-maleficence, justice – manifest in clinical practice in the form of communication, attitude, and bedside manner.[1-2] A physician of modern medicine is not only required to be clinically component but also be able to develop empathy and gain the confidence of his patients through interpersonal relationships. Doctor–patient relationship is imperative for delivery of high-quality medical care,[3] and has been shown to affect patient prognosis and outcome and workplace morale.[4-7] Effective treatment depends on the physician's understanding of the patient's preferences, concerns, pressures, and mental condition.

In India, corporatization of the healthcare delivery system has led to a rise in distrust and negative sentiments against doctors and modern medicine.[8-10] This is evident in the rising number of litigations and violence against the doctors.[11] The Indian Medical Association has reported that 75% of practicing doctors face verbal or physical abuse in hospital premises, and the fear of violence was the

most common cause for stress in 43% doctors.[12] Some government hospitals, in addition to a number of private ones, have also started employing bouncers to protect the doctors. In response, the NMC has published a series of rules outlining ethics and etiquette to be followed by doctors.[13]

However, there is a glaring disparity between the stated rules and routine practice. The complex medical care environment and the strained doctor-patient relationship is multifactorial: overburdened doctors, long working hours, stress filled environment, impatient patients, unrealistic expectations from the treatment, growth of health insurance and corporatization, expansion of literacy, and easy accessibility to unauthentic information via the internet and inadequate infrastructure.[8-9,11-12] With the increase in the number of patients accessing healthcare, the doctor-patient ratio has become skewed.[11] The mushrooming of private medical colleges is another important reason for the strained doctor-patient relationship.

Other societal factors have also contributed to the issue. Social media and sensational journalism, in its quest to sell news, highlights negative incidents.[12] Exposure to such sensationalized media reports further erodes trust in the health care delivery system.[8] The ever-increasing global consumerism and the explosion of information on the internet have resulted in an environment where patients are far more aware of their rights than their responsibilities. Exaggerated claims of medical success, fuelled by the corporate medical establishments, lack of proper communication by the doctors have all led to unreasonable expectations of patients.[12] This has resulted in aggressive behaviour when these expectations are not met.[11]

The judgments by various courts have placed greater emphasis upon a patient's right to know and offering them coherent choices, resulting in the consultation being looked upon as a business proposition.[10] Medical litigation has seen an exponential rise, and there is less tolerance toward even minor complications, which in turn generates distrust in the medical fraternity towards patients. Doctors have become less empathetic and have resorted to defensive medicine. The mutual distrust between patients and doctors - especially health professionals' distrust of patients and their relatives - leads to increased fear and self-protection by doctors, making good communication difficult, adding to the already heavy workload borne by health professionals. The burden of mistrust leads to poorer healthcare outcomes and increasingly discontented and angry patients.[14]

A study in Eastern India regarding doctors' perception of the doctor-patient relationship identified several areas of interest. Factors related to doctors included immense work pressure and related stress, lack of communication by doctors, and malpractice by some doctors. Patient factors included unrealistic expectations, incomplete knowledge gained through the internet, and a lack of understanding about the disease and its treatment. They also reported that doctors emphasised the largely negative role played by the media.[11]

On review of the literature, it was found that physicians and medical students mostly display patient-centric and empathetic attitudes, with some sociodemographic variations.[12,15-16] Then, the question arises whether the attitudes do not translate into clinical behaviour, leading to dissatisfaction. There is, hence, a need to identify and assess differences between theoretical proposals and attitudes, and practical application during routine practice.

We Identified 3 Areas for this Study: gender, experience, and work setting, to assess the standard of patient care and ethical practice. Evaluations were done in various settings with different patient loads and work pressure to allow us to compare factors that affect physician's ability to provide standard care. Aspects such as the experience of physicians, sensitisation as a part of education, and available infrastructure were considered. This allows for the formulation of the scope and methods of improvement.

MATERIALS AND METHOD

Five departments of the hospital with high physician involvement were identified for observation: General Medicine, Paediatrics, Obstetrics and Gynaecology, General Surgery, and Orthopaedics. A specially designed, standardised, and validated questionnaire prepared from literature[3,12] was used. The questionnaire focused on four components of physician behaviour: Patient care, Ethical behaviour, Professionalism, and Knowledge **(Table 1)**. Scoring was defined on a scale of 1 to 5, where 5 was the most ideal possible behaviour.

A cross-sectional survey was performed in the OPDs, wards, and emergency areas of the hospital. Convenience sampling was done. The demographics taken into consideration were gender, years of experience, and practice setting. Doctors were observed while on duty by

Table 1: Questionnaire
The questionnaire focused on four components of physician behaviour: Patient care, Ethical behaviour, Professionalism and Knowledge.

DETAILS
Gender
Year/ Experience
Workload
PATIENT CARE
actively listens and shows genuine care
takes time to address concerns without rushing
respects pt right to make informed decisions about t/t
treats pt with professionalism even when they are difficult to treat/non-compliant
is accessible for follow up and clarifications
ETHICS
provides truthful and complete information about diagnosis and t/t options
maintains patient confidentiality and sanctity
avoids unnecessary medication, tests and referrals
treats all patients fairly regardless of sex, race, socioeconomics, personal beliefs
PROFESSIONALISM
maintains professionalism in speech, behavior and dressing
clearly explains diagnosis, t/t, procedures so pt understands
acknowledges mistakes and takes corrective measures
collaborates with colleagues and paramedical staff
KNOWLEDGE
accepts new techniques and procedures

the three undergraduate students, trained through AETCOM modules focusing on different aspects of clinician behaviour, and were assigned pseudonyms as no identifying details were noted.

A total of 75 doctors, 15 from each department, were observed, and scores were noted separately by the three observers to eliminate observer bias. The individual scores were then compiled.

OBSERVATIONS

The sample of doctors to be observed was chosen by convenience sampling method. The demographic distribution of observed doctors is given in **Table 2**. Of the 75 physicians observed, 40 (53.4%) were male and 35 (46.6%) were female. The compiled results were analysed department-wise for all the demographic categories.

In the department of General Medicine **(Figure 1)**, male doctors demonstrated a higher level of professionalism with patient-centric care and adherence to ethical standards, as compared to their female counterparts. On the basis of experience, senior residents scored better than junior residents, followed by interns. However, interns displayed a higher degree of professionalism compared to junior residents. The best standards were seen in the out-patient setting, followed by the emergency area, and finally, the wards.

Table 2: Demographic Classification of Doctors
The demographics taken into consideration were gender, years of experience, and practice setting. This table provides the number of doctors observed from each demographic of the total 75 physicians.

Demographic Classification	
Gender	
Male	35
Female	40
Years of Experience	
Professor	17
SR	24
JR	30
Intern	4
Setting	
Emergency	30
OPD	28
Ward	17

In the department of Paediatrics **(Figure 2)**, female doctors scored better on the Patient Care and Professionalism aspects, but male doctors were more

adherent to the ethical criteria. In the scores reported for experience, professors did particularly well, followed by a negligible difference in the scores of the senior and junior residents. Optimal care was observed in the wards, followed by the outpatient setting, and then the emergency department. However, in the Professionalism aspect, the results were reversed – emergency, followed by OPD, and then the wards.

Figure 1: Performance Based on Years of Experience in the General Medicine Department.

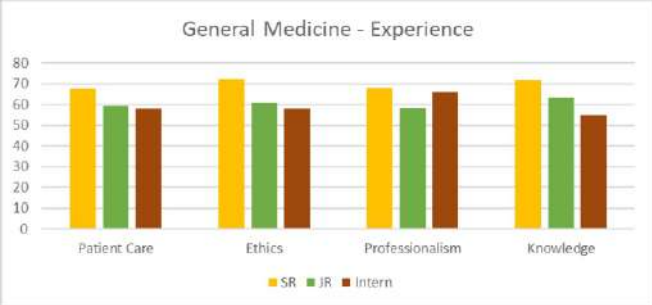
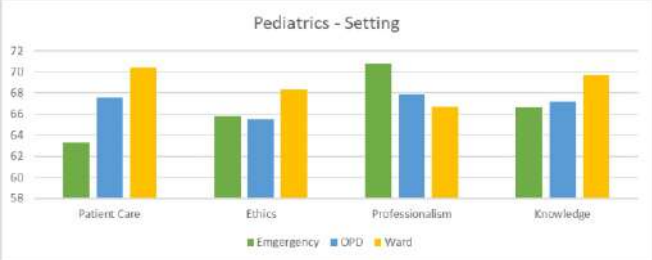


Figure 2: Performance Based on Setting in the Paediatrics Department.



In the Obstetrics and Gynaecology department **(Figure 3)**, the comparison on experience yielded almost progressively better scores with higher years of experience. Performance in the out-patient setting was markedly higher than in the emergency areas (which are also the areas where patients for labour report to).

In the department of General Surgery **(Figure 4)**, male doctors scored better on all components, with better performance in the Knowledge component. On the basis of experience, more years of experience seemingly counted towards better performance. However, interns scored better than junior residents on the Patient Care component, and displayed the most professionalism of all experience groups. In the category of settings, the doctors displayed the best performance in all aspects studied, in the emergency areas, followed by the wards.

In the department of Orthopaedics **(Figure 5)**, the professors and senior residents scored almost similar scores, with the senior residents doing marginally better on the Patient Care component, and professors being

Figure 3: Performance Based on Setting in the Obstetrics and Gynaecology Department.

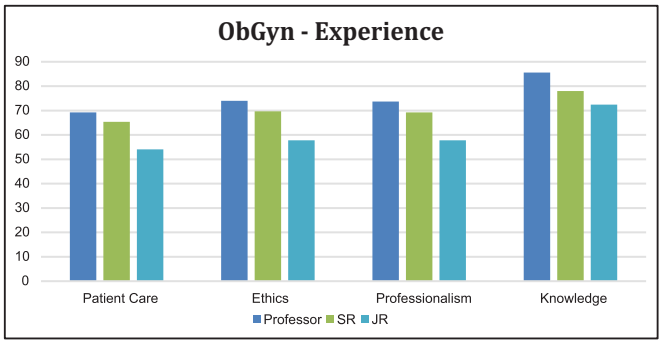


Figure 4: Performance Based on Years of Experience in the General Surgery Department.



better in the Knowledge component. Based on the setting, the overall performance of the doctors was the best in the out-patient setting, followed by the wards and then the emergency area. But the emergency area scored better than the wards on the Patient Care component.

On the overall analysis of 75 doctors across 5 departments, male physicians scored slightly better when compared to their female counterparts. On the basis of experience, there was no difference between the performance of professors and senior residents, both being in the higher ranges. The professors ranked remarkably high in the Knowledge aspect. It was followed by lower counts for junior residents and interns. However, interns did particularly well in the Professionalism aspect, scoring 66.3%. **(Figures 6, 7 & 8)**

The out-patient setting appears to be most conducive to patient centric practice, with the emergency area coming a close second, and then the wards. The performance of the doctors was below average in the wards on the Ethics and Professionalism components, scoring 48.8% and 48.6%, respectively.

DISCUSSION

The results of our study showed that patient-centric and ethical behaviour was more associated with the male gender, increased experience, and in the OPD settings.

Figure 5: Performance Based on Settings in the Orthopaedics Department.

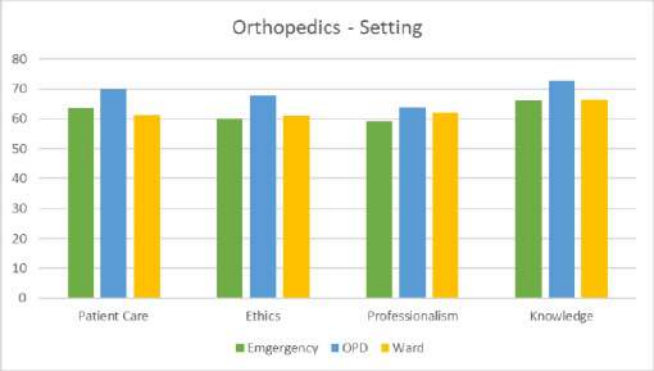


Figure 6: Overall Performance of 75 Doctors Based on Gender.

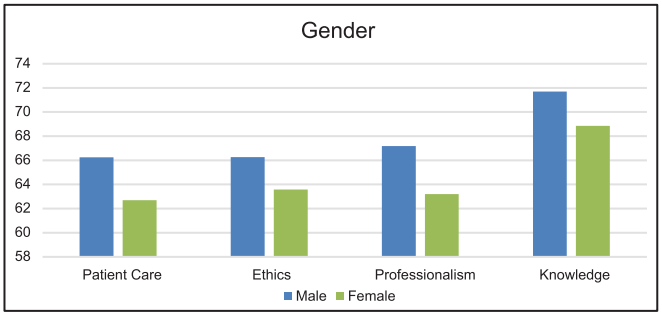


Figure 7: Overall Performance of 75 Doctors Based on Years of Experience.

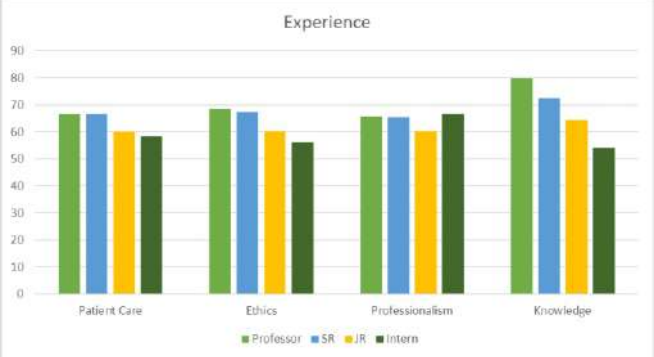
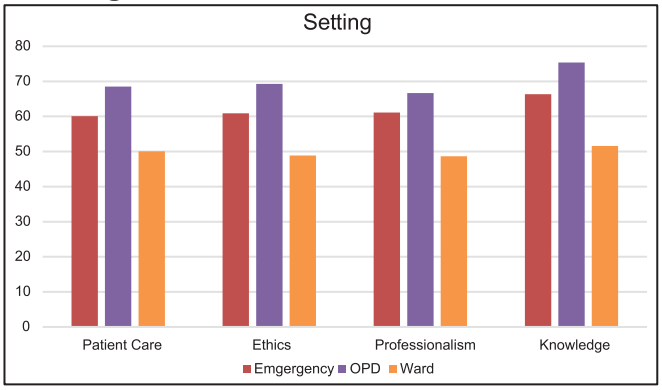


Figure 8: Overall Performance of 75 Doctors Based on Setting.



Bedside behaviour was directly proportional to the higher years of experience. However, the below average scores in wards were unanticipated, given the relatively calmer work environment and lower patient load.

On reviewing the literature and research regarding patient-centric care and ethical practices, the authors observed that most of the studies were based on surveys or interviews. This meant that studies usually revolved around ascertaining attitudes of physicians, patients, or medical students. There was a lack of studies involving the assessment of actual behaviour, based on observation.

On comparing with various previous studies based on attitude assessment, there seemed to be variations on reported results regarding some factors, with a consistent divergence as regards gender. An Austrian study on gender specific differences in attitudes towards doctor patient communication in students and physicians, conducted through self-response questionnaires, observed that female doctors and students associated more positive attributes to doctor-patient communication.[5] This differed from this study's finding of better patient communication exhibited by male physicians.

A cross-sectional study in Beijing, researching physicians' attitude towards patient-centric care, reported a positive association with female gender and intermediate titles.[15] We observed an increased patient centric behaviour in the male gender, and higher experience.

An American study on medical students' attitudes towards the doctor-patient relationship in the USA inferred that the idealistic attitude that existed at the beginning of students' medical careers declined with increasing years of experience in clinics. They concluded that the culture of medicine and the structure of medical education erode patient-centred attitudes.[7] In contrast, our observations showed that doctors with higher years of experience tend to exhibit more empathic and ethical behaviour.

However, a former Indian study assessing the attitude of doctors towards patients with substance use disorder observed that the sympathetic attitude of doctors towards substance use disorder was associated with increased years of experience.[16] This was in alignment with the observations made during this study, showing a positive correlation between patient-centric attitude and years of experience.

The study highlights several issues that warrant further study. The differences between results acquired from

studies based on attitudes and behaviours must be studied further. The most consistent variation was in the category of gender, with multiple studies based on attitude reporting that patient-centric care is positively associated with female gender. However, this study's results indicate that male physicians seemingly exhibit more patient-centric behaviour. These variations must be accounted for while planning training for physicians.

The performance lag exhibited by junior residents and interns implies that there is a need to support medical professionals at the beginning of their careers through skill development in communication and ethical behaviour. Attempts to teach students medical humanities[3,12,17] through AETCOM courses in undergraduate years have been made with good results.[18] Faculty must keep in mind that students generally have an unfavourable attitude about communication skills as a subject, especially during their initial years in the field.[19] This can be supplemented with interventions during internship[17] and postgraduate training.

The performance gap of all categories of doctors in the wards, compared to other work settings, could be due to various factors, but merits further research in this particular area. It is especially concerning, given the relatively calmer work environment and lower patient loads. It is important to identify problem areas so as to plan and implement potential interventions.

The negative perception of physicians, despite scores that indicate that physicians exhibit average to good bedside behaviour during daily practice, is potentially concerning for the state of the doctor-patient unit. The issue is multifactorial, involving doctors, patients, administration, judiciary, and the media at large. It is essential to identify and implement socio-culturally appropriate models of effective community engagement.[14] All stakeholders must play a part in this endeavour so that the rights, dignity, and interests of medical professionals and patients alike are recognized, safeguarded, and improved through better health and social institutions.[14]

LIMITATIONS

This study is a pilot study and has limitations which need to be considered when interpreting its results. The sample size was small, and the sampling strategy used in this study, convenience sampling, may be susceptible to sampling bias. The study should also have been conducted in a private setup to ascertain differences.

CONCLUSION

Ethical practice and professionalism are pivotal in the medical field and yet, gaps often exist between theoretical guidelines and practical application at bedsides. This study reported measures of patient-centric care through observation and compared differences with studies based on attitude of medical professionals. The capping factors in a government setting include overworked doctors and high patient load, resulting in reduced time per patient.

The study generates certain points that require further study. The difference in patient-centric care on the basis of gender denotes a need to bridge the gap between attitude in theory and practical application. The improvement associated with experience suggests that, along with the implementation of communication and ethics courses in the curriculum, such as the AETCOM modules in undergraduate and postgraduate courses, there is an immediate need for more practical exposure for students, starting early in their careers. The influence of settings on physician behaviour and the disparity in ward performance warrant further study.

Despite seemingly professional behaviour during clinical practice, there are reports of increasing violence against doctors. A change in perception of the doctor-patient relationship influenced by corporatization, consumer courts, and media can largely be blamed. This emphasises the need for all stakeholders to be involved in structural, social, and legal reforms that could benefit and protect the interests of both doctors and patients.

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Original Research Paper

Pattern of Skull Fractures in Fatal Road Traffic Accidents: A Retrospective Autopsy Study in and around Doda, Jammu and Kashmir

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ABSTRACT

Introduction: Traffic accidents on roads (RTAs) constitute a major public health problem worldwide and are one of the top causes of death in India. Fatal head injuries account for a substantial proportion of these deaths due to the vulnerability of the skull and intracranial contents.

Objective: The present study aimed to analyze the pattern and distribution of skull fractures and associated intracranial haemorrhages in fatal road traffic accidents occurring in and around Doda from January 2023 to December 2024.

Materials and Methods: A retrospective autopsy-based study was conducted on 153 fatal RTA cases in which death resulted primarily from cranio-cerebral injuries. Demographic details, type of vehicle involved, anatomical location of skull fractures, and patterns of intracranial haemorrhage were systematically documented and analyzed. IEC approval was taken from the IEC, GMC Doda.

Results: The highest number of fatalities was observed in the 21–30-year age group 37.2% (n=57) with a marked male predominance 90.2% (n=138). Motorcyclists constituted the most affected category 36% (n=55).The parieto-temporal region was the most frequently involved fracture site 18.3% (n=28). Combined subdural and subarachnoid haemorrhage was the most common intracranial finding 60.8% (n=93).

Conclusion: Young male two-wheeler riders represent the most vulnerable group for fatal head injuries in road traffic accidents in this region. Identification of prevalent fracture patterns provides valuable forensic and preventive insights.

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INTRODUCTION

Road traffic accidents continue to be a major contributor to morbidity and mortality worldwide, especially in developing nations. According to Mohan (2004), RTAs account for a significant proportion of preventable deaths in India.[1] Additionally, Hogarth[2] emphasized that external mechanical forces can damage the scalp, skull, or intracranial contents, even when the intensity of impact appears minimal. Head injury, identified as the principal fatal factor in many road accidents as noted by Chavli et al.,[3] remains a leading determinant of mortality due to the brain's limited capacity to withstand traumatic forces. Munro[4] reported that cranio cerebral injuries may occur following various forms of blunt force trauma, independent of force magnitude. These findings align with WHO reports highlighting the disproportionate burden of RTAs in developing countries.[5-6]

With the Rapid pace of urbanization, population growth, and increasing motorization have significantly contributed to the rising incidence of road traffic accidents, particularly in developing countries. In India, RTAs remain a major cause of unnatural deaths, accounting for a substantial loss of productive life years.[1] Head injury is the single most important determinant of fatality in road traffic accidents due to the critical functions of the brain and its limited tolerance to mechanical trauma. The similar finding was noted by Chavli et al.[3]

Head injuries result from the application of external mechanical forces leading to damage of the scalp, skull,

meninges, or intracranial structures. The severity of cranio-cerebral trauma does not always correlate directly with the magnitude of the external force, as even relatively minor impacts may produce fatal intracranial lesions depending on the mechanism and site of impact. Munro D reported similar finding.[4] Autopsy-based studies provide crucial information regarding injury patterns, helping to reconstruct events and guide preventive strategies.

The present study was undertaken to evaluate demographic characteristics, vehicle involvement, anatomical distribution of skull fractures, and patterns of intracranial haemorrhage in fatal road traffic accidents occurring in and around Doda region from January 2023 to December 2024.

MATERIALS AND METHODS

This retrospective study was conducted in the Department of Forensic Medicine, Govt. Medical College, Doda, over a period of two years. (January 2023 to December 2024) A total of 153 fatal road traffic accident cases were included.

Inclusion Criteria

- All victims of road traffic accidents subjected to medicolegal autopsy in whom death was attributed primarily to head injury.

Exclusion Criteria

- Decomposed bodies.
- Cases with uncertain or absent history of road traffic accident.
- Victims without evidence of head injury.

Detailed information regarding age, sex, type of vehicle involved, site of skull fractures, and intracranial haemorrhages were recorded during autopsy examination and analyzed.

RESULTS

Demographic Distribution

Out of 153 cases, males constituted 90.2% cases (n=138) and females 9.8% cases (n=15). The most affected age group was 21–30 years 37.2% (n=57), followed by 31–40 years 18.3% (n=28).

Vehicle Involvement

Motorcyclists formed the largest group of victims 36% (n=55), followed by cases with unknown vehicle history 28.8% (n=44) and four-wheeler occupants 19% (n=29).

Pattern of Skull Fractures

The parieto-temporal region was the most involved fracture site 18.3% (n=28), followed by fronto-parietal fractures 17% (n=26). Among isolated fractures, the occipital bone was most frequently affected 16.3% (n=25).

Intracranial Haemorrhages

Intracranial bleeding was observed in all cases. The most frequent pattern was combined subdural and subarachnoid haemorrhage 60.8% (n=93), followed by the presence of extradural, subdural, and subarachnoid haemorrhages together 22.9% (n=35).

DISCUSSION

The patterns observed in this study closely mirror long standing epidemiological trends in road traffic fatalities, yet they also highlight several region specific factors that merit emphasis. The marked predominance of male victims has been repeatedly documented in earlier studies,[7–11] and the present findings reaffirm this disparity. In the terrain of Doda, men constitute the majority of daily commuters and long distance travelers, often navigating challenging mountainous roads for work or personal obligations. These circumstances naturally place them at greater exposure to hazardous driving conditions.

The concentration of fatalities in the 21–30 year age group is particularly concerning. Numerous authors have reported similar age distributions,[10–13] and this reflects not only increased mobility in this demographic but also the tendency toward risk taking behaviour, including speeding, riding without helmets, and driving under time pressure. In hilly regions, even minor driver miscalculations can lead to severe consequences due to its sharp curves, inadequate lighting, and insufficient roadside protective barriers.

The anatomical distribution of skull fractures provides useful insights into the mechanics of injury. The parieto temporal region was the most frequently affected site, a pattern also described by Ranjit and Keoliya.[15] This region is often injured in side impact or oblique impact collisions, which are common on winding mountainous roads where vehicles may strike lateral surfaces or topple during sharp turns. The relatively high frequency of occipital fractures among isolated injuries may reflect backward impacts during falls or rear end collisions, both of which occur frequently in downhill driving scenarios.

The predominance of two wheeler users in the fatality profile is consistent with the work of Kumar M et al.[16] and aligns with observations made across various Indian states. Motorcyclists are inherently more vulnerable due to

the absence of external structural protection. In the present study area, many roads remain narrow and uneven, increasing the likelihood of skidding, head on collisions, and falls from height. Although helmet legislation exists, compliance in semi urban and rural areas remains inconsistent, contributing significantly to mortality.

The intracranial haemorrhage patterns further support the inference that victims were subjected to considerable acceleration–deceleration forces. The predominance of combined subdural and subarachnoid haemorrhage 60.8% (n=93) suggests widespread tearing of bridging veins and cortical vessels, injuries typically associated with rapid head movement rather than direct localized blows. This mechanism has been described extensively in forensic neuropathology and is commonly encountered in high energy vehicular trauma. Cases showing multiple haemorrhage types (EDH, SDH, SAH) likely represent especially high impact incidents, such as vehicular rollovers or collisions with rigid roadside structures.

An important aspect emerging from this study is the influence of the region's topography on injury severity. Doda's mountainous landscape often forces drivers to navigate steep gradients and narrow passages with limited manoeuvrability. In such settings, even low speed collisions can produce significant head injuries due to sudden changes in vehicle direction or falls onto hard surfaces. This geographical factor distinguishes the present findings from those of studies conducted in urban plains, where collision dynamics differ substantially.

Overall, the study underscores that fatal head injuries in this region result from a complex interaction of human behaviour, environmental challenges, and biomechanical forces. Understanding these interrelated factors is essential for designing targeted interventions, improving road engineering, and strengthening enforcement of protective measures such as helmet use.

CONCLUSION

Young males, aged 21–30 years, were the most common victims of fatal road traffic accidents in and around Doda region. Motorcyclists represent the highest risk group. Parieto-temporal skull fractures and combined subdural with subarachnoid haemorrhage are the most frequent fatal injury patterns. Focused road safety interventions, strict enforcement of helmet use, and targeted awareness programs for young two-wheeler riders are urgently required.

LIMITATIONS

This study has certain limitations that should be considered when interpreting the findings. As a retrospective autopsy-based analysis, the work relied entirely on existing records, which occasionally lacked detailed information regarding crash circumstances, protective measures, and pre-hospital events. Because the data were drawn from a single institution serving a predominantly hilly region, the results may not be fully representative of patterns seen in urban or geographically distinct areas. The study also included only fatal cases subjected to medicolegal autopsy, which may introduce selection bias and excludes individuals who survived long enough to receive treatment or whose cases did not undergo autopsy.

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Review Article

Age Estimation Through Skull Suture Evaluation: A Review of the Recent Literature

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ABSTRACT

Introduction: Age estimation is a critical component of forensic anthropology, assisting in the identification of unknown individuals. The degree of cranial suture closure has long been associated with chronological age. This review aims to highlight the variability in age estimation based on cranial suture closure, with a focus on differences across studies, populations, and methodologies.

Materials and Method: The literature from the last 12 years has been studied and summarised. Key studies focusing on age estimation techniques and proposed models were selected, reviewed, and compared to understand the accuracy and trends in age estimation methods.

Result: The reviewed literature indicates that the onset of sagittal, coronal, and lambdoid suture fusion begins in the third decade of life, the completion age can vary from the fifth to seventh decades Statistical models showed moderate to weak correlations with age.

Conclusion: The reliance on broad age interval estimates is a limitation in establishing precise age determinations in forensic science. The variability in reported fusion ages may be attributed to differences in population characteristics, sample sizes, methodological approaches, and scoring systems, which warrants population-specific models for forensic age estimation.

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INTRODUCTION

As criminal laws grow increasingly sensitive to age-related legal thresholds, and defence challenges and courtroom scrutiny intensify, the demand for precise, scientifically validated, robust and legally defensible methods of age estimation in forensic investigations have become more critical than ever before. There are different age estimation systems used to determine chronological age, dental age, skeletal age and sexual age.

Skeletal or bone age assessment is considered one of the most accurate approaches.[1] If an X-ray of a foetus is taken at eleven weeks, the bone tissue is visible at 806 different places, referred to as centers of bone growth. At birth, these reduce to 450 centers, and the adult skeleton is known to have a total of 206 bones. This implies that approximately 600 bone centers unite with adjacent centers to form adult bone. The process of the appearance of bone centers and their union has a sequence, and it occurs in a certain age range that can be approximated.[2] The age range, instead of a specific age, is determined because the union is a process and not an event.

X-rays of the elbow, wrist, clavicle, shoulder joints, pelvis, knee and ankle joints are usually recommended for determining age before 25 years of age.[3-4] The closure of the skull sutures is considered to be a reliable index of age estimation above 25 years of age.

Bone age evaluation provides a roughly accurate estimate of an individual's biological age through comparison with established reference standards. These reference standards include the application of radiological and

scoring methods for evaluating the skeletal maturity of an individual for age estimation.

The Greulich & Pyle atlas and the Tanner-Whitehouse method are two of the most commonly used radiographic techniques to estimate age in children and adolescents. The Greulich & Pyle atlas method was developed using data from North American Caucasian children, whereas the Tanner- Whitehouse method was based on a European population.[5] While these methods are used worldwide, one important drawback is that these atlases were developed for specific populations. When these techniques are applied to children from different ethnic or geographic backgrounds, the results may not always be accurate. Factors such as climate, socioeconomic background, genetics, diet, and geography influence the growth and maturation of bones.

As a result, using the same standards across diverse populations can lead to errors in age estimation. Several validation studies have been conducted on the Greulich & Pyle atlas in countries such as Australia, Portugal, India, Turkey, South Africa, and Ethiopia. These studies have consistently shown that the Greulich & Pyle atlas is not well- suited for accurately estimating age in these populations.[5] This highlights the need for population-specific reference data to ensure more accurate and reliable assessments.

In addition to radiographic and scoring-based techniques, standard anatomical references and developmental charts, which provide timelines for the appearance and fusion of ossification centers, epiphyseal unions, and cranial sutures, are commonly found in osteology and forensic medicine textbooks.

Cranial Sutures: Cranial suture fusion is a key parameter in age estimation and is widely utilized in forensic anthropology and medico-legal investigations. Sutures are the fibrous joints that interlock the edges of the skull bones, binding them together. This interlocking structure provides stability and strength to the skull, which allows it to protect the brain effectively.[6] During infancy and childhood, the brain grows rapidly, and the skull needs to expand accordingly.

Sutures allow this expansion by remaining flexible, which accommodates the growth of the skull bones.[7] As a person ages, the sutures gradually ossify, which eventually fuses the skull bones permanently. This process allows the skull to form a stable cranial structure by adulthood. The extent of suture closure can be used as an indicator of age in skeletal remains. Forensic scientists and anthropologists can estimate the age of an individual by examining the degree of fusion of the sutures[8].

Historical Background: The use of cranial suture closure as an indicator of age has a long and evolving history in forensic anthropology. Early anatomists, such as Fallopius and Eustachius in the 16th century, were among the first to observe that sagittal suture tends to begin closure earlier than other sutures.[9] By the 19th century, Topinard proposed a sequential pattern of closure beginning with the sagittal suture, followed by the coronal suture and then the squamosal suture. These insights were further refined by Frederic, who distinguished between the timing of endocranial and ectocranial closure.[10]

With the landmark study performed by Todd and Lyon in 192, systematic research began on this subject. They introduced a five-point scoring system to study the endocranial and ectocranial aspects of various sutures on crania of known age. The study emphasized the importance of segmenting sutures for analysis and revealed that endocranial sutures tended to close more consistently than ectocranial sutures. However, their method received criticism for introducing sampling bias by rejecting the samples that were supposed to be outliers.[7]

In 1957, McKern and Stewart conducted a study on 375 skeletal remains of American soldiers from the Korean War. The method included analyzing the facial sutures to formulate a regression for age estimation. The conclusions highlighted the unreliability of closure as a sole indicator for age, particularly in younger individuals, as most sutures had not started to close in their sample.[11] In 1970, Acsádi and Nemeskéri examined 352 Hungarian crania and focused exclusively on endocranial sutures.

They developed age estimation tables on the basis of regression analysis. Their work demonstrated that closure generally proceeds from the inner table to the outer table of the skull.[12] In 1984, Perizonius validated and refined their method with a Dutch sample, proposing separate indices for younger and older individuals. His results confirmed stronger correlations in the younger population and highlighted the benefit of selecting age-appropriate suture segments to enhance predictive accuracy.[13]

These foundational studies collectively shaped modern perspectives on cranial suture closure, highlighting its potential as a supportive tool in age estimation while also highlighting the significant interindividual and

interpopulation variability that limits its standalone reliability.

Methodologies for Suture Closure Assessment

Several scoring systems have been developed to assess cranial suture closure, each offering unique approaches for evaluating the degree of fusion. The most commonly utilized systems include the following:

A. Frederic Rating Scale: This scale involves a detailed classification of cranial suture closures, such as sagittal, coronal and lambdoid sutures, on the basis of macroscopic examination, often aided by CT imaging. It evaluates the degree of fusion through ranging from open (no fusion) to completely fused, and the scale assigns numerical values to each stage, allowing for a standardized comparison across individuals.[14]

B. Acsadi-Nemeskeri Scale: This scale is a part of the Acsadi-Nemeskari complex, which is a multifactorial method used to estimate age from multiple skeletal features, such as cranial suture closure, the internal structural changes observable in the proximal humerus and femur and age-related changes to the pubic symphyseal surface.[15] It divides the suture into three segments: anterior, middle, and posterior.

Each segment of the suture is analyzed and scored separately, as fusion of the segments might vary from each other. The scale uses a range of stages from open to completely fused, with specific descriptors for partial fusion stages, allowing for a detailed examination of the suture's progression over time.[16] This method was devised using an endocranial suture but has also been used to assess ectocranial suture closures.[17]

C. Meindl and Lovejoy Methods: This method focuses on evaluating the extent of fusion at ten specific sites of ectocranial sutures across the surface of the skull. Each site is scored on a 0-3 scale. The total score is then compared to referenced data to produce an estimated age.[17] This method is particularly useful for studying cranial sutures in skeletal remains, where the extent of fusion can vary across different parts of the suture.

MATERIALS AND METHOD

A narrative review was conducted by manually searching peer-reviewed articles published over the last 12 years. The studies were selected based on their relevance to cranial suture fusion and age estimation, particularly those that used measurable scoring systems, radiological techniques, or regression models. Both gross anatomical and imaging-based studies were included, with emphasis on sagittal, coronal, and lambdoid sutures. The selected studies were then summarized and compared to identify patterns, variability, and methodological approaches.

RESULT

Study-wise Observations

Chiba et al. (2013) conducted a study on 125 subjects who underwent Postmortem-Computed Tomography at the Department of Legal Medicine at Chiba University. The study applied a 7-stage scoring system to investigate the correlation between the degree of fusion of the sagittal suture and age. The Pearson correlation coefficient was 0.71 for men and 0.85 for women.[18] Khandare et al. (2014) carried out a study on cranial sutures of 100 male cases brought to the mortuary of Grant Government Medical College & Sir Jamsetjee Jeejeebhoy Hospital, Mumbai, to view the fusion of sutures and correlate it with age by computed tomography.

The study applied Frederic's rating scale to examine the sagittal, coronal, lambdoid, and temporoparietal sutures. The sagittal suture begins fusing between ages 25-30 and completely closes between ages 61-65 years. The coronal suture begins fusing between ages 25-30 and completely closes between ages 66–70. The lambdoid suture begins fusing between ages 25- 30 and completely closes after age 70. The temporoparietal suture begins fusing between ages 56-60 and completely closes after age 70.[19]

Kampan et al. (2014) conducted a study on the skulls of 100 Thai individuals aged 15-96 years from the Forensic Osteology Research Center at Chiang Mai University. The sagittal, coronal and lambdoid sutures were photographed with a Canon EOS Rebel XSi camera and traced onto clear plastic sheets. The linear lengths of the sutures were measured with a measuring tape.

The images were enhanced with Adobe Photoshop CS4 for better visibility and analyzed with ImageJ software to measure the number of pixels in each suture. Stepwise linear regression was employed to develop an age estimation model. The standard error for this model is 13.9 years.[20] Malik et. al. (2016) aimed to determine the age of deceased individuals by analyzing the degree of closure of sagittal sutures.

The study was conducted on 90 dead bodies brought for postmortem examination in the Department of Forensic Medicine & Toxicology, King Edward Medical University, Lahore. The degree of closure was scored according to the

Acsadi– Nemeskari scale. The most significant correlation was observed in the 31-40 years age group. Suture closure begins as early as 21 years endocranially and 28 years ectocranially. In the 41- 50 years age group, no segment of the sagittal suture remained unclosed endocranially. Ectocranially, complete closure was observed in all the subjects over 60 years old. Determining age from the sagittal suture of the cranium is reliable in individuals over 30 years old and in cases of putrefaction.[17]

Bhengra et. al. (2016) conducted a study on 100 cadavers in the Department of Forensic Medicine and Toxicology, Rajendra Institute of Medical Sciences, Ranchi. In this study, the endocranial sagittal suture begins to close between 20 and 29 years of age, with full fusion occurring by 40-49 years for S1, S2, and S3 and by 30-39 years for S4. The endocranial coronal suture starts closing in the same age range (20-29 years) and completes fusion by 40-49 years for C1 and C2, whereas C3 reaches completion by 50-59 years. The lambdoid suture begins fusion at 20-29 years of age and attains full closure by 50-59 years for L1 and L2, but the ectocranial L3 shows delayed union.[21]

Tiwari et al. (2018) conducted a study on 500 cadavers in the Department of Forensic Medicine & Toxicology, Gandhi Medical College, Bhopal. The suture closure of the coronal, sagittal and lambdoid sutures was assessed for the inner table as well as for the outer table. The process of sutural closure is more uniform and faster in the endocranial sutures than in the ectocranial sutures. The closure of these sutures commences in 20-29 years of age endocranially, and complete closure occurs at 40-49 years of age in the sagittal and coronal sutures and at 50-59 years of age in the lambdoid suture.[22]

Oza et al. (2019) conducted an autopsy-based study on 170 cases of postmortem examination in the Department of Forensic Medicine, Sir Sayajirao General Hospital, Baroda. The skull sutures were examined with the Acsadi–Nemeskari scale. In this study, endocranial fusion of sagittal suture began between 21 and 30 years of age and was completed by 51-60 years of age. For the coronal suture, fusion was detected as early as 21-30 years of age. The complete union observed was at the age of 50 years ectocranially and 35 years endocranially. Endocranial fusion of the lambdoid suture starts at 21-30 years of age, with the earliest complete union observed at 39 years of age ectocranially and 32 years of age endocranially.[16]

Upreti (2019) performed a study to estimate age from the fusion of skull vault sutures. A significant difference was observed between the age groups and suture closure. The coronal suture was the first to fuse, followed by the sagittal suture. Lambdoid sutures start fusing at the age of 25-30 years endocranially. Closure occurred earlier in females than in males.[23]

Kalsi et al. (2019) conducted a study to evaluate the extent of sagittal suture fusion on medicolegal postmortem cases for age estimation. The study was based on analyzing the sagittal suture macroscopically on 100 cases brought to Government Medical College and Hospital, Chandigarh. It was observed that the sagittal suture starts to fuse at 18-25 years ectocranially as well as endocranially. Endocranially, complete closure occurs at more 66-75 years of age in S1 and S4 and at more than 76 years of age in S2 and S3.[24]

The purpose of the Fan et al. (2020) study was to determine whether adult age assessment in Chinese Han persons can be aided by computed tomography of the cranial sutures. The study was based on cranial computed tomography scans of 230 Chinese Han males aged 23.33-76.93 years. The degree of suture closure was associated with adult age. The minimum inaccuracy of the test set was 7.73 years according to linear support vector regression, whereas the inaccuracies of the previous simple linear regression models were 13.09 and 10.97 years. The accuracy was greater in the 40.00-59.99 years age group than in the other age groups.[25]

Thakur et al. (2020) conducted a study in the Department of Forensic Medicine and Toxicology at Tertiary set up in Western India. A total of 221 roentgenograms of the skull were collected, and the degree of fusion of the coronal suture was graded. The study revealed that the coronal suture begins to fuse at approximately 31 years of age, with complete fusion observed as early as 50 years of age. The study also revealed a positive correlation between suture closure and age.[26]

Rodriguez et. al. (2021) analyzed cranial suture fusion patterns at Virginia Tech Carilion School of Medicine. The majority of cranial base sutures appear as early as the second decade and fuse around the eighth decade. Some sutures, such as the occipitomastoid, appeared >90% open even as late as the ninth decade. By then, most other sutures, including the frontozygomatic and sphenosquamosal sutures, have fused.[27]

Madon et. al. (2022) conducted a study to review Meindl and Lovejoy's age estimation method from cranial sutures in the Malaysian population. A total of 106 subjects were scanned using PM-CT and evaluated with ML scoring.

There was a positive correlation ($r = 0.370$; $p<0.05$) between suture closure and age.[28]

Akbar et al. (2023) aimed to investigate three-dimensional computed tomography scans of 263 individuals to assess the degree of suture closure. Suture closure was evaluated using a three-stage scoring method. The study revealed a statistically significant correlation between chronological age and cranial suture obliteration score ($p < 0.001$). In males, the highest correlation coefficient was observed for the obliteration score of the second part of the sagittal suture ($R = 0.512$; $p < 0.001$). In females, the obliteration score of the first part of the sagittal suture showed the highest correlation with chronological age ($R = 0.499$; $p < 0.001$).[29]

Hussaini et. al. (2023) conducted a study on 50 cases which were above the age of 20 years at the mortuary of the Department of Forensic Medicine, Government Medical College, Ratlam. The sagittal, coronal & lambdoid sutures were studied with Acsadi-Nemeskeri scale endocranially. Endocranial union begins in the 20–29 years of age, but its progression is not uniform. Complete closure of the sagittal and lambdoid sutures occurred in the 60-69 years, whereas the coronal suture achieved complete closure in the 50-59 years.[30]

Chawla et. al. (2023) carried out a study on 100 cases brought for medicolegal autopsy at Shaheed Hasan Khan Mewati Government Medical College, Nalhar, Nuh. The coronal, sagittal, and lambdoid sutures were examined ectocranially, and the degree of closure was graded according to the Acsadi-Nemeskeri scale. This study examined the obliteration patterns of cranial sutures, both ectocranially and endocranially. The sagittal suture tends to obliterate first, followed by the coronal suture and then the lambdoid suture. The Spearman rank correlation coefficient revealed a highly significant correlation between the number of sutures (sagittal, right and left coronal, and lambdoid) and age at death across all the cases.[31]

Callistus (2023) executed a study to estimate the age of the skull using endocranial suture of 114 Nigerian skulls with reference to the Ascadi-Nemeskeri scale system for aging the skull. The coronal, sagittal, and lambdoid sutures were examined. The coronal suture closes almost entirely between the ages of 40 and 50, the sagittal suture closes almost entirely between the ages of 45 and 75, and the lambdoid suture closes almost entirely between the ages of 45 and 55.

The significant anatomical marker that can be utilized to determine an individual's age is the cranial suture, particularly when the person's age is unknown. This study revealed a statistically significant correlation between skull age and cranial suture.[32]

Varghese et. al. (2023) analyzed the sequence and pattern of fusion of cranial sutures to corelate suture closure and age of the individual. In the study, the sagittal suture showed progressive closure from the middle portion (S2) towards the ends (S3, S4, and S1), with S1 fusing the slowest, especially after 55 years of age. The coronal suture fused earliest at its central part (C1), though complete closure was uncommon. A positive and statistically significant correlation between age and suture closure was observed. The sagittal and coronal sutures tended to close earlier than the lambdoid suture, both ectocranially and endocranially.[33]

Assil et al. (2024) aimed to determine the average age of closure of each cranial suture (coronal, sagittal, and lambdoid) and to correlate the association between age and closure by CT imaging in Egyptians with Frederic Rating Scale. This study revealed a positive correlation between age and cranial suture closure. The sagittal suture closed earlier and began closure first through its second part (S2) at approximately 38.82±11.62, followed by the coronal suture where the second part (C2) was the first to start closure, with a mean age of 43.29±16.58, and then the lambdoid suture.[34]

Wei et. al. (2024) explored the significance of cranial sutures to estimate age in Chinese Han adults. The 132 head CT samples collected were scrutinized for score grading of the coronal, sagittal and lambdoid sutures. A linear regression model and four machine learning models-gradient boosting regression, support vector regression, decision tree regression, and Bayesian ridge regression-were developed. The results revealed a positive correlation between each cranial suture closure grade and age, with the sagittal suture showing the highest correlation. Among the models, the support vector regression model demonstrated the highest accuracy, with a mean absolute error of 9.542 years.[35]

Fusion trends by suture: Most of the studies reported the onset of fusion of the sagittal suture during the third decade of life, but the completion of fusion of the suture varies across studies. Bhengra[21] & Tiwari (2018)[22] reported the completion of suture in the fifth decade, Malik[17] & Oza[16] in the sixth decade, and Khandare[19],

Upreti[23] and Hussaini[30] in the seventh decade. Callistus[32] documented a much broader fusion range, which is 15-40 years for commencement and 45-75 years for completion. Furthermore, Assil[34] reported a mean age of onset of 42.9 ± 9.92 years and a mean age of completion of 50.39 ±13.82 years.

Bhengra[21] and Tiwari[22] reported the commencement of the fusion of coronal suture between 20–29 years of age, and Oza[16] reported an onset window of 21–30 years. Upreti[23] and Khandare[19] placed the commencement at approximately 25–30 years. However, some findings deviate from this trend; Hussaini[30] reported a later onset in the fourth decade (40–49 years), and Callistus[32] documented a particularly wide onset range of 15–40 years, suggesting significant interindividual variation.

The age of fusion completion also varies widely. Tiwari[22] reported fusion completion of coronal suture as early as the fourth decade (40–49 years), whereas Bhengra[21] reported fusion completion slightly later in the fifth decade (50– 59 years). Upreti[23] reported that the completion occurred in the sixth decade, whereas Khandare[19] reported that completion extended into the late seventh decade (66–70 years). Callistus,[32] again highlighting broad variability, reported fusion completion between 35–65 years. Assil[34] reported a mean age of onset of 45.23 ± 10.18 years and completion of 50.99 ± 13.29 years.

The lambdoid suture exhibits a fusion pattern that generally begins in the third decade of life. Bhengra,[21] Tiwari,[22] and Hussaini[30] reported the commencement of lambdoid suture fusion between 20–29 years of age, similar to Oza,[16] who reported onset at 21–30 years of age. Upreti[23] and Khandare[19] placed the beginning of fusion at 25–30 years. Callistus[32] again presents a much broader commencement range, from 15–40 years. The completion of fusion varies significantly across the studies.

Tiwari,[22] Bhengra,[21] and Hussaini[30] reported fusion completion in the fifth decade (50–59 years), whereas Upreti[23] reported it between 65–70 years. Khandare[19] reported fusion only after 70 years, indicating the latest fusion completion among the studies. Callistus[32] provides a wide completion range of 35– 65 years. Assil,[34] using statistical analysis, reported a mean commencement age of 47.24 ± 12.53 years and completion age of 55.09 ± 12.45 years, suggesting considerable variation.

Table 1: Comparison Table: Key observations on the estimate of the age when skull suture fusion commences and is complete[16,17,19,21,23,24,32,34]

Table 1: Key Observations on the Estimate of the Age When Skull Suture Fusion Commences and is Complete.

Author	Scale	Sample	Sagittal Suture		Coronal Suture		Lambdoid Suture	
			Fusion Commencement	Fusion Completion	Fusion Commencement	Fusion Completion	Fusion Commencement	Fusion Completion
Khandare (2014)	FRS	Indian (100 male)	25-30 years	61-65 years	25-30 years	66-70 years	25-30 years	After 70 years
Malik (2016)	ANS	Pakistani (90 cadaver)	21-30 years	51-60 years	-	-	-	-
Bhengra (2016)	ANS	Indian (100 cadaver)	20-29 years	40-49 years	20-29 years	50-59 years	20-29 years	50-59 years
Tiwari (2018)	FRS	Indian (500 cadaver)	20-29 years	40-49 years	20-29 years	40-49 years	20-29 years	50-59 years
Oza (2019)	ANS	Indian (170 cadaver)	21-30 years	51-60 years	21-30 years		21-30 years	
Upreti (2019)	ANS	Nepalese (90 individual)	25-30 years	60-65 years	25-30 years	55-60 years	25-30 years	65-70 years
Kalsi (2019)	ANS	Indian (100 cadaver)	18-25 years	66-75 years	-	-	-	-
Hussaini (2023)	ANS	Indian (50 cadaver)	20-29 years	60-69 years	40-49 years	50-59 years	20-29 years	50-59 years
Callistus (2023)	ANS	Nigerian (114 skull)	15-40 years	45-75 years	15-40 years	35-65 years	15-40 years	35-65 years
Assil (2024)	FRS	Egyptian (100 individuals)	42.9 ± 9.92 Years	50.39 ± 13.82 years	45.23 ± 10.18 years	50.99 ± 13.29 years	47.24 ± 12.53 years	55.09 ± 12.45 years

List of Abbreviations.

Sr. No.	Sr. No.	Sr. No.
1	CT	Computed Tomography
2	S1	Pars Bregmatica (i.e. first part) of Sagittal Suture
3	S2	Pars Vertices (i.e. second part) of Sagittal Suture
4	S3	Pars Obelica (i.e. third part) of Sagittal Suture
5	S4	Pars Lambdica (i.e. fourth part) of Sagittal Suture
6	L1	Pars Lambdica (i.e. first part) of Lambdoid Suture
7	L2	Pars Intermedia (i.e. second part) of Lambdoid Suture
8	L3	Pars Asterica (i.e. third part) of Lambdoid Suture
9	C1	Pars Bregmatica (i.e. first part) of Coronal Suture
10	C2	Pars Complicata (i.e. second part) of Coronal Suture
11	C3	Pars Pterica (i.e. third part) of Coronal Suture
12	PMCT	Post Mortem Computed Tomography
13	ML	Meindl Lovejoy
14	ANS	Ascadi-Nemeskari Scale
15	FRS	Frederic Rating Scale

Regression Models: Often offer quantitative precision over traditional scoring systems. Kampan[20] provided a pixel-based model (SEE ±13.9 years), whereas Madon[28] used the Meindl & Lovejoy method but reported a weak correlation (r = 0.37, SEE ±15.9 years). Chiba et al.[18] applied multidetector CT to assess sagittal suture and reported a positive correlation between the closure stage and age with predictive equations. Thakur[26] confirmed a strong correlation for coronal suture fusion through radiographic analysis but lacked a predictive equation.

Limitations: Since this literature review was a narrative review and not conducted as a systematic review; therefore, it may be influenced by selection bias in the choice of studies. Most of the available studies on cranial suture fusion differ widely in methodology, imaging technique, sample size, and the population studied thus, this variation limits direct comparison and reduces the ability to draw uniform inferences. The review could not include a quantitative synthesis or meta-analysis due to the heterogeneity of data and reporting formats. Hence, the conclusions drawn suggest the possible pattern of suture fusion & cannot be taken as absolute findings.

CONCLUSION

Cranial suture closure remains a valuable component in forensic age estimation, particularly when combined with other biological indicators. This review has examined the patterns of cranial suture fusion, focusing on sagittal, coronal, and lambdoid sutures, and highlighted the variations across studies, populations, and methodologies. A common challenge across the literature is the reliance on broad age intervals, typically spanning ten years, which limits the precision needed in forensic contexts. Adding to the complexity, studies that use statistical measures often report high standard deviations, which makes it harder to pinpoint age accurately.

This wide variation can be attributed to biological differences as well as regional or environmental factors. Given these inconsistencies, it becomes clear that a one-size-fits-all approach is inadequate. The variability in reported fusion ages may be attributed to differences in population characteristics, sample sizes, methodological approaches, and scoring systems. These findings underscore the need for standardized methodologies and more granular age categorization in future research to improve the reliability of age estimation based on cranial suture fusion.

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Review Article

From Crime Scene to Courtroom: The Legal Journey of Forensic Evidence through Chain of Custody

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ABSTRACT

The role of forensic evidence in the criminal justice system is vital, substantially affecting investigations and adjudicatory processes. Critical to its admissibility and acceptance is the evidence's chain of custody as it helps in establishing the path from crime scene to court house, making sure it was untouched, properly managed and accurately identified. Interruptions in the chain due to handling, misidentification, theft or tampering can result in either the conviction of an innocent person or acquittal of the guilty. This article seeks to critically discuss the protocols, processes, methods and ethical considerations particularly in relation to safe-guarding the integrity of evidence through cases where evidence was rejected by courts because of weak documentation as well as poor forensic management. It also looks at forensic-processing backlogs, adjudication gaps and lack of infrastructure in what has broken up criminal trials in India and where do the United States of America (US), United Kingdom (UK) and Germany stand in forensic analysis. In addition, evidence related to other emerging technologies such as Artificial Intelligence (AI), block chain technology, digital labelling, automated tracking systems and automated forensic databases are reviewed for their potential to enhance transparency, integrity and efficiencies of evidence management. Finally, the article concludes that better crime scene practices as well as an application of technology use with proper legal boundaries and a good chain of custody are necessary to secure the right to fair trial, protect against wrongful convictions, and promote public faith in our legal system.

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INTRODUCTION

Forensic Science has transformed the role of criminal investigation in recent times by providing scientific methods to confirm or refute existence or guilt, and thus ensure the innocent are acquitted. The quality, credibility and reliability of forensic evidences considered in the course of the trial is not merely based on scientific presentation, it is also anchored on legal concepts, most especially in chain of custody. Secure transport and documentation of forensic evidence, known as chain of custody, is vital in order to prevent tampering with, destruction or compromise of the collection, which prevents it from being admissible in the court.[1] An intact chain of evidence guarantees the judge that the evidence is uncontaminated, unaltered and undamaged; therefore providing veracity in a court room.[2]

While forensic work is foundational to the justice system, systemic flaws in the way it is handled cast a shadow over criminal trials more than a decade after its landmark role was questioned. A break in such a chain of custody whether from mislabelling, failure to maintain proper documentation, unauthorised access or lengthy delays in forensic analysis can cause crucial evidence to be dismissed.[3] This not only undermines the prosecution's case, but can also result in innocent people being wrongfully convicted or guilty people being

acquitted.[4]

Many high-profile cases around the world show that justice can go awry if forensic evidence has been tampered with, the procedure was not followed correctly, or forensic samples were lost, and that a more systemic solution through legal and scientific reforms are needed in terms of answering forensic questions.[5]

Due to overburdened caseloads, out-dated equipment and shortage of manpower, many forensic laboratories have virtually ground to a halt when it comes to the examination and producibility in court.[6] In a country like India, which is already burdened with huge piles of pending cases, not being able to dispose forensic evidence in time contributes to the delay in justice.[7] The heterogeneity of protocols and inconsistencies in documentation of evidence, however, can render forensic data vulnerable to attack in court wherein the genuineness is questioned by bringing attention to limitations of manual record-keeping.

Various new technologies have emerged in recent years as potential game-changers in the management of forensic evidence. These services would thus create immutable records of forensic samples on a blockchain, minimising possibilities of sample tampering or misplacement. With each of these mentioned points, Artificial Intelligence (AI) forensic analysis can enhance the crime scene processing remarkably.[8] This improves real-time tracking of evidence movement with the help of automated barcodes and RFID (Radio Frequency Identification) tracking, thus reducing human errors in keeping records. However, successful implementation of these technologies depends on adapting the existing laws, investing in infrastructure, and training forensic personnel in order to adapt these technologies to current forensic systems.[9]

Following a comparative analysis of global best practices on forensic evidence management systems (as seen in countries like the United States of America (US), the United Kingdom (UK), and Germany), this study aims to probe into the Indian forensic chain-of-custody framework while making certain reform suggestions that could eventually bolster the credibility of the forensic evidence process in a criminal trial. Improving how forensic evidence is handled is about more than streamlining procedures; it is a critical requirement if the legal system is to function properly, avoid wrongful convictions, and regain public confidence in the criminal justice system. A properly documented and legally acceptable forensic chain of custody is crucial to achieving the goals of allowing science and law to utilise each for justice in an objective, transparent, and reliable way.[10]

DISCUSSION

The First Link: Capturing Truth at Crime Scenes

How forensic evidence is collected, recorded, and preserved at crime scenes: The collection of forensic evidence at a crime scene is a crucial step, all of which has a direct bearing on the integrity and reliability of an investigation. Trained forensic scientists and first responders adhere to stringent protocols for identifying, collecting, and documenting all types of evidence types (biological samples like blood, hair, and saliva), trace evidence (fibres, gunshot residue), and physical evidence (weapons, fingerprints). Normal protocols require that they wear protective clothing to avoid contamination, use special instruments for collection and place evidence in tamper-proof containers.[11]

Documentation is one of the most important pieces of the forensic investigation process. Each piece of evidence that is to be removed from the crime scene has to be photographed, tagged and logged in detail. Gen detectives sketch detailed crime scene overviews with observations and then digitally map the evidence for precision. This detailed documentation creates a timeline that connects evidence to accused individuals and events, serving as a basis for potential legal action.

Any missteps in these protocol things like not packaging evidence correctly, not documenting what was done when, or failing to store evidence properly can cause the evidence to be challenged or even to be ruled inadmissible in court. Global forensic agencies adhere to rigorous protocols and training programs for crime scene investigators to minimise associated risks.

Role of forensic experts, first responders, and legal safeguards in preventing contamination: Forensic experts rely on the work of first responders, who are often the first on the scene and help to preserve a crime scene before investigators with expertise in forensic science arrive.[12] The immediate actions containing the perimeter, limiting access to unauthorised personnel, and not touch things that do not need to be touched mean that the risks of contamination are minimised. The integrity of forensic evidence can be compromised by environmental exposure, or human interference, which will make it unfit for

consideration by a court of law.[13]

When forensic staff gets to work, they use systematic evidence collection method. They wear gloves, masks and sterilised tools while handling samples to ensure that the DNA transfer or outside pollutants do not mix with the evidence. Blood samples, gunshot residue and bodily fluids, for example, are kept in temperature-controlled containers, to ensure their chemical stability.

Legal protections exist to ensure continuity of the collection of forensic evidence. Forensic compliance in India is overseen by the Ministry of Home Affairs which is governed by the Central Forensic Science Laboratory (CFSL). Globally, agencies such as the Federal Bureau of Investigation (FBI) and INTERPOL offer forensic protocols in order to maintain international best practices. Forensic experts, who are required to give evidence to the court, are also governed by legal frameworks exposed to for scrutiny their methods of evidence collection.[14]

Forensic integrity, however, is a concept that is increasingly dependent on technology. Investigators wear body-worn cameras which documents them collecting evidence in real time. Evidence management systems such as those based on barcoding and digital scanning prevent human error due to mislabelling or logging items of evidence.[15] For instance, the CODIS (Combined DNA Index System) in the US and NATIS (National Automated Fingerprint Identification System) in India among others are forensic databases that allow for cross-referencing of forensic data resulting in more accurate investigation of crime.[16]

The forensic integrity of the crime scene is a multi-disciplinary practice designed to maintain the integrity of all evidence. The collaboration of police investigators, forensic scientists and judicial officers as well as continued education and the respect of the law can improve the quality of forensic science. With advances in forensic technology, another route in using blockchain based forensic tracking and AI-driven crime scene analysis can be used to improve security and accuracy of evidence collection, while lowering the odds of forensic evidence being challenged in court.

Paper Trails and Pitfalls: Documenting the chain of custody

The forensic chain of custody does not conclude with the evidence being collected but as equally important is it travels through documentation, handling and transfer.[17] It is important to have proper documentation of forensic evidence so that it maintains the chain of custody and can withstand legal examination in a court of law.[18] Used as an audit tool, the chain of custody log is the official record and includes every stage of the forensic process, from the time a piece of evidence is collected from the crime scene to its presentation in the courtroom. This document is a clear record of the evidence, which includes the time, date, location and the person responsible for handling the evidence at every stage. Even a small crack in keeping an accurate log can lead to legal nuisance and threaten the credibility of forensic results.[19]

Exhaustive record-keeping is essential to maintain forensic integrity. Failure to properly document evidence will lead to potential claims of tampering, contamination, or mismanagement.[20] In criminal cases, defense attorneys frequently examine the chain of custody for identifying strengths or weaknesses in a chain that could cause evidence to be deemed inadmissible. Accurate documentation is very important in forensic institutions around the globe and they are investing in new technologies to help minimise human errors. Technologies such as Electronic Evidence Tracking Systems, including barcode based logging and Radio Frequency Identification (RFID) technology, track movements in real time, reducing inconsistencies.[21] In addition, some jurisdictions have implemented blockchain based forensic tracking, which provides an immutable ledger of every transaction involving forensic evidence, effectively removing any concerns regarding tampering.

Despite such advancements, lapses in documentation have historically resulted in the dismissal of critical evidence in highly publicised criminal matters. One of them, perhaps the most notorious, is the O. J. Simpson Case, [People of the State of California v. Orenthal James Simpson, No. BA097211 (Cal. Super. Ct. Oct 3, 1995)][22] in the US. The DNA evidence was key to the prosecution's case, but allegations of mishandling and contamination gave the defense a chance to point to inconsistencies in the chain of custody.[23] The defense succeeded in arguing that the police investigators had not kept proper documentation, and that holes in the evidence log made clear that the evidence was not authentic. These errors had seriously undermined the prosecution's case and helped lead to Simpson's acquittal.

In India, a comparable event of contamination of the crime scene took place in the Aarushi Talwar Murder Case, MANU/SC/0009/2012.[24] Critical forensic evidence which

was washed away, unsealed or mishandled in ways cast serious doubt on its credibility. The break in the chain of custody meant that most essential evidence could not be clearly linked to the accused.[25] These mistakes were instrumental in overturning the conviction at some later date.

The Duke Lacrosse Case (2006)[26] in the US highlighted the adverse effects from mismanagement of forensic DNA records, e.g., incomplete documentation and refusal to share it with defence attorneys, whereas tainted forensic work was exposed as a cause of delay in Nirbhaya Case,[27] [Mukesh & Anr. v. State for NCT of Delhi & Ors., (2017) 6 SCC 1] in India. Though at last the aggrieved party in Nirbhaya case got justice, but it eventually highlighted the shortcomings within the system especially the forensic practice used in India.

In order to address these issues the forensic institutes in India should come forward with a rigorous documentary process of evidence. The government should take the initiative to provide periodic regular training for police, forensic scientist and advocates to minimise the error. Digital forensic logs, AI based forensic trajectory and block chain based authentication can be new techniques to upgrade the trustworthiness of digital evidence documentation.[28] Courts need to take a harder line on forensic evidence so police sloppiness does not stand in the way of justice. This will enhance the credibility of Forensic evidence; consequently forensic evidence would become a strong contributing factor in justice deliverance as keeping proper records will be explicitly stressed.

Vanishing Evidence: The dark side of mishandled forensics

Improper handling of evidence can lead to lost, contaminated, or tampered evidence, which is one of the most serious threats to the integrity of forensic investigations. Forensic chain of custody ensures that each evidence keeps its originality and is not tampered through the course of the investigation and the legal process. But they have a great effect on the legal process when an error, usually by omission, occurs; leading to erroneous convictions or acquittals.

At several points along the chain of custody, forensic evidence can be corrupted. This can be due to inappropriate storage conditions, unauthorised access, deliberate modification, or unintentional contamination. Mistakes in forensics have caused devastating consequences for a number of well-publicised cases in which justice has been delayed. One example is the Dwayne Allen Dail (North Carolina, 1987) who was convicted and forensic evidence that could have provided him with an alibi was lost for years due to mishandling. Convicted of rape he did not commit, and after 18 years in prison the DNA was eventually found and kept under the right conditions.[29]

Again the impact of forensic lapses was even too extensive in the Nithari serial killings case, MANU/SC/0119/2011 in India.[30] Failure to collect or preserve evidence properly or inconsistencies in forensic reports undermine key forensic results.[31] This resulted in delays for justice, and questions to be raised about the forensic results that had been presented. These are some of the most egregious examples of the kind of damage the forensic mismanagement can inflict, not only to the legal process, but also to public confidence in the justice system.

One of the major reasons why forensic mistakes are committed is due to inadequate training of both police officers and forensic workers. Many forensic facilities are underfinanced, undertreated, especially in developing countries and lack of technological infrastructure required for effective evidence-handling protocols.[32] Inadequate training may lead to improper forensic protocols, which means that there is a higher probability of contamination or loss during evidence collection.

The most terrifying part of it is when the law enforcement or forensics decides to intentionally manipulate their evidence such as altering the results or planting fake evidence to wrongfully persecute people, and/or covering up facts. One such example was the Rampart Scandal (Los Angeles, 1990s),[33] in which corrupt policemen tampered with forensic evidence and incarcerated numerous innocent people through use of this forged evidence. Though we cannot deny the evidentiary value of forensic science in the courtroom, the above illustrated cases reflected a totally different picture where well defined robust legal framework, autonomous scientific oversight and liability can stop abuse.[34]

To overcome such threats most of the countries around the world came up with strict laws related to forensic evidence. For example, in US the National Institute of Standards and Technology (NIST) have introduced detailed guidelines for forensic experts to increase the standard of competence in forensics. National Forensic Science University (NFSU), which is at the helm of forensic

infrastructure development through advanced forensic laboratories and evidence management. Under the NFSU guidelines, India has also been working hard on forensic infrastructure development.

New technologies plan to fortify the chain of devotion including blockchain-based forensic tracking, AI-driven proof verification, and automated forensic audits. These technologies can reduce human risks in maintaining tamper-proof evidence through its life cycle.[35] Careful forensic quality control checks and frequent forensic training programs can avoid the misinterpretation of forensic evidence and prevent irregularities in a large and reliable forensic system. In order to deliver a fair and impartial justice system, it is very important to make sure that none of the forensic evidence is compromised during the process of collecting, preserving, or analysing. The problem of vanishing evidence undermining the judicial process has to be addressed with multi-prolonged approach which includes the legal reforms, integration of technology with criminal justice and strengthening of forensic accountability through adequate oversight.

Justice delayed, Justice denied: How legal loopholes undermine forensic integrity

In cases where the forensic evidence is not supplied in time, the impact is severe and quite adverse to the receipt of a right to a speedy trial. Lack of evidence collection or even lack of evidence examination can lead to years of a case being outstanding, a suspect being in pre-trial detention awaiting a court ruling, a wrongful conviction or an innocent individual being detained due to insufficient evidence.[36] Criminal justice is hampered by cases that take months and even years to get through in jurisdictions across the world and lead to delay in cases before court through court proceedings as well as inefficiency in the criminal justice system. Often exacerbated with insufficient funding, lack of specialists and out-dated forensic technology, the challenge is how they manage to match an ever-expanding demand for forensic services.

A prominent example of how delays in forensics can thwart justice is the 2008 Noida Double Murder Case, MANU/SC/0009/2012[37] in India. Not all critical forensic evidence was collected in time, and much of it went through a slow processing path, introducing ambiguity as to the prosecution. It was delays and other faults with handling evidence that led to the conviction being overturned, highlighting the need for both the timely forensic testing and accuracy of this forensic testing. Likewise, the Houston Crime Lab Scandal[38] in the US revealed massive backlogs in DNA and evidence processing that created wrongful convictions and the non-admissibility of key evidence because it was contaminated.[39]

The UK courts have suggested that trial fairness may be affected by forensic delay more than ever before. It's judicial precedents have reiterated that forensic evidence is to be collected and analysed in a timely manner for the reliability and admissibility. The forensic analysis of Nirbhaya Case,[40] [Mukesh & Anr. v. State for NCT of Delhi & Ors., (2017) 6 SCC 1] was carried out at rapid pace in India because it was a high-profile crime. The hearing was taken through a fast track court ensuring convicts were convicted at the earliest within legal limits. Conversely, over the many other cases, the prosecution may have lost a strong case from delayed forensic reporting and continued push for procedural reforms are now badly needed.[41]

These delays are further exacerbated by the lack of legally mandating timely forensic processing. Poor handling of cases and the larger judicial system has resulted in the numberless rotting away in purgatory at the forensic investigation level with no guarantee when they would be taken through to trial. Such issues must be avoided by a number of policy solutions such as the statutory time limits on the processing of forensic evidence and the compulsory independent audits on forensic labs and designated financial and personal resources. This method would enhance the quality and rapidity of the forensic response even in low-resource environments.[42]

Technology can effectively be implemented to fight forensic backlogs by accelerating the processing of evidence such as automated DNA profiling, blockchain-based AI and tracking systems.[43] Evidence processing can be tracked in real-time by utilising digital forensic case management systems as it eliminates human errors and administrative burdens. Besides, the coordination between the forensic laboratories, law enforcement and courts should also be imperative in order to eliminate any instances of procedural bottlenecks to enable timely delivery of quality forensics.[44]

Delay in justice is considered to be justice denied particularly when there is long processing time in forensic evidence. Forensic science institutions should be more accountable through legislative reforms and organisational restructure to reduce the lag time between

the collection, examination, certification and trial presentation of forensic evidence. These reforms should encourage further harmonisation of forensic services with the modern technology and transparent procedure. The policy and the legislative agenda should include increased judiciary control of forensic analysis, rigid deadlines on examination, reporting & massive growth in forensic infrastructure and capacity to access. Interventions such as these are essential to a working, reliable criminal justice and policing system that should be perceived to be legit and fair by the community.

Tech to the Rescue: Can AI and Blockchain Secure Forensic Evidence ?

The fast moving developments in technology are providing forensic science with newer tools to improve reliability and security of forensic evidence.[45] AI-powered forensic analysis and block chain-based evidence tracking are two powerful solutions that address the challenges that stem from mismanagement, tampering, and inefficiencies in the chain of custody. AI enhances forensic investigations through automatically analysing processes, integrating information and data, assisting with crime scene evidence patterns, and combining complex forensic examinations such as matching DNA and fingerprints.[46] Anomalies in forensic reports can be detected by machine learning algorithms and this will reduce human error as well as bias when investigating. Moreover, facial recognition and voice scanning technologies powered by Artificial Intelligence are incorporated into forensic activities, which helps in more accurate identification of the suspects and others involved and checking for the evidence related to them.

In essence, the decentralised and immutable ledger system of blockchain technology offers a truly ground breaking way to preserve forensic evidence.[47] Block chain creates a tamper-proof digital record of every transaction associated with forensic evidence, from collection to transfer to storage to analysis, and makes it publicly available. Every single piece of itemised evidence can be allocated to its own, time-stamped and encrypted digital fingerprint that cannot be tampered. Some countries, including the US and the UK, are already piloting the use of blockchain-based forensic tracking to improve forensic integrity. In turn, the adoption of smart contracts within the blockchain systems also guarantees that the evidence is accessed and transferred under legal conditions limiting the risk of tampering or contamination.

While AI and Blockchain have not been implemented in the Indian legal system as of now, International best practices can be a guiding light for future implementation in managing forensic evidence. Some countries like Estonia, Switzerland has already introduced blockchain technology as part of legal and forensic documentation to set benchmarks using digital evidence. AI forensic case management introducing in agencies such as the Central Forensic Science Laboratory (CFSL) in India will be a game changer to impede the current backlog and for tracking evidence. Furthermore, partnerships between forensic scientists, the police, and technology companies could lead to blockchain forensic databases with an unchangeable record of how evidence has been handled.[48]

Adoption of these technologies in the Indian forensic and judicial system remains challenging. Barriers include problems with the digital infrastructure, concerns regarding cyber security threats to such infrastructure, along with the tentative, and uneven development of regulatory frameworks for AI and blockchain use applications in forensic science. Gradual transition to a technology-driven forensic system can be achieved through legislative amendments in evidence laws, building technological capacity and foundation of pilot programs in digital forensic labs. Judicial bodies should prepare and regulate Standard Operating Procedures (SOPs) for the integration of AI and blockchain technologies, ensuring that digital forensic evidence secured through digital fingerprints and forensic tools is duly recognised and accepted in courts.[49]

Persistent revolutionising has endless potential when using AI and blockchain for forensic evaluations and chain of custody.[50] Adopting these technologies allows law enforcement or judicial systems to address forensic inefficiencies and maintain evidentiary integrity whilst strengthening citizen confidence in criminal justice systems. AI-driven forensic automation and blockchain-based evidence tracking can minimise risks posed by human error, evidence tampering, procedural lapses, and create a justice system that is both efficient and transparent.[51]

Lessons from the World: Global Perspectives on Chain of Custody

For the purpose of establishing the highest level of reliability in criminal justice, forensic evidence management systems have been standardised in different parts of the world. Illustrative of this fact are jurisdictions

such as US, UK, and Germany where chain of custody between the stages of scientific intervention (development; testing; registration; use), as well as additional stages (investigation; trial; adjudication) are subject to strict protocols.[52] Forensic evidence digital tracking mechanisms such as Radio Frequency Identification (RFID) technology and barcoding systems are implemented in the US enabling precise documentation of all evidence movements. The Federal Bureau of Investigation (FBI) and state-level forensic agencies have set strict guidelines that help close the gaps in documentation and strengthen forensic evidence in court.[53]

The UK is governed by a strong framework overseen by the Forensic Science Regulator (FSR), which requires forensic labs to be accredited by the UK Accreditation Service (UKAS).[54] Moreover, forensic specialists have to adhere to the Criminal Procedure Rules, which highlight the significance of unbroken chain of evidence. In the UK, the adoption of systems for electronic management of evidence minimises errors that occur in manual documentation.[55]

In contrast, the forensic infrastructure in Germany is regulated by the Bundeskriminalamt (BKA), whose forensic investigations take place with strict conformity to the requirements of the law, as well as theoretical research, but subject to the BKA's own scientific standards.[56] Forensic evidence is widely used in German Courts and evidence tracking is very precise and systematic, as any challenge to the authenticity of evidence will be significant.[57]

This comparative study of the diverse systems pinpoints crucial areas of reform within the Indian forensic apparatus. The backlogs in forensic processing, inadequate infrastructural and non-uniform digital tracking system put India on backfoot and hampered its growth in forensic science context.[58] The inconsistency in between the specific fields of forensic science and their grey area with other disciplines as well as lack of a national accreditation body for laboratories has led to inconsistent approach in evidence handling from one state to another. The US has a national level digital forensic database known as CODIS, that can be replicated in India and, block chain based record keeping systems will enhance the transparency and authenticity of forensic evidences in India.[59] Moreover, the implementation of training standards for forensic personnel as practised in Germany might strengthen the binding force of such guidelines. Since custody of samples is to be maintained from crime scene to court, such standard would shape training on who is authorised to seize materials and on how to package them.

The best practices from these international models would enable the country to enhance its forensic practices, reduce cases of wrongful convictions, and gain public trust in the criminal justice system. The need of the hour for India is to proceed further into contemporary forensic age, where scientific evidence is unbiasedly used in our judiciary. India should amends its laws pertaining to forensic science and investing in the development of forensic technology infrastructure at grassroot level.

CONCLUSION

The forensic chain of the evidence custody is critical, to maintain the integrity and admissibility of evidence in the criminal justice system. The article demonstrates how procedural deficiencies, scientific errors and legal vacuities contributes to undermine forensic evidence leading to wrong convictions or unwarranted acquittals and finally, to jeopardise people's confidence in the public administration of justice. These challenges are further compounded by inadequate practices in forensic documentation, mishandling of evidence and systemic forensic processing delays that usually deny victims access to remedy. Experiences in other countries, including the US, the UK and Germany also show that stable forensic management system supported by core technologies such as AI, block chain evidence tracing and automatic traceability balanced system can largely improve evidential reliability. For India, the need of the hour is to adopt these contemporary practices in terms of better infrastructure and enhanced cooperation between the investigating agencies and the forensic experts. Such an integrated approach would help to ensure not only the science integrity but also legal admissibility, ultimately enabling forensic evidence to contribute to a fair and just criminal justice system.

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Review Article

Microplastics in Human Biological Matrices: Forensic Integration of Toxicological and Medico-Legal Evidence

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ABSTRACT

Introduction: Microplastics (MPs) have emerged as a pervasive environmental contaminant with growing evidence of human internal exposure. Their persistence, ability to cross biological barriers, and potential c additives raise significant concerns in forensic toxicology and public health.

Aim: To critically synthesize global evidence on the occurrence, toxicokinetics, and forensic relevance of MPs detected in human biological samples, highlighting their potential value in medico-legal investigations.

Methods: A narrative review of over 40 peer-reviewed studies (2019–2024) was undertaken using PubMed, Scopus, and Web of Science. Studies reporting MPs in human matrices, including blood, placenta, breast milk, lungs, feces, urine, semen, thrombi, and cerebrospinal fluid, were evaluated for analytical methods, particle characteristics, and quality-control rigor.

Results: MPs were consistently detected in 12 different human matrices, with particle sizes ranging from <1 µm to 500 µm, predominantly as fibers and fragments of polyethylene, polypropylene, and polyethylene terephthalate. Documented biological effects include oxidative stress, inflammation, immune dysregulation, and possible neurotoxicity. From a forensic perspective, polymer-specific signatures and pigment residues can serve as trace indicators of exposure, supporting medico-legal investigations in cases of environmental or occupational disease.

Conclusion: The detection of MPs in human biological samples underscores their dual toxicological and forensic significance. Standardized analytical protocols and forensic validation are essential to ensure evidentiary reliability and to interpret microplastic exposure in a medico-legal context.

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INTRODUCTION

Plastics are integral to modern life owing to their versatility, durability, and low cost. However, the exponential rise in global plastic production, exceeding 400 million tonnes annually, and inadequate waste management have led to the widespread contamination of terrestrial, aquatic, and atmospheric environments, resulting in the formation of microplastics (MPs; <5 mm) and nanoplastics (NPs; <1 µm) through fragmentation and weathering processes.[1–3]

Initially recognized as an environmental concern in marine systems, MPs have since been detected across freshwater, soil, and air matrices, highlighting their ubiquity and persistence.[4–10] Their minute size facilitates bioavailability and entry into human exposure pathways such as ingestion, inhalation, and dermal contact.[11–16] Once internalized, MPs have been detected in human stool, blood, and placenta, and experimental studies indicate their ability to cross biological barriers and reach organs such as the liver, kidneys, and brain.[17–20]

From a forensic toxicology standpoint, this pervasive exposure introduces emerging challenges. MPs and NPs have recently been identified in vital human matrices, including blood, placenta, lungs, semen, and cerebrospinal fluid, indicating systemic penetration.[19,18,21-25]

Such findings raise important forensic questions: Can the type, morphology, or polymer profile of detected MPs be linked to a specific environmental or occupational source? Could their accumulation or associated additives contribute to the cause of death or disease etiology in

medico-legal cases? And can these particles serve as trace evidence in reconstructing exposure histories?

Despite growing toxicological awareness, forensic integration remains limited. Current forensic practice lacks standardized protocols for sampling, contamination control, or analytical validation during post-mortem or clinical toxicological investigations. Moreover, medico-legal interpretation frameworks for MPs are absent; no threshold criteria exist to differentiate incidental exposure from potential toxic or causative levels.[26–28]

Therefore, this review critically synthesizes global evidence on the detection of MPs and NPs in human biological matrices, emphasizing analytical methods, particle characteristics, toxicokinetics, and health implications. It further highlights their potential forensic value in toxicological assessment and medico-legal interpretation, aiming to bridge the gap between environmental exposure science and forensic investigation.

MATERIALS AND METHOD

A narrative literature synthesis was conducted to evaluate peer-reviewed reports detecting MPs or NPs in human biological matrices. The search covered studies published between January 2000 and March 2024 to include both early foundational and contemporary evidence.

Databases and Search Strategy

The electronic databases PubMed, Scopus, and Web of Science were systematically searched using Boolean combinations of the following keywords: "microplastics" OR "nanoplastics" AND "human" AND ("blood" OR "placenta" OR "breast milk" OR "lung" OR "urine" OR "semen" OR "cerebrospinal fluid" OR "thrombi" OR "tissue").

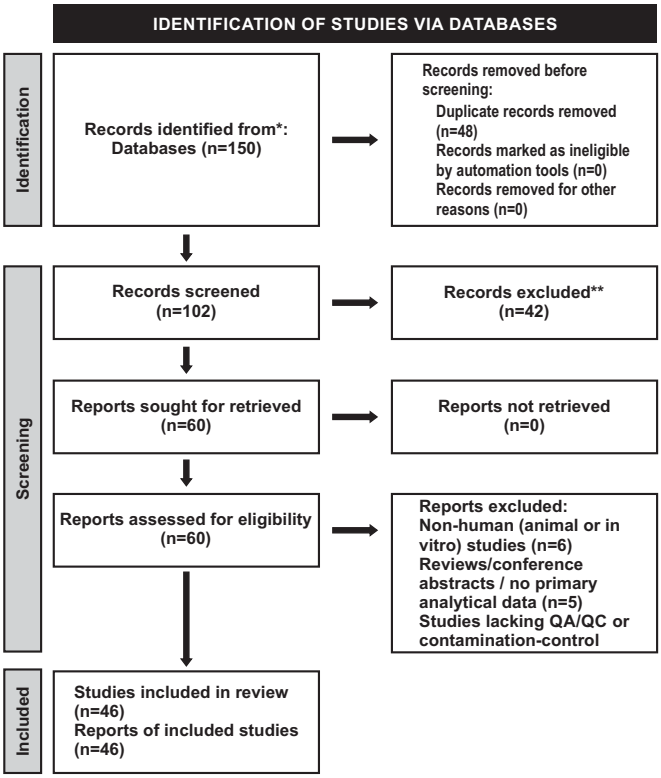
Screening and Inclusion Framework

Although this review followed a narrative synthesis design, elements of the PRISMA 2020 guidelines were incorporated to ensure transparency. After removing duplicates, 102 studies were screened by title and abstract. Of these, 46 studies met the inclusion criteria and reported detecting MPs or NPs in at least one verified human biological matrix. A PRISMA-style flow overview **(Figure 1)** summarizes the selection process.

Inclusion Criteria:

1. Peer-reviewed articles published in English between 2000–2024.

Figure 1: PRISMA flow diagram summarizing study identification, screening, and inclusion for the review on microplastics in human biological matrices. A total of 150 records were identified, 48 duplicates were removed, and 102 records were screened. After full-text assessment of 60 articles, 46 studies were finally included, providing the evidence base for forensic and toxicological interpretation.



2. Studies reporting micro- or nanoplastic detection in human tissues, organs, or biological fluids.
3. Articles using validated identification techniques such as Raman spectroscopy, μFTIR, Py-GC/MS, or SEM-EDX.
4. Studies employing contamination-control or blank analyses.

Exclusion Criteria:

- Non-human (animal or in vitro) or purely environmental studies.
- Reviews, conference abstracts, or reports lacking primary analytical data.
- Studies without clear QA/QC or contamination control information.

Data Extraction and Analysis

Extracted variables included country, biological matrix, digestion and identification methods, polymer types, particle morphology, and size range. Quality assurance and

Figure 2: Sources and Exposure Routes of Microplastics in Humans. The schematic illustrates major environmental sources (plastic waste, textiles, packaging, food, bottled water, indoor and outdoor air), primary exposure pathways (ingestion, inhalation, dermal contact), and potential biological effects (oxidative stress, inflammation, immune dysregulation, endocrine disruption).

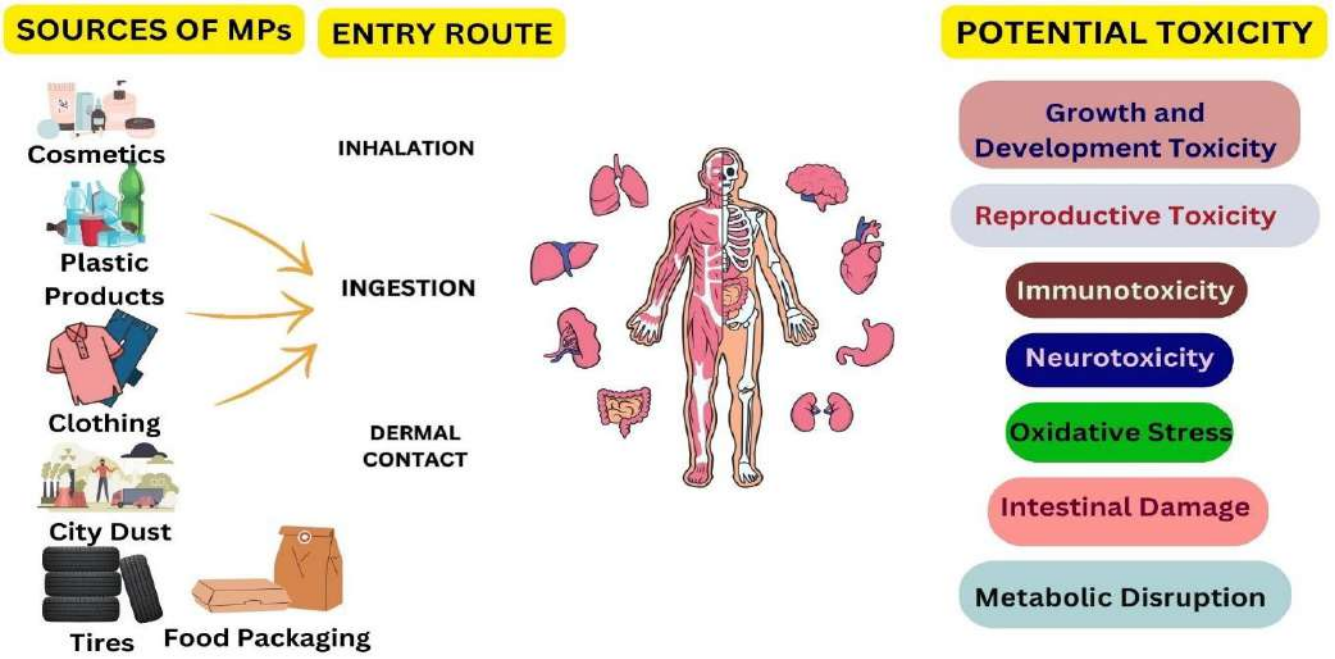


Figure 3: Proposed absorption, distribution, metabolism, and excretion (ADME) of microplastics in the human body. Diagram shows ingestion leading to gastrointestinal uptake and systemic circulation, inhalation leading to pulmonary deposition and translocation, subsequent organ deposition (liver, spleen, kidney, lung, brain), and excretion routes (feces, urine).

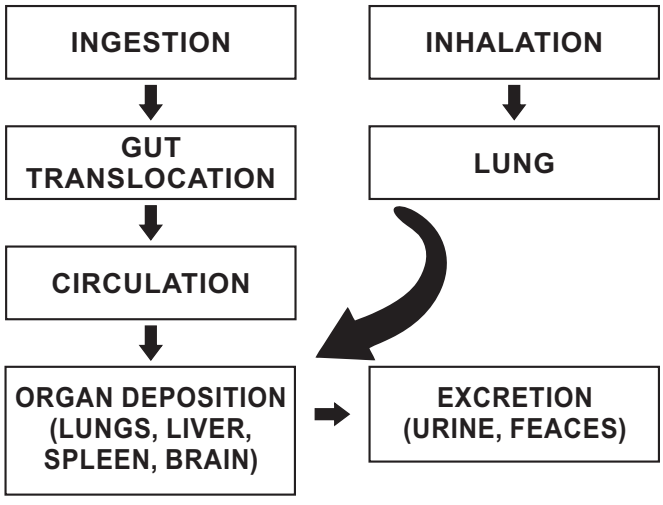
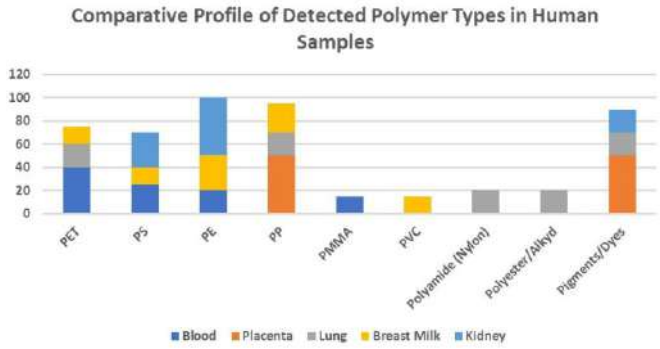


Figure 4: Comparative profile of polymer types detected in human biological matrices. Relative abundance of polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), polystyrene (PS), and other polymers reported across blood, placenta, lungs, breast milk, and kidney samples. Data are approximate and subject to analytical limitations.



contamination control procedures were carefully documented for forensic interpretation.

Ethical Considerations

This study was reviewed and approved by the Institutional Ethics Committee of the Institute of Medical Sciences, Banaras Hindu University. The approval reference number is ECR/526/Inst/UP/2014/RR-20, dated February 28, 2024. All procedures were conducted in accordance with relevant ethical guidelines and regulations.

SOURCES AND EXPOSURE ROUTES

MPs originate from primary sources (manufactured microbeads, industrial pellets) and secondary sources (fragmentation and wear of larger plastic items and

synthetic textiles). Major environmental reservoirs include marine and freshwater systems, sediments, soils, and indoor/outdoor air.[29-31]

Human exposure primarily occurs through ingestion (contaminated food, bottled water, and table salt), inhalation of airborne fibers and fragments, and dermal contact (with textiles and cosmetics).[32-37]

ANALYTICAL METHODS AND QUALITY CONTROL

Detection of MPs in biological samples involves (i) sample collection with contamination precautions, (ii) organic-matter digestion (acidic, alkaline, oxidative, or enzymatic), (iii) density separation and filtration onto appropriate membranes, and (iv) particle identification using μFTIR, Raman microspectroscopy, Py-GC/MS, or SEM-EDX.

Key methodological challenges include variations in digestion protocols, filter types, and pore sizes (0.02–50 μm), the risk of airborne or laboratory contamination, and limitations in detecting particles at the nanoscale.

Robust QA/QC (including field and procedural blanks, pre-cleaned glassware, clean-air benches, and procedural replicates) is essential for maintaining forensic credibility.

For best-practice guidance, see Brander et al. and Engler.[13,38]

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION (ADME)

Understanding ADME is crucial to evaluating toxic effects. Particle size, shape, surface chemistry, and associated chemicals all play a role in determining the translocation of substances across biological barriers. Studies indicate:

- Particles <150 μm can cross the gastrointestinal epithelium; particles <10 μm may enter circulation and translocate to distant organs.
- Inhaled fibers and fragments may deposit in upper and lower airways, with smaller particles reaching alveoli and translocating across the alveolar–capillary barrier.
- Smaller nanoscale particles (<0.1 μm) have potential for prolonged systemic residence and organ accumulation (liver, spleen, kidneys).

Table 1. Selected Studies Reporting Microplastics in Human Biological Samples.

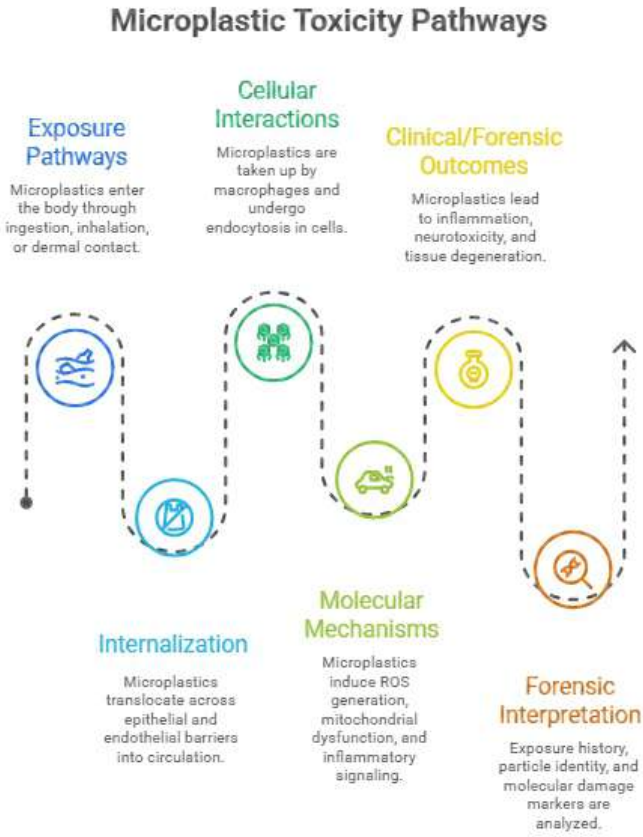
Sr. No.	Study (Author, Year)	Country / Matrix	Digestion / Method	Dominant Polymer Types	Key Findings
1	Leslie et al., 2022	Netherlands / Blood	Proteinase K, Py-GC/MS	PMMA, PP, PS, PE, PET	Measured mass concentrations of plastic particles in blood, suggesting systemic exposure.
2	Ragusa et al., 2021	Italy / Placenta	10% KOH digestion, Raman	PP	MPs detected in placenta (5–10 μm), indicating potential fetal exposure.
3	Jenner et al., 2022	UK / Lung tissue	H2O2 digestion, μFTIR	PET, PP, PS, PTFE	MPs detected in lung tissues, supporting inhalation exposure.
4	Horvatits et al., 2022	Germany / Liver (cirrhosis)	KOH & H2O2, Raman	PET, PMMA, PP, PS	Higher MP levels in cirrhotic livers compared to controls, possibly due to accumulation in disease.

Note: Data compiled from selected peer-reviewed studies on MPs in human biological matrices.

Table 2. Summary of Microplastic Detection Across Human Biological Matrices, Showing Reported Detection Frequencies, Dominant Polymer Types, Size Ranges and Analytical Methods used in Published Studies.

Biological Matrix	Detection Frequency (%)	Dominant Polymer Types	Size Range (μm)	Analytical Method
Blood	80–100	PET, PE, PS	2–30	Py-GC/MS
Placenta	100	PP, PE, PVC	5–50	Raman, μFTIR
Lung/BALF/Sputum	70–100	PP, PE, PET	1–40	μFTIR, Raman
Feces	100	PE, PP	20–500	Raman
Urine	40–60	PET, PS	1–35	Raman
Semen	75	PET, PE	1–20	Raman
CSF	40–60	PP, Pigmented MPs	50–70	Raman

Figure 5: Conceptual framework illustrating microplastic toxicity pathways and their forensic relevance. Microplastics enter the human body through ingestion, inhalation, or dermal contact (Exposure Pathways) and translocate across epithelial and endothelial barriers into circulation (Internalization). Once internalized, they are taken up by macrophages and epithelial or neuronal cells (Cellular Interactions), where they induce oxidative stress, mitochondrial dysfunction, and inflammatory signaling (Molecular Mechanisms). These processes may culminate in neurotoxicity, tissue degeneration, and inflammatory outcomes (Clinical/Forensic Outcomes). In forensic interpretation, polymer identity, molecular damage markers, and exposure history can aid medico-legal reconstruction of exposure or disease etiology.



These mechanisms have been reported in toxicological and human studies.[39-42]

OCCURRENCE IN HUMAN BIOLOGICAL SAMPLES

A range of human matrices has been sampled and reported to contain MPs. **Table 1** provides a concise overview of key studies (selected representative studies). Detailed methodological parameters are summarized in the table to aid forensic interpretation.

Blood

Detection of plastic particle mass in human blood using Py-GC/MS has been reported, indicating that particles or polymer-associated compounds reach the circulatory system. Studies have highlighted the need for mass-based and particle-based reporting for comparability.[18,23]

Placenta, Fetal and Neonatal Matrices

Multiple investigations have found MPs in placenta, meconium, breast milk, and infant feces, suggesting prenatal and early-life exposure pathways. These observations raised concerns regarding potential developmental and immunological effects.[19,25,43]

Respiratory Tract (Lungs, Sputum, BALF)

Airborne MPs and fibers have been identified in bronchoalveolar lavage fluid and lung tissue; sputum studies show variable loads that correlate with smoking and invasive airway procedures.[21,44,45]

Gastrointestinal Tract and Faeces

Fecal studies report abundant MPs, representative of dietary exposure. Comparative studies have shown higher loads in patients with inflammatory bowel disease in certain cohorts.[46-48]

Urine and Semen

Preliminary studies have reported the presence of MPs in urine and semen using Raman and μFTIR spectroscopy, indicating systemic distribution and potential reproductive implications.[22,49]

Liver, Thrombi, and Vascular Tissue

MPs have been found in cirrhotic livers and in thrombi. Vascular tissue studies (saphenous vein) indicate the presence of MPs within the vascular wall, suggesting both translocation and retention.[50-52]

Cerebrospinal Fluid (CSF) and Neural Tissue

Emerging reports (including pilot and case studies) indicate the detection of microplastics and pigment microparticles in thrombi and enclosed body fluids; CSF detection is rare but has been reported, warranting further forensic attention in unexplained neurological conditions.[23,24]

RESULTS

To provide a concise overview, we summarized the occurrence data across reported human matrices. A total of 46 peer-reviewed studies (2000–2024) documented the presence of MPs and NPs in at least 12 distinct human matrices, including blood, placenta, breast milk, lungs, feces, urine, semen, thrombi, and CSF.

The majority of studies employed μFTIR, Raman spectroscopy, or Py-GC/MS for polymer identification. Table 2 provides a consolidated summary of microplastic findings in human blood, placenta, lung tissues, feces, urine, semen, and cerebrospinal fluid, illustrating both the widespread presence of MPs and the analytical heterogeneity across studies.

Quantitative Overview

Reported detection rates vary by biological matrix, with the highest detection frequencies in blood, placenta, and fecal samples (80–100%) and relatively lower detection in urine and CSF (40–60%) due to smaller sample volumes and stringent contamination controls. Mean particle loads, where available, range between 1–20 particles per sample or up to 1.6 μg/mL in blood.[18,23,24]

Nano-scale Detection and Forensic Implications

Recent studies indicate that NPs (<1 μm) may cross epithelial and blood–brain barriers, contributing to systemic exposure and potential neurotoxicity. However, current spectroscopic limits (0.5–1 μm) hinder reliable detection of true nanoscale particles. This presents forensic challenges during case evaluations, as nano-MPs may remain undetected in routine analyses. Development of validated trace-detection methods and forensic QA/QC frameworks is therefore essential for medico-legal reliability.

MORPHOLOGY, SIZE DISTRIBUTION, AND POLYMER TYPES

Across reported human samples, fibers and fragments predominate. Coloration is often transparent or white, with blue being the most frequent color. Reported particle sizes range from >5 mm to submicron scales; many studies report that most human-associated MPs are in the few-micrometer range.

Dominant polymers reported include polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), polystyrene (PS), polyvinyl chloride (PVC), polyurethane (PU), and polyamide (PA).

TOXICOLOGICAL IMPLICATIONS AND POTENTIAL MECHANISMS

MPs exposure induces complex toxicological effects, with emerging evidence linking particle size, polymer chemistry, and surface-bound additives to oxidative stress, inflammation, and cellular dysfunction. Recent molecular studies demonstrate that polystyrene and polyethylene MPs trigger excessive reactive oxygen species (ROS) production, mitochondrial depolarization, and DNA strand breaks, suggesting oxidative DNA damage and mitochondrial dysfunction as key mechanistic pathways.

However, current dose–response relationships remain uncertain due to discrepancies between high-dose experimental models and chronic low-level human exposures. Confounding factors such as co-exposure to adsorbed toxicants, age, disease status, and lifestyle influence observed outcomes and should be considered when interpreting mechanistic data.

Collectively, oxidative and genotoxic mechanisms provide biological plausibility for observed associations between MP presence and tissue inflammation or degeneration, with potential forensic relevance in linking polymer-specific exposure to pathological outcomes in occupational or environmental contexts.

A visual overview of these mechanistic and interpretive relationships is presented in **Figure 5**, which summarizes the continuum from exposure and cellular interaction to molecular injury and medico-legal interpretation. These mechanistic insights are supported by cumulative evidence from recent toxicological and molecular studies.[20,52–60]

FORENSIC AND MEDICO-LEGAL IMPLICATIONS

From a forensic toxicology perspective, the presence of MPs in human tissues has several potential applications and implications:

1. Trace evidence of exposure: Polymer identification (type, morphology, color) can indicate occupational or environmental contact and help reconstruct exposure histories.

2. Medico-legal interpretation of disease: In unexplained chronic respiratory, hepatic, or neurological pathology, MP detection may support hypotheses of environmental contribution, if supported by consistent exposure history and QA/QC-protected analytical findings.

3. Autopsy practice and sample handling: Forensic labs should adopt MP-aware protocols (clean-air collection, procedural blanks, standardized digestion) to avoid contamination and preserve evidentiary value.

4. Legal implications: Demonstrable body burdens of MPs and associated chemicals could inform regulatory, occupational health, and compensation claims; however, establishing causation will require large epidemiological and mechanistic datasets.

Expanded Forensic and Medico-Legal Context

Forensic interpretation of detected MPs requires a strict chain of custody, contamination control, and method validation. Samples should be collected in pre-cleaned glass/metal containers, sealed with tamper-evident tape, and accompanied by field and procedural blanks; processing must occur in clean-air benches with documented recovery experiments and orthogonal polymer confirmation (μFTIR/Raman or Py-GC/MS). Inter-laboratory comparisons and ISO/IEC 17025–style validation improve evidentiary reliability.

In medico-legal contexts, MP data can corroborate exposure histories; for example, polymer-specific MPs in lung tissue may support chronic occupational inhalation, while pigmented MPs in cerebrospinal fluid could suggest workplace or environmental exposure with possible neurotoxic implications. However, such findings should be presented as corroborative rather than conclusive.

For court admissibility, methods must be validated, contamination control demonstrated, and uncertainties clearly stated (e.g., limits of detection, recovery rates). Until standardized forensic thresholds are established, MP evidence best functions as supporting evidence in occupational or environmental claims.[23,24,27,38,51,61]

LIMITATIONS AND RESEARCH GAPS

Current research on MPs in human matrices is constrained by small sample sizes, heterogeneous analytical protocols, and limited nanoscale detection capacity. To strengthen forensic reliability, future work should prioritize the creation of centralized reference databases containing verified Raman and μFTIR spectra, polymer morphology profiles, and contamination fingerprints.

Such repositories would allow laboratories to cross-validate findings and distinguish genuine biological inclusions from artefacts, enhancing inter-laboratory comparability and evidentiary credibility. Progress in this field will also require interdisciplinary collaboration among toxicologists, analytical chemists, materials scientists, and forensic pathologists to integrate exposure assessment with mechanistic and medico-legal interpretation.

Joint research networks and training initiatives could harmonize methods, develop certified reference materials, and establish consensus guidelines for forensic microplastic analysis. Together, these coordinated efforts will move the field from descriptive detection toward standardized, legally defensible interpretation.

CONCLUSION

MPs are now unequivocally detectable in multiple human biological matrices, underscoring their emergence as a new class of environmental toxicants with growing forensic and medico-legal significance. Although current data confirm systemic exposure and tissue deposition, causal relationships with specific diseases remain to be firmly established. Going forward, forensic toxicology must adopt validated analytical protocols, strict contamination control, and robust QA/QC standards to ensure evidentiary credibility.

The development of centralized reference databases for polymer spectra and morphology, alongside interdisciplinary collaboration among toxicologists, chemists, and forensic pathologists, will be vital to translate laboratory findings into medico-legal interpretation. Until exposure thresholds and mechanistic pathways are standardized, MP detection should be viewed as supportive rather than conclusive evidence, guiding future regulatory and forensic frameworks.

Microplastics may soon define a new frontier in forensic toxicology, bridging environmental exposure science with evidentiary investigation.

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Review Article

Cheiloscopy in Criminal Justice: Evaluating Population-Based Uniqueness and Legal Challenges in India

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ABSTRACT

Introduction: Cheiloscopy is the study of the characteristic patterns on the lip created by the sulci labiorum, or wrinkles and grooves on the labial mucosa. Lip prints have been identified as available supplementary tool in forensic science, given their distinct patterns and wide range of applicability, similar to fingerprints. It can be crucial in positively identifying an individual's identity, a person's presence or absence at the crime scene. They are specific to each individual, with the exception of monozygotic twins, and they remain unalterable even after death. This discipline, together with fingerprint analysis and dental record comparison, forms a cornerstone of scientific forensic identification methods.

Materials and Method: A comprehensive literature review was conducted using a targeted search strategy in the PubMed/Medline database, focusing on full-text articles containing the keywords "lip," "lip print," and "Human identification." Relevant data were extracted, categorized under several headings like journal, country of authors, study, aims, outcomes, tool, sample, etc. This information was analyzed, with further examination of cited references.

Discussion: The most widely accepted classification for lip prints is the Suzuki and Tsuchihashi Classification, which provides a clear and comprehensive description of common lip patterns. Lip prints found on diverse surfaces-such as binding tape, glasses, cigarette butts, or windows-can serve as vital evidence in numerous crimes. These can be registered using various methods such as direct photography, usage of lipstick or using substances like aluminum powder or lysochrome dyes. Accurate transfer and recording of lip prints using suitable media is crucial for their validity as reliable forensic evidence.

Conclusion: The stability and individuality of lip prints, unaffected by age and shared genetic factors, render them a valuable biometric marker, complementing fingerprints for personal identification.

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INTRODUCTION

The labial mucosa is not smooth, instead has wrinkles and grooves on it called as sulci labiorum. They form a characteristic pattern which is unique for every individual. It is known as "Lip prints" and the study is known as "Cheiloscopy.[1-2] The word Cheiloscopy is derived from the Greek word 'Cheilos' means 'Lips' and 'scopy' means 'to examine'.[3] In general, cheiloscopy is defined as 'a method of identifying an individual based on the arrangement of lines on the red part of the lips' or 'a science dealing with the various lines appearing on the red part of the lips.[4]

Lip patterns can be identified as early as 6th week of intrauterine life.[3,5] They are unique to each person except monozygotic twins. They are permanent, do not change throughout life with the advancing age and unchangeable even after death.[5] It has been noticed that the lip prints of parents and children and those of siblings have resemblance.[3] In cases of inflammation, trauma or disease like Herpes, Cold sore, Erythema Multiforme etc, the pattern on lips usually recover to normal pattern

eventually.[3-4] In fact, smoking also does not influence the alterations and changes of lip pattern. However, in certain conditions like genetic deformities, some surgical marks or occupations like blowing musical instruments, alterations on the lip patterns may occur.[4]

A likewise even lip prints is unique for an individual and hence bears the potential for identification purpose.[5] Lip prints can be a reliable tool in identifying a person positively and can be useful to verify whether a person was present or not present at the scene of crime.[2]

Historical Background

The pattern of furrows on the red part of the lips was first described by Fischer in 1902. Later in 1932, lip prints were used by Edmond Locard in criminalization and identification of a person.[1-2,6] The first research in Europe was carried out in 1961 on the subjects of lip prints in Hungary. It started at a crime scene when lip traces were found on a glass door.[5] In 1967, Suzuki made an elaborate study on features of lip prints and brought out the features that could be used in forensics.[6]

Since then, Cheiloscopy has evolved tremendously till date through various studied conducted world over and has become an important aspect in Forensic identification.[7] Just like similar other types of forensic evidences, Cheiloscopy has a comparable value for personal identification and sex determination.[4]

In order to identify population-specific variations and highlight gaps in the existing literature where standardized, large-scale comparative data are scarce, this review aims to synthesize and critically evaluate the distribution of lip print patterns in the Indian population using the widely accepted Suzuki and Tsuchihashi classification. In order to better understand pattern prevalence, sexual dimorphism, and forensic application, this review addresses multi-population evidence within the Indian context and highlights cheiloscopic patterns specific to India and their potential forensic utility.

MATERIALS AND METHOD

A comprehensive literature review was conducted to evaluate the forensic application of cheiloscopy in human identification. The review followed a structured and targeted search strategy using electronic databases to ensure the inclusion of high-quality and relevant studies.

Search Strategy: For wider coverage, Google Scholar and ScienceDirect were added to the systematic search, which was conducted mainly using PubMed/Medline. Original research studies released between January 2010 and May 2024 by Indian authors were included in the search.

The terms "Lip," "Lip print," "Cheiloscopy," "Human identification," were employed, along with their Boolean combinations.

Inclusion Criteria

- Research studies done by Indian authors only, that was published between 2014 and 2024 in peer-reviewed journals.
- Articles focusing on original research studies on cheiloscopy in the context of forensic human identification.
- Original research studies with defined methodology and outcomes with different populations dataset of India.
- Research studies with minimum 90 sample size having both male and female ratio almost equal and age groups varying 15-80 years.
- Articles that provided information on methods for collecting lip prints, classification schemes, methods of analysis, and/or findings regarding sexual dimorphism or identification that could be validated on repeating the study .

Exclusion Criteria

- Studies not related to Indian population data set by Indian authors.
- Abstract-only publications, editorials, letters to the editor, and opinion pieces.
- Research that only looks at the non-forensic, dermatological, or cosmetic features of lips.
- Studies that are duplicates, have insufficient information, or produce conflicting findings.

RESULTS

Data Extraction and Categorization: 8 relevant studies 8-15 of cheiloscopy in various Indian populations, mainly concentrating on the distribution of lip print patterns and their role in sexual dimorphism, based on the data shown in **Table 1**. With sample sizes ranging from 100 to 300 people, the majority of studies covered a broad age range and employed the Tsuchihashi classification method.6 Interestingly, some studies found statistically significant differences between males and females, especially in the third and fourth lip quadrants.[8-9,11–15] This suggests that lip prints could be used as an additional tool for forensic

Table 1: Cheiloscopy Studies done in India.

Author	Population Studied	Age (Years)	Sample Size	Methodology	Results Discussed
Suraj Multani et al 2014[8]	Rajnandgaon, Chhattisgarh	15-55	Males: 100 Females: 100	Dark shaded, non-glossy lipstick, cellophane tape	**Males:** Type 3 most common pattern **Females:** Type 1' most common pattern
Ashish Gupta et al 2016[9]	Uttar pradesh	17-24	Males: 200 Females: 200	Lip impression was made on cellophane tape using dark red lipstick. Statistical analysis was done by Chi-Square test to compare proportions	Different quadrant shows different types of patterns in males as well as in females which reveals that lip print is unique for each individual.
Avanindra Kumar et al 2016[10]	Bihar	15-57	Males: 45 Females: 45	Bright red lipstick on white bond paper	Type 4 was most frequent in both sexes. Lip prints also show racial differences.
Pooja Ahuja et al 2018[11]	Gujarat	18- 25	Males: 50 Females: 50	Dark brown lipstick, cellophane tape, white bond paper	Type 2 (29.57%) was predominant in Gujarati population. **Males:** Branched pattern Type 2 (35.60%) **Females:** Long Vertical Type 1 (28.28%)
Tim Peter Thermadam et al 2020[12]	Calicut, Kerela	15 - 60	Males: 1056 Females: 1056	Lip impression was made on cellophane tape using dark red lipstick.	**Males:** predominance on Type 1' & Type 1 grooves patterns. **Females:** Predominance on Type 4 & Type 5 lip grooves pattern.
Pallavi Mishra et al 2022[13]	Odisha, West Bengal, Bihar	18 -25	Males :151 Females: 149	Lip impression was made on cellophane tape using dark red or pink, non glossy, non metallic Lipstick.	Difference was noted b/w Males and females for 3rd quadrant (P=0.003) & 4th quadrant (P = 0.014). Bihar & West Bengal: most common Type 2 Odisha: most common Type 1
Dr Mrinal Haloi et al 2024[14]	Assam	21-78	Males: 50 Females: 50	Dark non-glossy lipstick brush A red-coloured lipstick	**Males:** Predominant pattern Type 4 **Females:** Predominant pattern Type 1'
Dahiya P, et al 2025[15]	Gujarat	18-50	Males: 105 Females: 105		Most common pattern: Type 2 Second most common pattern:- **Males:** Type 1 **Females:** Type 3

identification when determining gender.

DISCUSSION

The field of forensic odontology can be greatly expanded by the methodical collection and registration of lip prints, especially in crime scene investigations where other identifiers might be compromised. Furthermore, lip prints can be found on surfaces like skin, clothing, or glass, and they are vital proof in criminal cases like theft or assault. Lip print records' role in forensic investigations would be strengthened by their integration into dental and forensic databases, which would guarantee quick retrieval, comparison, and legal validity. For lip print evidence to be as reliable and admissible as possible in medico-legal settings, standardized procedures for collection, classification, and storage are necessary. When lip prints are properly registered, a reference database is created that can help with identity verification, personal identification, and even determining gender by analyzing patterns.[16-18]

Registration of Lip Prints:

Lip prints can be captured through various methods:-

1. Direct photography if the prints are present on a glass surface.
2. Photographing the suspects lips.
3. Applying lipstick, lip rouge, or other suitable transfer mediums to the lips.
4. Using numerous substances such as Aluminium powder, Silver metallic powder, Silver Nitrate powder, etc.
5. All lip prints contain lipids which make their development possible by using Lysochromes dyes.

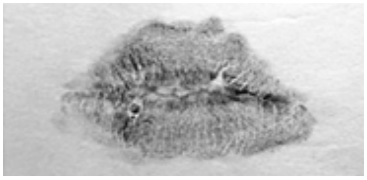
Collection of Lip Prints:

Crime scene lip prints can be categorized into three types:

1. **Visible Lip Prints:** Colored lip prints left behind by lipstick.
2. **Persistent Lip Prints:** Invisible lip prints on surfaces, retrievable days later using agents like aluminum or magnetic powder.
3. **Latent Lip Prints:** Prints formed by the secretion of oil and moisture from minor salivary glands, sebaceous glands and sweat glands present at edges of the lips that are detectable through development techniques.

Lip prints found on diverse surfaces-such as binding tape,

Figure 1: Collection of Lip Prints.

Type	Image	Description
Visible Lip Print		Colored lip prints left behind by lipstick
Latent Lip Print		Prints formed by the secretion of oil and moisture from minor salivary glands, sebaceous glands and sweat glands present at edges of the lips that are detectable through development techniques.
Persistent Lip Print		Invisible lip prints on surface, retrievable days later using agents like aluminum or magnetic powder.

Lip prints found on diverse surface-such as binding tape, glasses, cigarette butts or windows - can serve as vital evidence in numerous crimes, aiding investigators in cracking cases.

glasses, cigarette butts, or windows-can serve as vital evidence in numerous crimes, aiding investigators in cracking cases.

Procedure of lip print collection and registration is shown in **(Figure 1 & Figure 2)**

Merits and Demerits of Cheiloscopy

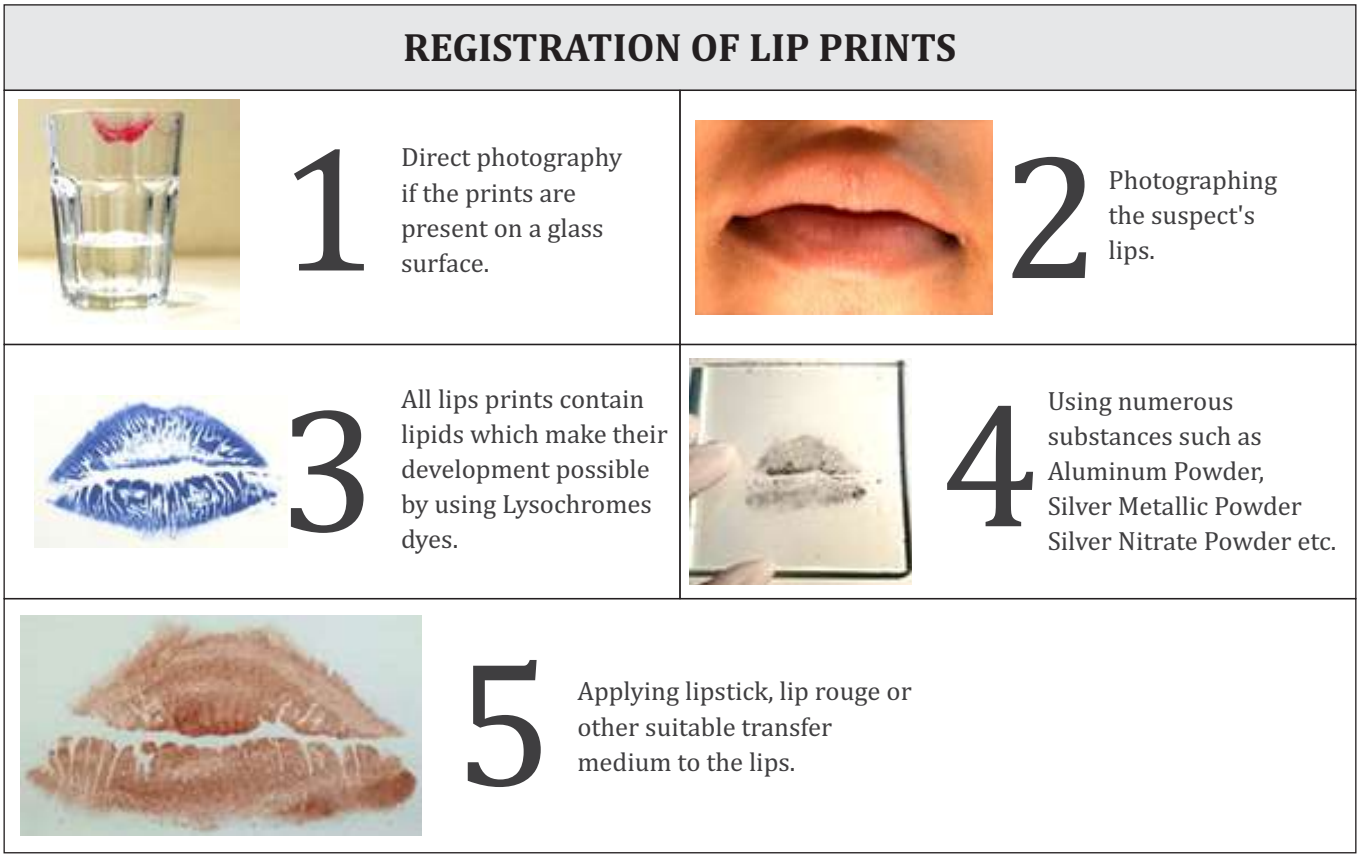
Lip prints can reveal crucial investigative details, such as the number of people present at a scene, gender determination, and possible clues about cosmetic use, personal habits (like smoking), and professional traits. The method is economical, non-invasive, and helpful in situations where other identification techniques might not be appropriate. Despite its potential, cheiloscopy has a number of drawbacks that may compromise its accuracy. Variations in pressure, direction, and technique used during recording or collection can result in different lip print patterns. Excessive or insufficient lipstick application can compromise analysis by producing smudged, distorted, or incomplete prints. Additionally, a number of pathological conditions can change the morphology of the lips and make identification less accurate, including lymphangiomas, congenital lip defects, fistulas, scleroderma, Melkersson–Rosenthal syndrome, syphilis, and cheilitis. Variability in results is also influenced by environmental factors and the absence of standardized protocols.[19]

Legal Framework on Lip Print Analysis In India

Indian laws provide the necessary legal basis for the collection of evidence from a forensic expert, including lip prints, under Bharatiya Sakshya Adhiniyam, 2023 or (oldIndian Evidence Act, 1872), and the Bharatiya Nagarik Suraksha Sanhita, 2023 (Code of Criminal Procedure, 1973).[20] Section 45 of the Indian Evidence Act deals "with expert testimony, permits courts to admit the opinion of persons skilled in science or art when the subject matter is beyond the comprehension of laypersons". This provision has become the principal gateway for introducing lip print analysis in courts through trained forensic odontologists or crime scene specialists.[21] Section 73 relating to the court's ability to compare disputed signatures and writings has also been read broadly by some of the lower courts to allow for comparisons of additional biometric traces, including lip impressions, though such use is limited and has been admitted only sparingly and at considerable discretion.[22]

There are few precedents under which Indian courts have

Figure 2: Procedure of Lip Print Registration.



either admitted or rejected evidence based on lip prints with a degree of doctrinal consistency. In various lower court decisions, lip print evidence has been admissible as corroborating evidence as opposed to substantive, particularly in cases where lip prints have been seen on glasses, garments and bodies and later compared with known suspects. There have been no such cases in High Court or Supreme Court judgments which define any jurisprudence on status of the scientific admissibility and value of proving lip print analysis under the law of India. This legal reluctance arises mainly from the absence of a standard classification. Fingerprints and DNA have historically been given a priority by the courts because they have more scientific and evidentiary strength.[23] Thus the burden is still on the forensic and legal system to prove the authenticity and standardization of cheiloscopy for broader judicial acceptance. It is also interesting to consider this matter from the IPR angle as well.[24]

The concepts of the lip print analysis and the IPR law in India could be an interesting. Lip prints being biological means cannot be protected directly by Indian IPR law. But inventions pertaining to instruments, software and algorithms employed in recording, scanning, categorizing and matching lip prints are potentially patentable under the Patents Act 1970 of India.[25] Forensic biometric systems which use artificial intelligence or machine learning approach to automate the detection and identification of lip prints, thereby reducing the load on examiner encompassing novelty, inventive step and industrial applicability are likely to be considered as patentable. Several other technologies, including those developed by Indian universities and start-ups have either filed for patents or are seeking technology transfer in the forensic space.[26] The systems of classification or records of lip print impressions may be considered original compilations and thus could be considered protected under copyright law. For the use of personal biometric data such as the lip prints, the morality and legal issues of personal rights trigger the raising of the protection for those, including the Digital Personal Data Protection Act, 2023 (DPDPA).[27] This law makes biometric data, but not limited to facial and lip features, sensitive personal data and therefore a subject to explicit consent for collection and processing, purpose limitation and use of measures to ensure accountability. The law enforcement units and crime labs that store lip prints should observe privacy protections even when that data represents victims who are considered vulnerable or is computerized. India is a signatory to the TRIPS Agreement of the WTO; by signing the treaty, the nation ensures that the combating of the rights on intellectual property and maintaining confidentiality in data is carried out according to the international standards, which creates the basis of the future introduction of cheiloscopic technologies to the system of the more general protection of the intellectual property and lawful compliance. The prevailing supreme court xprision in Justice K.S. Puttaswamy and another vs. The regulation of privacy based on state-level decisions is the defining line of the prime privacy jurisprudence, which establishes a mandatory equilibrium of forensic innovation and fundamental rights (Union of India and Others, 2017, 10 SCC1).[28] In that regard, Indian courts will be mandatory to make seminal decisions to align the evidentiary worth, science accuracy, and constitutional admissibility of lip-print analysis. This jurisprudential type of leadership will necessarily play a critical role in the promotion of the emergence of lip-print examination based on forensic analysis as an investigative tool into a legally admissible, constitutionally sound and ethically regulated practice. Through ongoing developments in the field of forensic science, India is on the brink of integrating cheiloscopy into an effective legal-technological framework providing recognition to innovation, protection of rights and delivered justice.

CONCLUSION

The forensic value of cheiloscopy as a trustworthy, non-invasive method for human identification is supported by the examination of lip print patterns from different Indian populations studies reviewed in this paper. Lip prints must be transferred and recorded accurately using the right media in order to be accepted as trustworthy forensic evidence. Developments in cheiloscopic research have great potential to make it a useful supplement to current identification methods. To improve its forensic validity, standardization and additional population-based research are necessary.

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A Case Series

Forensic Sights Into Cracker Blast Injuries During Festive Settings: A Case Series

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ABSTRACT

Introduction: Firecracker-related injuries during festival celebrations like Diwali are of important public health concern. These injuries vary from minor wounds to severe amputations and vision loss, often hurting young individuals. Here, in these case series we discuss about three such blast injury cases emphasizing the urgent need for preventive measures, prompt trauma care, and attention to medicolegal aspects.

Case-History:

Case-1: A 13-year-old boy suffered from traumatic amputation of his right hand at the level of carpal bones along with minor abrasions on the neck, left thigh, and face with hemodynamic stability after a firecracker blast on November 1, 2024.

Case-2: A 19-year-old male sustained superficial burns on his face, hands, and left foot along with third-degree corneal chemical burn in his left eye, leading to vision loss while mixing firecracker powder at home. Despite normal vital signs, he was needed immediate medical and ophthalmologic care for his injuries.

Case-3: 64-year-old male during an attempt to assemble a gas cylinder in his home sustained severe and complex injuries resulting from a devastating gas cylinder blast incident by a gas leak ignited by nearby fire crackers as a part of Diwali celebrations. The ignition caused a powerful explosion, leading to extensive trauma to multiple regions on the patient's body. After the explosion the patient became unconscious and was immediately transported to the hospital. Upon admission, he was in hypovolemic shock with unrecordable blood pressure needing aggressive fluid resuscitation to stabilize his condition.

Discussion: These cases emphasise the hazards of improper firecracker use. The first case shows the severe impact of high-energy blasts resulting in limb amputation. In complement, the second case shows the outcomes of performing experiments with explosive materials, leading to significant ocular injury. The third case highlights about the correlation of multiple factors—a gas leak, festive firecracker ignition, and severe blast injuries—occurring all together. All cases call attention to the importance of safety precautions, public awareness, and the requirement for swift medical intervention. They also highlight medicolegal issues, like adhering with safety regulations and addressing negligence.

Conclusion: Firecracker injuries can be prevented on a largescale with the help of adequate public education, stricter safety laws, and responsible supervision by adults especially during the festival seasons. From a forensic stand, detailed description of the injuries like traumatic amputation and chemical burns, are necessary for forensic analysis of the remnants of firecrackers or explosive materials.

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INTRODUCTION

In a Forensic context, Explosion refers to the rapid release of previously contained energy due to the conversion of solid or liquid components into gases, followed by the emission of significant amounts of heat and sound waves.[1] Trauma caused by explosions occurs on a regular basis all

over the world possessing a challenge out-of-hospital personnel, emergency physicians, and trauma surgeons.[2] The BNS (Bharatiya Nyaya Sanhita) addresses injuries from explosives primarily under Sections 288 and 326. Section 288 punishes negligent conduct with explosives that endangers human life or causes injury, while Section 326 covers mischief involving an explosive substance that results in serious consequences, such as causing grievous hurt or destroying property.

Explosions occur from Variety of mechanism broadly categorised as broadly categorized as accidental, intentional, or criminal. Common causes include chemical reactions (Combustion etc), Electrical faults (Short circuit), Mechanical failures (over pressurised vessels, gas cylinders), Natural causes (Lightning, earthquakes, Volcanic eruptions) and human activities which may be intentional (terrorism), occupational (mining demolition) and Accidental causes includes gas leaks, factory blasts and mishandling or experimentation with fireworks.[3-4] Each category presents unique forensic challenges in determining the exact circumstances surrounding the injury.

The injuries caused by fireworks increases especially during the festival seasons like Diwali. The festival of lights (Diwali) is celebrated with fireworks by Indians across the globe leading to injuries varying by prevalence, period of occurrence, sex and age variation, adult supervision, causative fireworks, mode of lighting, age groups prone to injury, patterns of injury caused by individual fireworks.[5] The annual incidence of firecracker-related injuries in India is 7 in every 100,000 population.[6]

The types of injuries caused during this festival season depends on the age, type of firework, use behaviour whether the person takes place actively or a bystander and the region of body involved.[7] This article highlights the various types of mechanism and injuries of the firework directly or as a consequence of firework causing like burns (thermal, chemical), eye injuries, ear damage, limb injuries (amputation, fractures) and blast injuries leading to burns, soft tissue and fractures.

These cases highlight the dangers of improper firecracker use. The first case demonstrates the severe impact of high-energy blasts leading to limb amputation. In complement, the second case shows the outcomes of performing experiments with explosive materials, leading to significant ocular injury. The third case highlights about the correlation of multiple factors-a gas leak, festive firecracker ignition, and severe blast injuries-occurring all together.

All cases call attention to the importance of safety precautions, public awareness, and the requirement for swift medical intervention. They also highlight medicolegal issues, like adhering with safety regulations and addressing negligence.

CASE SERIES:

Case 1: The 13-year-old male patient sustained injuries on the left side of his face, right hand, and left thigh while attempting to fire and throw an explosive firecracker. It exploded while in his hand. On arrival, his vital signs were stable. Routine blood investigations were normal.

On examination the patient had, Abrasion of size 1.2x1.2cm present over left side of the face with reddish brown scab, Abrasion of size 1.2x1.2cm present over middle part of front of neck with reddish brown scab **(Figure 1)**, Traumatic amputation of right hand at the level of wrist bones **(Figure 3)** and Multiple contused abrasion of size ranging from 0.2x0.2cm to 2.4x1.2cm present over the anterior aspect of left thigh **(Figure 2)**.

The patient was managed with IV fluids, antibiotics, analgesics and surgical debridement and wound wash with disarticulation.

Figure : 1, 2 and 3



Case 2: The 19-year-old male patient sustained superficial burns on the left side of his face, left hand, and left foot. The incident occurred during the mixing process with a wooden stick when an unexpected explosion. Upon admission, his vital signs were stable.

Routine blood investigations were normal. On examination, Superficial burns involving left side of face and left eye. Upon Ophthalmic evaluation he was diagnosed with 3rd degree chemical burn **(Figure 4)**, Total Body Surface Area (TBSA): 6% superficial flame burns. Superficial burns on lateral aspect of the left hand **(Figure 5)**. The injuries were Managed Conservatively.

Figure : 4 and 5



Case 3: A 64-year-old male during an attempt to assemble a gas cylinder in his home sustained severe and complex injuries resulting from a devastating gas cylinder blast incident by a gas leak ignited by nearby fire crackers as a part of Diwali celebrations.

The ignition caused a powerful explosion, leading to extensive trauma to multiple regions on the patient's body. After the explosion the patient became unconscious and was immediately transported to the hospital. Upon admission, he was in hypovolemic shock with unrecordable blood pressure needing aggressive fluid resuscitation to stabilize his condition.

A detailed examination revealed the following injuries. Extensive second-degree burns to the face and eyes. A deep laceration measuring 16×7cm, muscles deep on the left forearm. Blast injury to the left hand, resulting in near-total amputation of the second, third, and fourth fingers at the metacarpal level, traumatic amputation of the thumb at the proximal phalanx, and complete amputation of the left little finger at the carpal level.

Additional lacerations included a 4×3cm muscle-deep wound on the lower third of the right forearm and a 6×4cm muscle-deep wound on the lower one-third of the right leg. Multiple minor superficial abrasions across the chest, forearms, and legs were also found. The X-ray of the left hand and forearm was shoeing fractures of radius and ulna with fractures of all metacarpal bones with shattering effect of tissues. **(Figure 6-7)**

Figure : 6 and 7



Angiogram was conducted to see the viability of the tissues and it was found that the arterial supply was not sufficient in distal area **(Figure 8)**. The patient underwent emergency surgical intervention, including a disarticulation surgery at the left wrist level **(Figure 9)**. The patient was discharged after a period postoperative care and medications from SICU.The patient was advised physiotherapy to aid the recovery process and improve functional outcomes.

Figure : 8 and 9



DISCUSSION

A chemical explosion can result in various kinds of injuries, ranging from small burns to serious ones like amputation of body parts, deep wounds, or even death. These injuries mainly happen because a huge amount of energy is released very quickly, creating a strong blast wave. Explosives are grouped into two types based on how they are made: manufactured and improvised.

Manufactured explosives made by the military are carefully tested for safety and quality whereas Improvised explosives, on the other hand, are made in small amounts using common weapons or chemicals for purposes other than what they were originally meant for. Accidental explosions can occur during the process of manufacturing of improvised explosive devices and its improper handling.[8]

Firecrackers are basically built from cardboard or plastic casings and contain a mixture of chemicals such as flash powder, cordite, and either smokeless or black powder as the propellant. In addition, components may contain match heads and combustible substances such as kerosene or lighter fluid. For ideal performance, the contents of a firecracker must be tightly packed. However, defects caused while manufacturing and improper handling during usage can make firecrackers potentially dangerous[9]

Injuries resulting from Explosions are classified into four main types: primary, secondary, tertiary, and quaternary. Primary blast injuries occur because of overpressure or under pressure waves, primarily impacting gas-filled organs. Secondary injuries arise from flying debris and bomb fragments and represent the leading cause of death and morbidity in explosion incidents.

These injuries commonly involve penetrating or blunt trauma, leading to fractures, traumatic amputations, and soft tissue damage. Tertiary injuries occur when individuals are displaced by the blast wind, resulting in additional blunt or penetrating trauma. Finally, quaternary injuries encompass all other explosion-related effects not included in the previous categories, such as burns, inhalational injuries, toxic exposures, and crush injuries.[10] The classic triad of abrasion, contusion and punctuate lacerations are Marshall triad which are diagnostic and characteristically seen in explosion injuries.[26]

In Cases 1 and 3, a high-energy blast led to the complete amputation of Patients limbs, posing medical and psychological challenges. Injuries caused due to traumatic amputation are Avulsion, degloving and laceration.[11] Amputations due to explosive injuries often results in jagged, irregular wound edges **(Figure 6 & 7)**. Musculoskeletal injury is exceedingly common in victims of blast events and accounts for >80% of all surgical procedures in those that survive as a consequence of primary blast injury and Traumatic amputations, or limb avulsions occur in 1% to 3%of blast victims.[12]

Hand is the most common part affected during the explosions because of its proximity while lighting the firework or malpractice of holding the firework in the hand while lighting.[13] Proximal amputations resulting from blast injuries, occurring above the wrist or ankle, are indicative of severe internal trauma and poor prognosis. Recovered amputated segments are seldom viable for reattachment, limiting their forensic and reconstructive value.[14] Scene investigation is critical for gathering clues about the cause and manner of amputation, including the recovery and preservation of severed parts and related evidence, such as blood patterns and fire work used.

In case 2 the material used in this firecracker was Gandhak (Sulphur) and the patient sustained eye injuries and burns while assisting an individual in handling inflammable materials, specifically firecracker powder and yellow sulphur (gandhak). Sulfur has inherent flammable properties and is very sensitive to friction.[15] Gandhak Potash mixture is usually used by farmers in an improvised iron gun to produce a sound blast that deters birds, pests, and wildlife animals from damaging their harvest.[16] Exposure to this chemical produces a combination of chemical and thermal injuries, closely resembling firecracker-related blast trauma, and results in significant damage to the ocular surface and it is usually because of primary and secondary injuries[17] as seen in above mentioned case. The severity of injuries ranges from mild corneal abrasions to more serious complications like traumatic cataract and conjunctival-scleral tears.[18]

Krishan Gopal et al, have described the pattern, severity, and demographic distribution of burn injuries caused by firecrackers during the Diwali festival highlighting that most victims are young males, often injured while lighting or being near fireworks. Common injury sites include the hands, face, and eyes, with varying degrees of burns many requiring hospitalization as seen above case 2 or surgical intervention emphasizing a clear correlation between inadequate safety measures, lack of awareness, and the high incidence of injuries during the festive period calling for stronger public education, stricter regulation on firecracker use, and promotion of safer celebration practices to reduce preventable harm.[19]

Studies have shown the male predominance and usual age of patients was less than 30 years with increasing in number over the consequent years.[20] Tandon R et al in 2012 highlighted the rising cases of blast injuries during Diwali, urging authorities to assess the situation and implement preventive measures and attention on public education through digital platforms, social media, and traditional awareness methods.[21] Firework safety interventions included distributing multimedia disks and brochures, sending school mailings to parents, encouraging budget limits, and peer-to-peer education by trained students.[22]

In India, unrestricted access to firecrackers increases the risk of burns and other serious injuries. Many countries have enacted laws to control firework use. In the UK, the Fireworks Act 2003 prohibits possessing or setting off fireworks in public places and restricts their use between 11 p.m. and 7 a.m., with significant penalties for violations. In Canada, the Fireworks Act allows the sale and use of fireworks only between October 24 and November 1, and prohibits sales to minors without written parental consent.[23]

It is important to analyse consumer fireworks in order to

know their composition for quality control. Usage of appropriate analytical method and extraction technique for fire accelerant residue in suspicious homicidal cases for determining the manner of injuries.[25]

Furthermore, there is a high forensic interest in the analysis of post-blast residues (PBRs) and pre-blast devices, Identifying pre- and post-blast fireworks and reagents and in Analyzing basic components (oxidizers, fuels, binders, colorants) from incidents for finding cases that are related to misuse, devices being altered or fireworks being made by non-professional users using legal handbooks in a home laboratory[24] for medicolegal purposes such as Mechanism (accidental or intentional) criminal negligence, insurance claims, compensation, Product liability, public safety laws and Violations of safety regulations

CONCLUSION

Fatal injuries can be caused by fires. Fire cracker and explosive related injuries remain a significant challenge and medicolegal issue particularly among young males causing injuries varying from burns, ocular injuries to severe musculoskeletal trauma, amputations and decapitations in severe cases. Younger age, male sex and risky behaviour patterns are associated with these injuries requiring incident reconstruction and forensic evaluation by newer methods. There is a need of stricter enforcement of new laws, safety regulations, awareness and coordinated efforts between healthcare workers and policy makers to reduce such incidents and for ensuring public safety.

LIMITATIONS

Cases might have been underreported when treated on outpatient basis in hospital settings. Cultural, regional, socio-economic characteristics are not fully exposed overlooking complex behavioural patterns. Delayed information regarding the cases after treatment makes difficult for forensic evaluation of injuries

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A Case Report

White Spot Lesions on Teeth as Forensic Identifier in a Decomposed Body: An Odontology Forensic Case Report

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ABSTRACT

Background: Victim identification in disaster scenarios is challenging, especially when remains are decomposed, burned, or fragmented. Forensic odontology plays a key role by using the unique and durable features of teeth for identification. Teeth resist decomposition and high temperatures, making them reliable markers in incidents such as accidents, fires, or natural disasters, particularly when other identifiers are unavailable.

Purpose: This case report aims to highlight the utility of smile photograph superimposition and the forensic value of white spot lesions as unique identifiers in disaster victim identification, particularly when conventional dental records are unavailable.

Case: A decomposed adolescent body was recovered from a river without available fingerprint and dental records.

Case Management: The body was recovered and examined for external injuries and identifying features. During autopsy, dental examination revealed white spot lesions on the maxillary anterior teeth. Forensic odontologists performed smile photograph superimposition using ante-mortem images from social media. The white spot patterns and superimposition matched, and DNA analysis later confirmed the identity.

Conclusion: This case highlights the value of white spot lesions as individualizing dental features. When combined with photographic superimposition, such enamel defects can support victim identification in the absence of formal dental records. It demonstrates how subtle dental anomalies, when carefully analyzed, can aid confirmation of identity through a multidisciplinary forensic approach.

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INTRODUCTION

Sudden or unnatural deaths require a medico-legal investigation to determine the cause of death, assist legal processes, and identify the deceased. Identification is crucial not only for legal and administrative reasons, such as issuing death certificates and accessing insurance, but also for emotional closure of the family and cultural practices like proper burial.[1] Unidentified bodies may also pose public health risks, especially in controlling infectious diseases and supporting epidemiological efforts.[2]

In Indonesia, the right to identification is supported by the Indonesian Law Number 17 of 2023 on Health.[3] The law includes medical services for legal purposes as part of public healthcare (Article 22), affirms citizens' rights to such services (Article 154), and assigns responsibility to both central and local governments (Article 155). Article 157 mandates that all deceased individuals must be identified, with standardized procedures followed to determine identity and cause of death.[3]

Forensic odontology, originating from the Latin terms forensis (relating to the court) and odontology (the study of teeth), is a fundamental branch of criminal investigation that applies dental evidence to legal matters and is firmly grounded in the exceptional durability of dental tissues.[4]

Owing to their high mineral content and robust enamel composition, teeth can withstand decomposition, burning, dismemberment, and a wide range of environmental extremes with minimal structural degradation, thereby often guiding the direction of an investigation. As a result, in many cold or long-standing forensic cases, the skull, and particularly the dentition, remains exceptionally well preserved and provides a dependable source of biological information.[5] These dental structures can assist in estimating age, sex, and ancestry, thereby playing a critical role in the process of human identification. Identification is commonly established by comparing known dental records of a missing person (ante-mortem data) with dental findings from an unidentified body (post-mortem data).[6-7]

Dental records, along with DNA and fingerprints, are accepted as primary scientific methods of human identification.[8-9] This comparative process is essential in both routine cases and Disaster Victim Identification (DVI) scenarios. In such events, forensic odontology offers a reliable, efficient, and scientifically validated approach to establishing or excluding identity when traditional methods may not be possible. The purpose of this case report is to demonstrate the value of smile photograph superimposition and highlight white spot lesions as unique dental features that can assist in disaster victim identification, particularly when conventional dental records are not available.

CASE REPORT

On the morning of September 2024, civilian witness discovered seven heavily decomposed male adolescents (estimated age 15-19 years) floating in Bekasi River. By 09:00, The Regional Agency for Disaster Countermeasure had recovered all victims, noting severe edema and 2nd-degree skin slippage indicative of 12-18 hours submersion. The fingerprint could not be found in the Indonesia Automatic Fingerprint Identification System, possibly because the victim is underage and has not yet registered for an Indonesian National Identity Card, so his fingerprint is not in the database. During the afternoon autopsy at Bhayangkara Hospital Level I R. Said Sukanto, forensic odontologists observed distinctive white spot lesions on the maxillary anterior teeth of one of the victims.

This case highlights successful identification of one victim through smile photograph superimposition, where white spot lesions (early enamel demineralization) served as key identifiers. These hypomineralized defects showed identical patterns in ante-mortem social media photos and post-mortem dental documentation, proving crucial when formal records like ante-mortem odontograms or dental records were unavailable. A few days after the dental examination by the forensic odontologist, DNA analysis confirmed that the ante-mortem and post-mortem dental records matched. This validated the accuracy of the identification based on the white spot lesion and the dental superimposition on the victim's teeth.

CASE MANAGEMENT

Following the recovery of the decomposed adolescent body, an external examination was conducted to assess injuries and identify distinguishing features; however, due to advanced decomposition, second-degree skin slippage, and the absence of national ID registration, fingerprint identification failed, and no ante-mortem odontogram or dental records were available from a dentist.

During the autopsy, forensic odontologists conducted a detailed dental examination of all teeth **(Figure 1)** and recorded the findings on a post-mortem odontogram form. In the absence of formal dental records, investigators searched social media platforms and obtained multiple smile photographs of the presumed victim. The photographs were uploaded onto a computer and the superimposition was carried out by using Canva, a graphic design application software. With such software, the forensic odontology team performed photographic superimposition by overlaying the post-mortem dental

Figure 1: The Condition of the Teeth of the Decomposed Victim: (A) Visible from the Right Side, (B) Visible from the Front, (C) Visible from the Left Side.



Figure 2: Photographic Superimposition of the Maxillary Teeth: (A) 0% Transparency, (B) 40% Transparency, (C) 100% Transparency

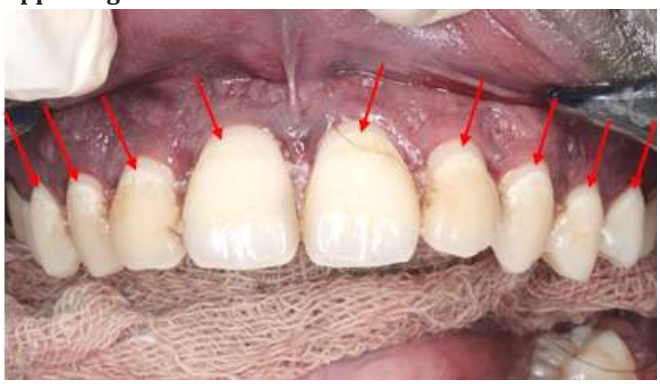


Figure 3: Photographic Superimposition of the Mandibular Teeth: (A) 0% Transparency, (B) 40% Transparency, (C) 100% Transparency.



images onto the ante-mortem smile photographs **(Figure 2 and Figure 3)**. The alignment was adjusted to match anatomical landmarks, including tooth shape, incisal edges, midline, and lip contour.[10-11]

Figure 4. Appearance of White Spot Lesions on the Cervical Third of the Upper Left Second Premolar to the Upper Right Second Premolar.



Distinctive white spot lesions were observed on the maxillary anterior teeth, specifically the central and lateral incisors and canines. White spot lesions were also found on the first and second upper premolars on both the right and left sides **(Figure 4)**. These enamel opacities, indicative of early demineralization, were recorded through high-resolution intraoral photography. The white spot lesions were then compared for shape, size, position, and symmetry between the ante-mortem and post-mortem images. These lesions appeared consistent in both images, showing identical patterns and locations, particularly on the upper central incisors **(Figure 5)**. White spot lesions were found in only one of the seven bodies, and the same was observed in the ante-mortem photos, confirming the identity of that individual. The distinctive nature of these enamel defects provided strong visual evidence

Figure 5. Comparative Photographs of White Spot Lesions on the Maxillary Anterior Teeth: (A) Ante-mortem Photograph, (B) Post-mortem Photograph.



supporting a positive identification.

Subsequently, DNA analysis was conducted and confirmed the match between the recovered body and the presumed identity. This multidisciplinary approach validated the use of smile photograph superimposition and white spot lesion analysis as reliable methods of forensic identification in the absence of traditional records.

DISCUSSION

Teeth are highly valuable in personal identification due to their considerable individual variation, making each person's dentition nearly unique. As a result, dental identification serves as a reliable method for comparing ante-mortem data, such as radiographs, dental casts, palatal rugae patterns, and notes from dental or medical records, with post-mortem findings**[7,12-13]**. However, obtaining complete ante-mortem dental records can be challenging, particularly in Indonesia, where routine dental visits are uncommon. Most individuals seek dental care only when experiencing pain, and formal dental documentation is often lacking, especially among lower-income or suburban populations, as seen in this case. In such instances, personal photographs provided by friends or family members may be the primary source of comparison, allowing for identification through the method of photographic superimposition.

Several case studies have highlighted the use of smiling photographs as a tool in dental identification. A range of both qualitative and quantitative methods has been documented in earlier research and case reports.[10-11,14] One of the most basic methods is direct visual comparison, which, despite lacking scientific precision, can provide valuable clues, such as lip shape or perioral scars, especially when the body is photographed prior to significant decomposition. Features like missing teeth, wear patterns, and restorations are also readily visible for side-by-side comparison.[15]

However, while this technique is simple and accessible, it relies heavily on the examiner's subjective judgment and expertise, making it prone to potential errors due to its lack of objective validation. For a more structured approach, comparisons can be made using incisal edge tracings. The contours of the incisal edges can be analyzed and compared to assess the degree of similarity between the dental features. Additionally, more advanced techniques include image superimposition, such as overlaying crown outlines for enhanced comparison accuracy.[10-11] This approach aligns with our case, in which software was used to perform photographic superimposition by overlaying post-mortem dental images onto ante-mortem smile photographs. The alignment was carefully adjusted based on anatomical landmarks, such as tooth morphology, incisal edge contours, the dental midline, and the shape of the lips.

A study by Santoro et al. demonstrated the effectiveness of digital image analysis in dental comparison by using photographs of smiling individuals. The analysis was performed both homologously and heterologously using Facecomp software, which proved capable of detecting even minor differences in dental features to support positive identification. This approach holds practical value in forensic investigations, where post-mortem images of dental structures can be matched with ante-mortem photographs of missing persons.[14]

As illustrated by the previously discussed case reports, ante-mortem dental records are not always accessible. In many instances, especially with the widespread use of smartphones and internet connectivity, photographs, such as selfies, may serve as the only available ante-mortem material for comparison.[16-17] Photographic comparison offers several advantages: it is simple, cost-effective, convenient, and can be both objective and dependable.[10,16] While online smile photographs may sometimes lack high image quality, they can still significantly aid in narrowing down the identity of an individual and prove particularly useful in large-scale disaster scenarios.[18]

White spot lesions indicate areas of enamel demineralization beneath a surface layer that remains intact and highly mineralized. These lesions may progress to dental caries but do not always do so. Demineralization refers to the loss of minerals from the enamel, resulting from the acidic environment that dissolves hydroxyapatite crystals, leading to the formation of microscopic pores in the enamel structure. In areas affected by demineralization, the mineral content is reduced, leading to a loss of enamel translucency and resulting in a visibly opaque white appearance. For this reason, early-stage enamel lesions or smooth surface caries are commonly referred to as white spot lesions. White spot lesions are often the earliest visible indicators of caries development and are commonly regarded by clinicians as initial lesions. However, it's important to note that demineralization must reach a depth of at least 300–500μm before it becomes clinically detectable.[19]

From a clinical perspective, white spot lesions appear as

opaque white areas due to changes in light behavior caused by mineral loss and the difference in how air or water and enamel bend light. The increased porosity reduces translucency, resulting in a whitish discoloration. This uneven surface also disrupts light reflection, leading to a duller appearance with scattered, rather than direct, light reflection.[20-21] In clinical practice, distinguishing the underlying cause of white spot lesions is essential, as they may result from either dental caries or hereditary conditions. Caries-related lesions typically appear more opaque and chalky when the tooth surface is air-dried, due to the loss of enamel translucency caused by subsurface demineralization. In contrast, developmental lesions show little to no change in appearance when dried with air.[19]

Visual inspection and photographic analysis remain common methods for diagnosing white spot lesions, allowing clinicians to evaluate their depth and extent. In recent years, however, additional advanced techniques have been introduced, including fluorescence-based tools, such as the DIAGNOdent system, as well as microradiography and micro-computed tomography, which provide more precise assessments.[22] Visual examination remains the most commonly used diagnostic method in everyday dental practice. Through this approach, clinicians can assess whether a white spot lesion is active or inactive. An active lesion typically presents with a chalky, rough surface, whereas a smooth and glossy appearance suggests the lesion is inactive.[19]

For an accurate visual assessment, the tooth surface must be cleaned with pumice and air-dried for at least five seconds before being examined under proper lighting with a mirror. Light is essential, as enamel translucency is influenced by its refractive index (1.62). Demineralization increases enamel porosity, lowering this index. When enamel is wet, the pores fill with water (refractive index 1.33), making lesions less visible. Air-drying replaces water with air (refractive index 1.0), enhancing the contrast of white spot lesions.[19] In our case, we performed visual inspection following air-drying and illumination. The observed characteristics, porosity and a white opaque area on the cervical third of the anterior teeth, were consistent with typical white spot lesions.

White spot lesions (WSLs) were observed to be 2.5 times more common in maxillary teeth than in mandibular teeth. They showed a symmetrical distribution across the arches, with the maxillary lateral incisors being the most frequently affected, followed by maxillary canines and then mandibular canines.[23] The shape of white spot lesions is influenced by the distribution of biofilm and the orientation of enamel prisms.[24] As these factors vary among individuals, the resulting lesion shapes are unique and may serve as a useful tool for personal identification.

White spot lesions can serve as distinctive dental features valuable for forensic identification, particularly when formal dental records are unavailable. When combined with photographic superimposition, these enamel anomalies offer a viable alternative for confirming identity. This approach demonstrates the potential of subtle dental traits to function as reliable forensic markers when analyzed through a multidisciplinary lens, integrating dentistry, imaging, and forensic science. Such methods are especially relevant in challenging contexts, including mass disasters or unidentified remains, where conventional identification tools may be insufficient.

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A Case Report

Black and White in Toxicology, Virtual Autopsy Findings in Intentional Acid Ingestion

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ABSTRACT

Background: Analytical toxicology using postmortem viscera, blood, or urine samples is the established method for the detection of poison or intoxicating substances in cases of fatal poisoning. Medico-legal postmortem cases with undiagnosed/concurrent COVID-19 infection posed a challenge to the autopsy surgeons during the COVID-19 pandemic. However, during the initial pandemic, confusion was encountered related to the retrieval of samples for analysis from autopsy procedures. Until clear guidelines were issued, analytical laboratories were also reluctant to analyse the samples due to the spread of the virus. Hence, the authors intend to share the experience of solving an intentional acid ingestion during the initial pandemic, adhering to Minimal/Non-invasive methods. The feasibility of using advanced radiological techniques like PMCT was explored in determining the cause of death for legal purposes in these circumstantially confirmed poisoning cases due to corrosives.

Case Report: The author here describes two different deaths due to corrosive ingestion, whose autopsy was conducted in the Department of Forensic Medicine and Toxicology, of a tertiary institute. The police investigation and circumstantial evidence revealed the corrosive ingestion. In both scenarios, the deceased expired due to gastric perforation and related complications. The same findings were observed in Post Mortem Computed Tomography (PMCT) and corroborated with the conventional autopsy findings.

Conclusion: These cases highlight the scientific use of the Virtual Autopsy using PMCT in concluding the cause of death for corrosive poisoning cases with clear-cut circumstantial evidence supplanting or at times substituting the explorative dissection and analytical techniques on a case-by-case basis.

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INTRODUCTION

Corrosive ingestion is one of the commonest toxicological emergencies, if not intervened; leads to the demise of the patient due to the complications like the perforation of the stomach wall. In the autopsy examination, analytical toxicology using postmortem viscera, blood or urine samples is the established method for detection of poison or intoxicating substance in cases of fatal poisoning.

Medico-legal postmortem cases with undiagnosed/ concurrent COVID-19 infection posed a challenge to the autopsy surgeons during the COVID-19 pandemic. However, during the initial pandemic there were confusions encountered related to the retrieval of samples for analysis from autopsy procedure. Until clear guidelines were issued, analytical laboratories were also reluctant to analyse the samples due to the spread of the virus.

Minimal/Non-invasive methods could be adhered in establishing the cause of death for legal purposes as per the

existing guidelines in the country.[1-2] Simultaneously the use of Post-mortem Computed Tomography (PMCT) in the field of Forensic Medicine in the country was budding. Considering the uncertainty, the authors intend to explore the feasibility of using advanced radiological techniques like PMCT based virtual autopsy in determining the cause of death in certain circumstantially confirmed poisoning cases like corrosives. The findings at PMCT suggestive of perforation in a case of corrosives are the presence of extraluminal air due to pneumoperitoneum, fluid in the mediastinum and peritoneal cavity.

However, the implications of PMCT to identify the cause of the death in deaths due to acute caustic injury needs to be explored. Hence, the authors intend to explore the feasibility of using advanced radiological techniques like PMCT in determining the cause of death for legal purpose in this circumstantially confirmed poisoning cases due to corrosives.

The author here describes two different deaths due to corrosive ingestion, whose autopsy was conducted in the Department of Forensic Medicine and Toxicology of a tertiary institute. The survival period of the two cases differed. The police investigation and circumstantial evidence revealed the corrosive ingestion by the deceased. Both the deceased expired due to gastric perforation and related complications.

The same findings were observed in PMCT and corroborated with the conventional autopsy findings. In both cases, neither external nor internal inspection delivered findings suggestive of third-party involvement. There were no other injuries suggestive of blunt force/surface impact all over the body. The autopsy procedure of Virchow's dissection technique was adhered.

Case 1

Alleged history of a 40-year-old female ingested household acid kept for cleansing the washroom in the early morning hours at her residence around 01.00am. She developed abdomen pain and vomiting post was shifted immediately to the tertiary institute and expired in the course of treatment with a survival period of around 10hours. At autopsy, the abdomen cavity showed the presence of 200 ml of blood on the intestine coils **(Figure 1A)**. The stomach was adherent with the diaphragm superiorly and adnexal transverse colon anteriorly and small intestines inferiorly, posteriorly as well **(Figure 1B)**. The stomach wall was blackened and presented blood clots and, due to extensive chemical burning, was highly fragile and therefore difficult to dissect. On careful dissection, a rupture of size 9cm x 6cm was noted in pyloric end of the stomach.

Case 2

Alleged history of a 61year old male consumed toilet cleaner (acid) due to personal reasons. He was taken to nearby hospital and was referred to our tertiary institute for management. He survived for about 20 hours post consumption and expired during the course of treatment. At autopsy, multiple brownish discoloration around the lips and tongue suggestive of abrasions due to corrosive action of acid were present. About 600 ml of brownish colour fluid was present in the peritoneal cavity **(Figure 2A)**.

The stomach wall was black in color admixed with hemorrhage appearing as a mass (Fig 2B). On careful dissection a rupture measuring 5.5 cm X 5 cm was present on the greater curvature of the stomach. A duodenal perforation of size 1 cm X 1 cm was present, 1 cm proximal to doudeno-jejunal junction.

PMCT Findings of Both Cases: The stomach region in PMCT was seen hyperdense with difficulty in interpreting the stomach borders on the posterior aspect. The complete examination of the abdomen revealed multiple extraluminal air. **(Figure 1C & 2C)**

DISCUSSION

Computed tomography (CT) is a highly sensitive tool for evaluating the upper GI tract lesions in antemortem subjects. The findings interpreted at CT could be graded as normal appearing organs, wall lesions in the form of thickening, soft tissue infiltrations, extraluminal air in the mediastinum and peritoneal cavity as a sign of perforation. This grading is based on studies conducted in the antemortem subjects evaluating the importance on CT as a screening tool in acute caustic ingestion for further managements.[3]

Even though there are literatures regarding the efficacy of PMCT in deaths due to ingestion of Benzene, mercuric chloride, implications of PMCT in acute caustic injury is less explored.[4-5] Hence, the authors intended to discuss the implications of PMCT either to supplement or supplant the conventional autopsy procedure in corrosive deaths through above mentioned cases.

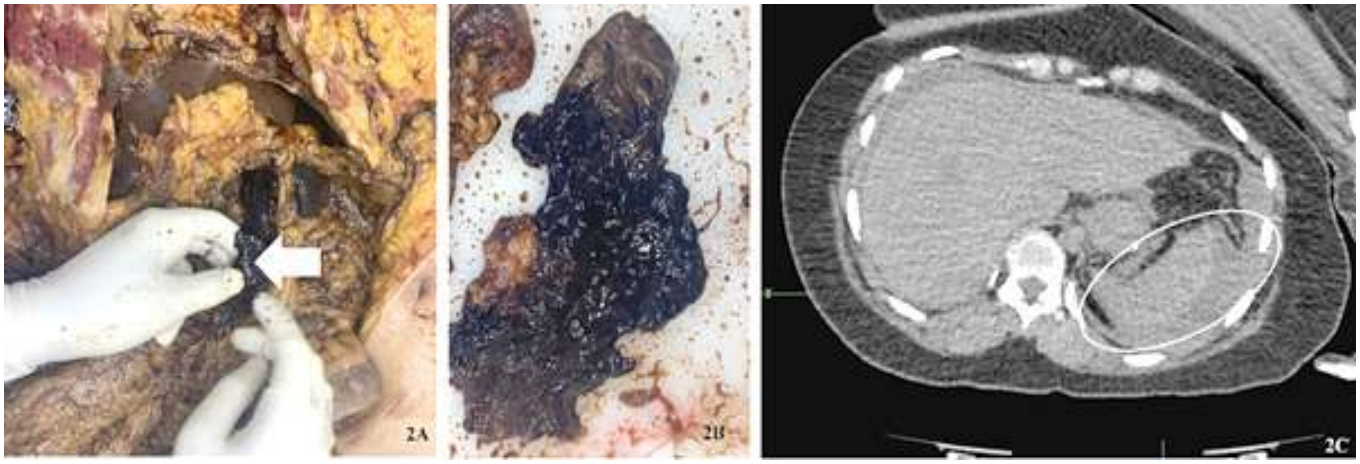
Robert in 1828 was first to report case report of isolated corrosive injury to the stomach from sulfuric acid ingestion.[6] Chatzaraki et al. studied the accuracy of PMCT and different radiological signs to identify the source of

Figure 1: Gross and PMCT Images of Case 1.



1A: Blood Collection Inside the Peritoneal Cavity,
1B: Hemorrhagic Stomach Wall with Clots,
1C: PMCT (coronal) Stomach is Hyperdense with Poorly Differentiated Borders.

Figure 2: Gross and PMCT images of Case 2.



2A: Blood Collection Inside Peritoneal Cavity Along with Stomach Rupture.
2B: Dissected Stomach with Hemorrhagic Walls and Adherent Clots.
2C: PMCT (axial) Stomach is Hyperdense with Poorly Differentiated Walls.

bleed in cases with hemoperitoneum confirmed at autopsy. The criteria analysed were sentinel clot sign, blood collection surrounding the related organ, sedimented blood around the organ, intraparenchymal abnormal gas distribution and combinations of mentioned signs.

They concluded that there is moderate sensitivity and high specificity to determine the bleeding source in cases with hemoperitoneum using PMCT.[7] In the present cases, the authors screened the body by studying PMCT images prior to autopsy conduction. PMCT in both the deceased showed areas of hyperdensity surrounding the stomach due to which the borders of the stomach were not clearly seen in addition to areas of hyperdensity in between the coils of the intestine. There was presence of air under the diaphragm in both cases which aroused suspicion of intestinal perforation, however the exact site of perforation can't be detected in PMCT. During autopsy, stomach wall haemorrhage admixed blood clots was present both inside and outside the stomach and upon careful dissection the perforation site was identified.

The corrosives comprise of both acids and alkalis but differ at the site to affect the gastro intestinal tract. Alkalis produces severe effects on the oesophagus producing liquefactive necrosis while the acids on the stomach produces coagulative necrosis.[8-9] The squamous epithelium of the oesophagus is spared from perforation owing to the short passage time. This makes the stomach more vulnerable, resulting in perforation.[10] The presentation of perforation peritonitis is never silent and it

is usually preceded by the symptoms like stomach pain, vomiting etc accompanied by signs of abdomen rigidity.

The autopsy examination on the other end is a multistep procedure that mandates a proper circumstantial history including the presentation of the deceased to the emergency. In deaths due to poisoning, samples like blood, urine, gastric lavage samples at emergency department or routine viscera at autopsy are collected as a part of protocol for analytical purposes. When the circumstantial evidences are clear; the findings observed externally and PMCT findings are congruent with the said history, the need to preserve such samples becomes less.

This case selective decision of preserving the samples also saves the time of overburdened forensic scientists and reduces the usage of resources required to perform the tests. Hence, a combination of detailed external examination and PMCT technique in such type of cases may be considered to conclude cause of death scientifically to answer the legal queries. Virtual Autopsy using PMCT may supplant the internal dissection in circumstantially confirmed corrosive ingestion. This will also be a better modality during the pandemic period curtailing the spread of infections and upholding the dignity to dead.

CONCLUSION

Both the deceased were COVID-negative and circumstantial evidence of corrosive ingestion was present. There was neither foul play alleged by the relatives nor the investigation officer demanded any samples for preservation. However, the viscera samples were kept for determining the nature of poisoning as per the established norms.

The authors successfully explored the scientific use of the Virtual Autopsy using PMCT in certifying the cause in the deaths following corrosive poisoning ingestion. We intend to suggest that, had the deceased been COVID positive; the same cause of death could have been certified by adhering to the Virtual autopsy using PMCT curtailing the sample preservation for toxicological analysis.

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A Case Report

Autopsy Findings and Toxicological Insights in a Rare Case of Bromisovalum (BVU) Poisoning

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ABSTRACT

Background: Bromisovalum (α-bromoisovalerylurea or BVU, also known as Bromisoval) is an uncommon cause of poisoning-related deaths in India. Once prescribed for its sedative effects, it carries a high risk of fatal overdose, whether accidental or intentional.

Case Presentation: A 32-year-old chronic alcoholic man presented to the hospital complaining of nausea, vomiting, and diarrhea with the alleged history of intake of some poisonous substance. He died within 24 hours despite supportive measures.

Autopsy Findings: Autopsy revealed acute intoxication signs like an edematous brain, congested and voluminous and pale lungs, on dissection of both the lungs, reddish colored fluid was coming out, and the stomach contained about 100 ml greenish-brown colored viscous material mixed with semi-digested food particles. The mucosa of the stomach was pale, and a few submucosal hemorrhages were present. Forensic toxicology confirmed bromisovalum in his system, alongside a blood alcohol concentration of 90.50 mg% ± 1.80%.

Conclusion: The fatal potential of bromisovalum and its significance were taken into consideration, as this case highlights unusual presentations. Regulation of its availability in India is necessary to stop similar incidents in the future.

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INTRODUCTION

Bromisovalum, a sedative-hypnotic, belongs to the bromoureide group and has been used in the past for its central nervous system (CNS) depressant effects. In several Asian countries, it is available over the counter, often combined with non-steroidal anti-inflammatory drugs (NSAIDs) under the brand names Brovarin and NARON ACE. In the combinations, NARON ACE contains BVU (100 mg), ibuprofen (72 mg), ethenzamide (42 mg), and anhydrous caffeine (25 mg). The serum half-life of BVU is approximately 12 days.[1–3]

Pharmacologically, bromisovalum is a monoureide derivative with reported anti-inflammatory properties and sedative-hypnotic. It inhibits both pro- and anti-inflammatory cytokines in macrophages stimulated by lipopolysaccharide (LPS) or interferon-gamma (IFN-γ). Comparable to dexamethasone, bromisoval suppresses Toll-like receptor (TLR)-mediated cytokine release but reportedly lacks the adverse effects typically associated with corticosteroids.[4]

Acute BVU intoxication often presents with lethargy, myoclonic jerks, respiratory failure and altered sensorium, even at serum levels as low as 67–110 mg/L, commonly associated with suicide attempts. In contrast, chronic

poisoning from long-term use manifests with delirium, ataxia, tremors, depression, and psychosis, typically at serum levels >500 mg/L. MRI in chronic cases may show cerebellar or pontine atrophy, while acute cases may show edematous changes or appear normal. The duration of exposure and concentration help distinguish between acute and chronic poisoning.[1,5]

Fatal BVU intoxication is rarely reported. To assist forensic and medical professionals in early detection and management, this case report focuses on the clinical, pathological, and toxicological characteristics of a rare fatal case of acute bromovalerylurea poisoning.

Case Presentation

A 32-year-old chronic alcoholic man died under suspicious circumstances with suggestive signs of ingestion of a poisonous substance. He lived with his family but was sleeping alone at the time of the incident.

In the evening, he developed symptoms of nausea, vomiting and diarrhea, which worsened overnight. The following morning, his parents rushed him to a tertiary care hospital, where he was admitted and received symptomatic treatment. Despite all resuscitation efforts, the patient developed cardiopulmonary arrest and was declared dead the next day. The body was brought to the autopsy complex for post-mortem examination, covered with a white cloth bought by Himachal Pradesh Police Officer Sub-Inspector with police inquest.

On examination, the deceased was identified as a moderately built and nourished male, 170 cm in length. He was dressed in a light yellow half-sleeved t-shirt, brown pajama and gray underwear, all of which were intact.

The eyes and mouth were partially open and bluish discoloration of the nails on both hands was noted indicating peripheral hypoxia. Fecal incontinence was present.

Rigor mortis developed throughout the body as the autopsy was carried out 13 hours after death. Post-mortem lividity was fixed and distributed posteriorly, consistent with the reported time since death. Signs of decomposition were not appreciable.

Internal Examination

Central Nervous System: The brain appeared edematous, with no evidence of intracranial hemorrhage.

Respiratory System: Both lungs were voluminous and pale. On sectioning, reddish fluid exuded, indicating pulmonary edema. The tracheal mucosa was pale and empty.

Cardiovascular System: The heart was grossly normal in size and configuration. Chambers contained clotted blood. No significant valvular or myocardial pathology was noted.

Gastrointestinal Tract: The stomach contained about 100ml of greenish-brown viscous material with semi-digested food particles. mucosa of stomach was pale and few sub-mucosal hemorrhages were present. The small intestine and its contents contain brownish colored fluid and large intestines contained fecal matter and gases.

Liver, Spleen, Kidneys and Gall Bladder: All solid organs were pale but grossly intact with no focal lesions. Gall bladder was partially distended.

Genitourinary System: The urinary bladder was intact and empty.

Toxicological Samples: Viscera and blood samples from the heart were preserved for chemical analysis and were sent to the Forensic Science Laboratory (FSL), through the police.

Toxicological Report and Cause of Death

Blood samples from the heart and viscera were preserved and sent to FSL for chemical analysis. Bromisovalum confirmed as the cause of death by the toxicology report. Bromine was detected in the samples using Gas Chromatography-Mass Spectrometry (GC-MS) method. Ethyl alcohol was detected in the blood at a concentration of 90.5±1.80 mg%. However, the alcohol level was below the lethal threshold i.e., >300 mg% and not deemed to be the primary cause of death, but synergistic CNS depression occurred with bromisovalum.

Based on autopsy findings and toxicological analysis, the cause of death was acute bromisovalum poisoning. The manner of death was ruled as accidental as per police inquest records.

DISCUSSION

This report presents a rare and fatal case of acute bromisovalum poisoning in a 32-year-old Indian male, likely India's first documented bromisovalum fatality. Following the alleged ingestion of a poisonous substance, he developed nausea, vomiting, diarrhea, and rapid respiratory failure. Despite symptomatic management, he succumbed to poisoning within 24 hours of symptom onset.

Bromisovalum, originally developed as a sedative-hypnotic, is still used in some industrial formulations and

over-the-counter sedative preparations such as NARON ACE in some countries in Asia (Kawakami et.al.).[1] It undergoes hepatic metabolism into bromine containing metabolites, which accumulate in the body and interfere with neuronal chloride transport mechanisms, resulting in CNS depression, altered sensorium and neuromuscular incoordination (Biyajima et.al).[6] Acute toxicity typically presents with nausea, vomiting, confusion, and respiratory failure (van Balkom et.al).[7] Bluish nail beds and fecal incontinence indicated hypoxic terminal events.

No external or internal injuries were excluded as mechanical causes, confirming a toxicological etiology. FSL analysis of gastric contents (100 ml greenish-brown material) and viscera confirmed bromisovalum via GC-MS. GC-MS reliability extends to decomposed remains, where femur marrow detected bromisovalum after 7 months, supporting our fresh tissue findings.[8]

Blood ethanol (90.5±1.80 mg%) sublethal likely potentiated CNS depression via GABAergic synergy (Kawakami et.al).[1]

Indian toxicology protocols rarely screen for bromisovalum, necessitating inclusion of obscure sedatives in routine forensic panels. Hyperchloremia from habitual OTC analgesic use signals chronic bromisovalum toxicity, treatable with saline diuresis though neurological sequelae often persist.[9] Clinicians must suspect bromisovalum in unexplained coma or respiratory failure with sedative exposure. (Ishikura et.al).[2] While our case represents acute fatality, chronic bromisovalum abuse manifests nystagmus and ataxia detectable by EDX bromine analysis, contrasting acute versus chronic toxicology.[10]

CONCLUSION

This rare Indian case of acute bromisovalum poisoning highlights its lethal potential. Autopsy and FSL toxicology confirmed bromisovalum, as the cause of death. Sublethal blood ethanol likely potentiated CNS depression. In cases of unexplained coma or collapse, clinicians and forensic specialists need to check for bromisovalum. Bromisovalum's unknown source here reflects its rarity in India, warranting stricter controls on its availability.

Conflict of interest: Nil

Informed Consent: Following medico-legal procedures, an autopsy was performed during a police inquest.

Ethical approval: N/A

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