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# Indian Journal of Forensic Medicine & Toxicology

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## Severe Lead Toxicity Simulating Myelodysplastic Neoplasm: A Case Report

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### Abstract

**Background:** Diagnosis of Myelodysplastic Syndromes (MDS) relies on the integration of morphology and genetics. While lead toxicity is a known cause of basophilic stippling, its ability to mimic the complex morphologic features of clonal myeloid malignancies – such as micromegakaryocytes – is often under-recognized in the era of Next-Generation Sequencing (NGS).

**Case Presentation:** A 54-year-old male presented with transfusion-dependent anemia, constipation, and cognitive decline. Bone marrow examination revealed profound trilineage dysplasia and micromegakaryocytes, highly suggestive of MDS. However, comprehensive molecular profiling (Karyotype, FISH, and NGS) was negative for clonal markers. A detailed history revealed long-term ingestion of an alternative medication preparation containing *Nag Bhasma* (lead ash). The blood lead level (BLL) was >100 µg/dL (normal levels < 5 µg/dL).

**Conclusion:** This case highlights “morphologic-molecular discordance” as a red flag. Withdrawal of the offending agent and chelation therapy resulted in rapid hematologic recovery. In the absence of clonal markers, heavy metal toxicity must be excluded before diagnosing MDS.

**Keywords:** Lead toxicity, Myelodysplastic Neoplasm (MDS), Basophilic stippling, alternative medication, Chelation therapy, Case report, Nag Bhasma.

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## Introduction

The latest WHO and International Consensus Classification (ICC) of myeloid neoplasms emphasize the integration of morphology and genetics.<sup>1</sup> While MDS is increasingly defined by specific mutations (e.g., *SF3B1*, *ASXL1*), “genetically negative” dysplasia remains a diagnostic challenge.<sup>2</sup> Lead poisoning (Plumbism) interferes with the heme biosynthetic pathway by inhibiting  $\delta$ -aminolevulinic acid dehydratase (ALAD) and ferrochelatase.<sup>3</sup> This report describes a unique case where toxic marrow suppression simulated a clonal malignancy with such morphologic precision that it necessitated a comprehensive genomic exclusion. This case underscores the diagnostic priority of environmental history over advanced genomics in cases of molecular-morphologic discordance.

## Patient Information

A 54-year-old male presented to the Hematology outpatient department with a 6-month history of progressive fatigue, severe constipation, and a 13 kg unintentional weight loss. Family members noted recent-onset cognitive impairment and irritability.

- Medical History: The patient had no history of hypertension. He had been diagnosed with Diabetes Mellitus shortly before presentation.
- Psychosocial History: The patient is a Chartered Accountant by profession. He denied any occupational exposure to heavy metals or industrial chemicals.

## Clinical Findings

On physical examination, the patient appeared pale and lethargic.

- Vitals: Stable.
- General Exam: Pallor was present. Notably, there was an absence of Burton’s line (lead line) on the gingiva. There was no lymphadenopathy.
- Systemic Exam: Abdominal examination was soft and non-tender. Neurological examination revealed no motor weakness and intact reflexes, despite the reported cognitive decline.

## Timeline

- 6 Months Prior: Patient begins taking alternative medication supplement.
- Month 1-5: Onset of fatigue, constipation, and gradual weight loss (13 kg).
- Presentation: Admitted for transfusion-dependent anemia (Hb 6.9 g/dL).
- Day 1: Bone Marrow Aspirate/Biopsy performed due to suspicion of MDS.
- Day 7: NGS and Cytogenetics return Negative. Toxin screen ordered.
- Day 9: Blood Lead Level confirmed at 104  $\mu$ g/dL. Alternative medication stopped.
- Day 10: Chelation therapy initiated with D-Penicillamine.
- Day 25: Rapid clinical improvement; Hb rose to 13.5 g/dL; Lead levels dropped to 38  $\mu$ g/dL.

## Diagnostic Assessment

- Laboratory Evaluation: Initial CBC showed Hemoglobin 6.9 g/dL (transfusion-dependent) and MCV 85.7 fL. Peripheral smear demonstrated significant coarse basophilic stippling.
- Bone Marrow Findings: Aspirate showed a hypercellular marrow with significant erythroid dyspoiesis (nuclear budding, fragmentation). The biopsy revealed marked megakaryocytic proliferation with a high frequency of micromegakaryocytes.
- Molecular Studies: Cytogenetics: Karyotype 46, XY. FISH: Negative for 5q, 7q, 20q deletions. NGS: A 54-gene Myeloid panel was negative for pathogenic variants.
- Confirmed Diagnosis: Severe Lead Toxicity (Blood Lead Level 104  $\mu$ g/dL) secondary to alternative medication intake.

## Therapeutic Intervention

The patient was advised to immediately stop the ingestion of the alternative medication. Oral

chelation therapy was initiated with D-Penicillamine at a dosage of 250 mg TDS for 1 month. He received supportive care for constipation and nutritional counseling.

### Follow-up and Outcomes

The patient showed a dramatic response to therapy. Within 15 days of initiating treatment, his Hemoglobin improved significantly to 13.5 g/dL. Concurrently, his blood lead levels dropped from 104 µg/dL to 38 µg/dL. The patient was compliant and tolerated the therapy well.

### Discussion

This case illustrates the capacity of lead toxicity to masquerade as Myelodysplastic Neoplasm (MDS). Our findings align with recent literature identifying alternative medication preparations as a significant source of non-occupational lead poisoning. A comparable case reported by Thomas et al. (2024) described a patient taking herbal medicines for diabetes who presented with anemia and basophilic stippling.<sup>5</sup>

Our case is distinct in the severity of the marrow dysplasia, specifically micromegakaryocytes, which necessitated a broad molecular workup (NGS) to rule out clonal malignancy. The absence of Burton's line in our patient supports the observation by Gidlow (2015) that classic physical signs of plumbism are frequently absent in subacute toxicity.<sup>7</sup> Furthermore, while traditional purification of *Bhasmas* (herbo-metallic preparations) is intended to detoxify metals, unregulated manufacturing often results in bioavailable lead persisting in the final product.<sup>8</sup>

### Conclusion

Hematologists must maintain a high index of suspicion for lead toxicity when faced with

molecular-negative trilineage dysplasia. A detailed drug history, including alternative medication, is as vital as the bone marrow biopsy. In cases of discordance between severe morphology and negative genetics, heavy metal screening is mandatory.

**Conflict of Interest:** Nil

**Source of Funding:** Nil

### Informed Consent

Written informed consent was obtained from the patient for the publication of this case report.

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## The Blue Enigma: Decoding A typical Organ Discoloration-A Case Report

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### Abstract

**Introduction:** Any unusual organ discoloration during autopsy should be carefully examined to exclude decomposition, poisoning or underlying diseases. We report such a case of turquoise discoloration of the brain and heart, rarely observed during routine autopsy, which posed a major diagnostic challenge.

**Case Summary:** A 50-year-old male was admitted to a nursing home with a history of head injury following a fall in the bathroom. He died after 15 days due to sepsis and refractory vasoplegic shock. At autopsy, the brain and heart appeared light bluish in colour. On cut section and exposure to atmospheric air, the organs rapidly turned turquoise-blue in colour, producing a distinctive “avatar-like” appearance.

**Discussion:** The rapid colour change did not match typical findings of asphyxia, methemoglobinemia, decomposition or hydrogen sulphide poisoning. Review of clinical records revealed use of methylene blue as rescue therapy for refractory septic shock. Methylene blue is reduced to colourless leucomethylene blue in tissues, which re-oxidizes upon exposure to oxygen, explaining the intense turquoise-blue colour change during autopsy.

**Conclusion:** This case highlights the importance of considering iatrogenic causes of abnormal discoloration of organs. Awareness of methylene-blue associated colour changes can prevent misinterpretation of autopsy findings as poisoning or decomposition.

**Keywords:** Methylene blue, Sepsis, Organ discoloration, Forensic pathology.

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## Introduction

In routine autopsy practice, visual inspection of internal organs provides valuable clues regarding the physiological and pathological condition of the deceased before death and may occasionally help the autopsy surgeon in estimating the time since death. Unusual organ discolorations are commonly found in cases of asphyxia, poisoning, decomposition or underlying pathological conditions. However, rapid and intense turquoise or bright blue discolorations are rare and create a diagnostic dilemma among the autopsy surgeons.<sup>1-3</sup>

Few authors have previously described greenish-blue or turquoise discoloration of the brain and other organs following therapeutic administration of methylene blue.<sup>1-4</sup> Nevertheless, this phenomenon is rarely encountered during routine autopsy practice and the rapid change of colour on exposure to atmospheric air is poorly documented in this part of the country. Hence, such findings are often misinterpreted as evidence of poisoning, decomposition, pathological conditions of organs or obscure organ findings, especially when clinical history is unavailable or not properly reviewed.

The present case describes an unusual turquoise blue discoloration of the brain and heart on exposure to air observed during autopsy examination in a patient who received methylene blue for refractory septic shock. By corroborating the post-mortem findings with the clinical history and available literature, this report aims to highlight an uncommon iatrogenic cause of organ discoloration and emphasize the importance of clinicopathological correlation in avoiding diagnostic error.

## Case Report

A 50-year-old hypertensive male was brought for medicolegal autopsy with a history of head injury following a fall in the bathroom and loss of consciousness. He was admitted and treated in a nursing home for 15 days, after which he expired in the ICU.

External examination revealed rigor mortis all over the body and fixed post-mortem staining over the back, without cyanosis, pallor, or external injuries. Internal examination revealed congestion of

all organs. The lungs showed patchy consolidation in both upper lobes with mucopurulent exudates oozing out from the parenchyma on cut section and the spleen was enlarged and soft in consistency. The heart showed left ventricular hypertrophy (wall thickness 1.7 cm) with atheromatous plaques at the inner aspect of the root of aorta.

The most notable finding involved the brain and heart. Initially, these organs appeared light bluish in colour. On sectioning and exposure to atmospheric air, the colour rapidly intensified into a vivid turquoise blue. This "avatar-like" appearance was confined specifically to the brain and heart. No features of decomposition, such as greenish discoloration of the iliac region or marbling, were present, and no gross findings suggestive of poisoning were identified. Blood and viscera were preserved for toxicological analysis, and representative tissues were sent for histopathological examination.

Relevant clinical records including Bed Head Ticket (BHT), were obtained and reviewed. On admission, the patient was hemodynamically stable (BP 110/76 mmHg) with a GCS of 12/15. His total leukocyte count (TLC =15,000/mm<sup>3</sup>) was raised. On day 7 he developed severe sepsis with a TLC of 22,000/mm<sup>3</sup> and C-reactive protein of 199 mg/L. A persistent fever was documented and cultures of endotracheal aspirate were positive for *Klebsiella pneumoniae* and *Acinetobacter*. Despite maximum inotropic support, he developed refractory vasoplegic shock on Day 12 (MAP 60 mmHg, arterial lactate 5.5 mmol/L, pH 7.18) and his infection proved resistant to all major antibiotics, including colistin. As a rescue strategy, intravenous methylene blue infusion was initiated. The patient survived another 3 days before his death on Day 15 from admission.

Toxicological analysis of preserved blood and viscera did not reveal any common heavy metals, agricultural poisons, volatile substances or cyanide compounds. Histopathological examination showed acute ischemic neuronal changes in the brain and patchy myocardial necrosis, consistent with septic shock. However, no pigment deposition or other specific histopathological correlation of the unusual discoloration was noted.

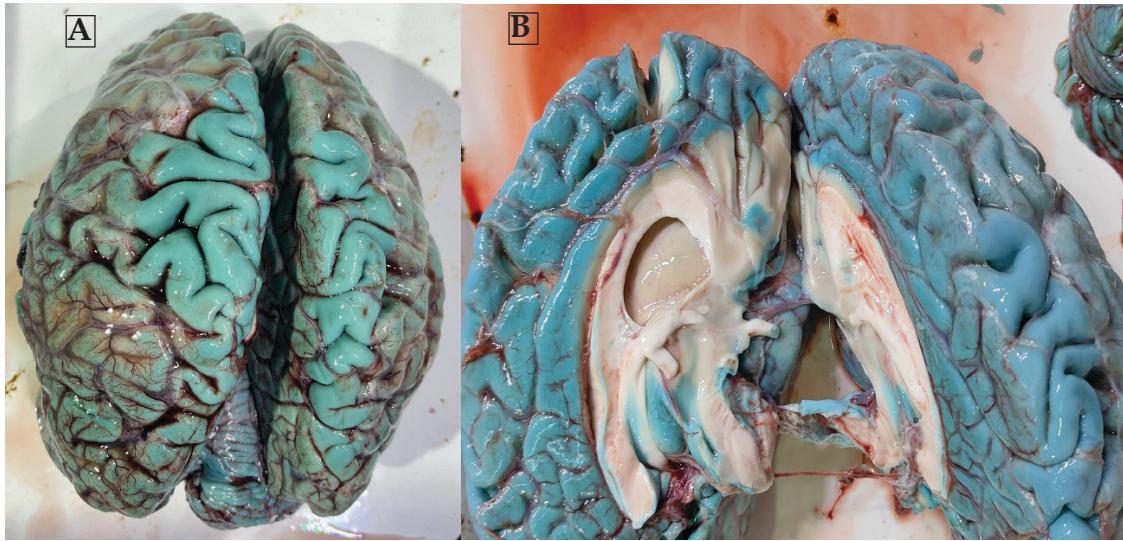
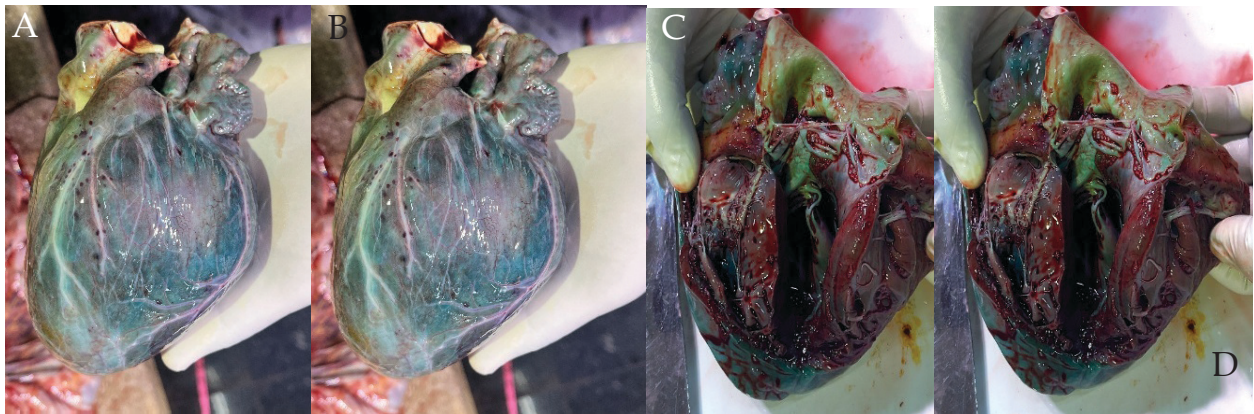
**Figure 1 :-**

Figure 1 (A) : Gross photograph of the brain (superior view) showing diffuse turquoise-blue discoloration of the cerebral surface, with colour intensification following exposure to atmospheric air at autopsy.

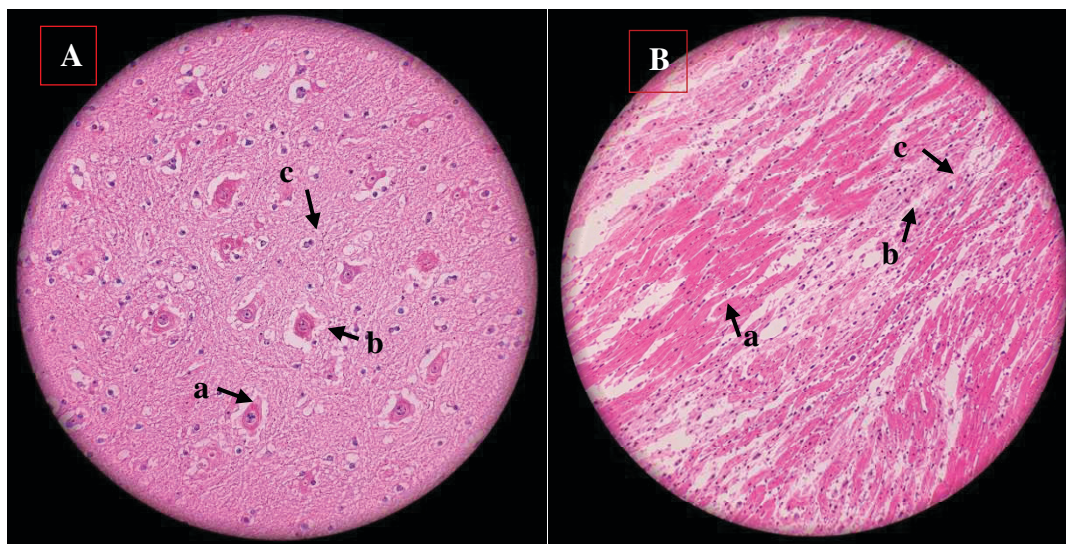
Figure 2 (A) : Coronal section of the brain demonstrating prominent turquoise-blue discoloration of the cerebral parenchyma after sectioning and air exposure, attributed to oxidation of methylene blue metabolites.

**Figure 2 :-**

Time →

Figure 2 (A, B, C) : Sequential gross photographs of the heart demonstrating gradual progression of discoloration from faint bluish hue to intense turquoise-blue appearance following exposure to atmospheric air, attributed to postmortem oxidation of methylene blue metabolites.

Figure 2 (D) : Cut section of the heart demonstrating turquoise-blue discoloration of the myocardium after air exposure at autopsy.

**Figure 3 :-**

**Figure 3 (A) : Histopathology of Brain; a - Perineuronal vacuolation (edema), b - Red neuron (eosinophilic cytoplasm), c - Pyknotic / shrunken nucleus**

**Figure 3 (B) : Histopathology of Heart; a - Interstitial edema with separation of muscle fibers, b - Loss of myocyte nuclei, c - Necrotic hypereosinophilic myocardial fibers**

### Discussion

Turquoise discoloration of internal organs at autopsy poses a significant diagnostic dilemma for autopsy surgeons. Moreover, the rapid colour change on cut sections and exposure to atmospheric air as noted in this case, was inconsistent with the commonly known forensic differentials. Several pathological and toxicological causes of such organ discoloration were considered and ruled out. Methemoglobinemia, often associated with nitrites, nitrates or aniline compounds, generally produces a slate-grey or bluish discoloration of organs consequent to oxidation of haemoglobin.<sup>5,6</sup> A diffuse greenish discoloration of all internal organs is seen in cases of hydrogen sulphide poisoning but the discoloration remains relatively stable and does not show rapid colour changes on exposure to air.<sup>7</sup> Pseudomelanosis is associated with black-green spots on the intestines.<sup>5,6</sup> Death due to asphyxia typically produces a dark reddish to bluish congestion from inadequate oxygenation.<sup>6</sup> In cases of death due to sepsis, the organs are congested and

occasionally show a pale greenish discoloration due to impaired perfusion and hypoxia.<sup>5</sup> Jaundice and hyperbilirubinemia generally produce a yellowish to yellowish-green discoloration, but it has no relation to exposure to air.<sup>7</sup>

Some postmortem causes and artefacts were also considered. Early decomposition produces greenish to greenish black discoloration, while hypostasis leads to bluish purple staining of the dependent parts of the body.<sup>5,6</sup> But none of these conditions show rapid colour changes on exposure to air.

The diagnostic breakthrough came only after reviewing the clinical records, which revealed intravenous administration of methylene blue (MB) for refractory vasoplegic shock secondary to severe sepsis. A similar green-blue discoloration of the brain was noted during the autopsy of a 16-year-old boy at Thiruvananthapuram who was administered MB to treat refractory shock. However, the study did not report any discoloration of the heart during autopsy.<sup>7</sup> Afzal et al and Durão et al have also

reported similar cases where MB was administered during treatment of septic shock, resulting in bluish-green or turquoise discoloration of the internal organs, particularly the brain, heart and lungs which intensified during sectioning and exposure to air.<sup>1,8</sup>

MB is a phenothiazine derivative used in the treatment of methemoglobinemia and as an emergency rescue therapy for refractory vasoplegic shock.<sup>7</sup> It plays a role in septic shock by inhibiting nitric oxide synthase (NOS) and soluble guanylate cyclase (sGC) to decrease cGMP-mediated vasodilation and help restore blood pressure when conventional vasopressors fail.<sup>9</sup>

The “avatar” appearance of the organs is explained by the unique biochemistry and high tissue affinity of MB. MB is reduced to a colourless form (leucomethylene blue) by reduction in the body, particularly in the liver and red blood cells.<sup>1</sup> This metabolite may accumulate in tissues during therapy, especially after prolonged administration. When the organs are dissected and exposed to atmospheric air during autopsy, leucomethylene blue is rapidly oxidised back to methylene blue, giving the characteristic turquoise-blue discoloration.<sup>4,10</sup>

Unlike earlier reports by Afzal et al and Durão et al,<sup>1,8</sup> the present case documents sequentially photographed progression of the intense discoloration with time, providing additional visual evidence in support of this oxygen-dependent oxidation of leucomethylene blue.

Recognition of this rare postmortem finding has important forensic and clinical implications. Methylene blue therapy causes such turquoise blue discoloration that mimics pathological, toxicological or postmortem causes of organ discoloration. Hence, a detailed review of the clinical records and corroboration with autopsy findings are essential to prevent misinterpretation of this iatrogenic phenomenon and unnecessary toxicological investigations.

However, as this is a single case report, the findings cannot be generalized to all patients receiving MB, as the typical discoloration of organs may depend on dosage, route and timing of administration, duration of therapy and interval between administration and death. Moreover, histopathological confirmation remains difficult as routine tissue fixation and processing washes out the dye. Further research should focus on developing standard protocols for documenting such time-lapse changes on exposure to air and on specialized tissue processing techniques which can allow for microscopic confirmation of MB in tissue samples.

## Conclusion

Administration of methylene blue for refractory septic shock may produce a turquoise discoloration of internal organs, which rapidly intensifies on cut sections of organs and exposure to air. Recognition of this uncommon iatrogenic phenomenon is important for accurate interpretation and to avoid diagnostic error, as it can closely mimic colour changes due to pathological or toxicological conditions.

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**Ethics Statement:** Institutional Ethics Committee approval was not required for publication of this anonymized single-patient case report according to institutional policy.

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# Sexual Assault Nurse Examiners (SANE) in Forensic Investigations: A Narrative Review

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## Abstract

Sexual assault inflicts profound physical and psychological distress on victims regardless of gender or age. Nurses often serve as the initial responders in healthcare facilities. The Nirbhaya case of December 2012 led to the enactment of the Criminal Law (Amendment) Act, 2013, which expanded the definition of rape and recognized the right of victims to receive medical and forensic care in India. The Protection of Children against Sexual Offences (POCSO) Act, 2012 further strengthened safeguards for child victims. Forensic nursing is a specialized field that applies nursing principles to care for individuals affected by violence, trauma, or criminal acts. These nurses collaborate with law enforcement, legal experts, and healthcare facilities for assessment, treatment, evidence collection, injury documentation, emotional support, and legal testimony. Sexual Assault Nurse Examiners (SANE) are specially trained professionals authorized to conduct Sexual Assault Forensic Examinations and are integral members of Sexual Assault Response Teams. This article explores their roles, responsibilities, and scope in the Indian context.

**Keywords:** Forensic Nursing, Sexual Assault, Sexual Assault Forensic Examinations, Sexual Assault Nurse Examiner, Sexual Assault Response Team

## Introduction

Sexual violence creates immense physical and psychological distress in victims shattering their sense of safety, and trust, often resulting in diminished

quality of life.<sup>1</sup> Contrary to societal myths, young males can also become victims of sexual abuse, along with girls and women.<sup>2</sup> Global estimates of sexual violence incidents highlight a higher prevalence of

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such crimes against women compared to men.<sup>3</sup> The initial responder to such cases in healthcare facilities is often a nurse. Nurses are the preferable professionals to address the challenging and constantly evolving requirements of caring for sexual assault victims while navigating the difficult legal system.<sup>4</sup>

Sexual violence profoundly impacts multiple corners of the victim's life. The trauma that the victims receive from faulty systems can add to the suffering they are already experiencing.<sup>1</sup> Unfortunately, there are many instances of victim blaming, directly or indirectly, by the medical, legal, and law enforcement agencies and society at large.<sup>5</sup> This renders the victims feeling responsible for the assault, subjecting them to further victimization. Prosecuting sexual offense cases poses inherent challenges, as these crimes typically lack eyewitnesses, relying predominantly on scientific evidence.<sup>4</sup>

## Methodology

This narrative review article explores the role of Sexual Assault Nurse Examiners (SANEs) in conducting forensic examinations and caring for sexual assault survivors. A comprehensive literature search from January 1995 to January 2024 was performed using databases like Medline, PubMed, Scopus, and Google Scholar, focusing on SANEs' responsibilities and forensic examination, sample handling, and the role of SANE in sexual examination. A secondary search examined the pathway to becoming a SANE and nurses' expanded roles.

## Results

After removing duplicates and screening titles, abstracts, and characteristics, 54 relevant studies were identified. Studies that did not meet the inclusion criteria were excluded. Ultimately, 43 full-text studies were included in the discussion.

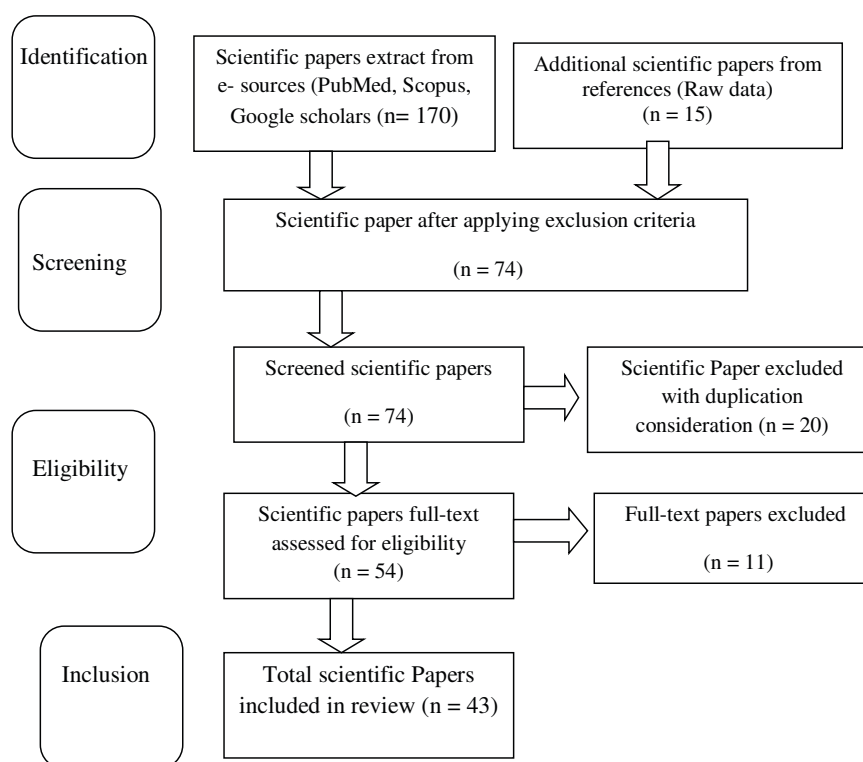


Figure 1: PRISMA search strategy and article selection process

## **Sexual Violence and the Epidemiology and Legal Aspects**

Sexual violence is a serious crime that has major harmful effects on the victim's well-being.<sup>2</sup> Sexual violence affects individuals of all age groups, though, studies have reported the majority of victims belong to the age group of 13 to 20 years.<sup>6</sup> In 2019, the National Crime Record Bureau, India (NCRB) in its annual report, noted that 405,861 crimes against women were recorded, with around 8% being cases of sexual assault.<sup>7</sup> Despite the government taking multiple initiatives and making popular laws, the crime against women in India has increased by almost 6%; from 62.4 % in 2019, up from 58.8 in 2018.<sup>8</sup>

The horrific New Delhi Nirbhaya case of December 2012, where a female student was brutally gang raped in a running bus in New Delhi led to the enactment of the Criminal Law (Amendment) Act 2013. This act gave a broader definition to rape and acknowledged that all the victims of sexual abuse had the right to get medical and forensic attention.<sup>9</sup> The Protection of Children from Sexual Offences (POCSO) Act (2012), makes it clear that a female doctor must examine female pediatric victims, and the lack of an available female doctor can cause delays in the examination and evidence collection.<sup>10</sup>

## **Origins of Forensic Nursing**

The role of the nurse in medical-legal cases was initially recognized by Dr John Bud the chief medical examiner of Alberta, Canada in the mid-1970s.<sup>11</sup> He established that forensic nurse examiners could represent the forensic pathologist at the crime scene for death investigations. Further consolidation of forensic nursing as a specialty occurred in the 1980s, pioneered by the studies of Ann Wolbert Burgess, and Virginia Lynch, who proposed the conceptual model of integrated practice of Forensic nursing science.<sup>12</sup>

## **The Forensic Nurse**

Forensic nurses, play a crucial part in bridging the gap between the distinct domains of law and medicine.<sup>4</sup> The scope of forensic nursing is quite extensive and includes the provision of direct client

services, consultation services to nursing, medical, and law-related agencies, and provision of expert court testimony with the evidence of assault, etc.<sup>13</sup> In countries where Forensic nursing is already accepted as a specialty, multiple subspecialty areas have emerged, for example, Sexual Assault Nurse Examiner (SANE).<sup>14</sup>

## **Sexual Assault Nurse Examiner (SANE)**

SANE is a relatively new concept introduced in many countries where nursing professionals are trained for a dedicated role in the medico-legal examination of sexual assault cases.<sup>15</sup> With the rising concern over increasing crime against women, female forensic nurses can easily build trust with these victims. Their sensitivity to victims' needs helps build trust, making it easier to gather medical history and forensic evidence. Furthermore, Forensic nurses have the necessary qualifications and training to provide skillful management of sexual assault victims.<sup>16</sup>

In India, the concept of SANE is gradually gaining traction. This article is an attempt to explain the nuances of the roles, and the need to accept SANE in the Indian healthcare system. The SANE program was first developed when the health care providers and other stakeholders working with sexual assault victims noted the inadequacy of services for the victims.<sup>17</sup> In the Indian healthcare system, most sexual abuse victims are treated in emergency departments where specialized forensic experts are often unavailable. The Indian healthcare delivery system has been resource-limited for ages.<sup>7</sup> Most sexual assault cases are handled by emergency departments that lack healthcare professionals trained in forensic science, fewer female doctors available and the situation is similar in both government and private hospitals.<sup>18</sup> In the absence of a dedicated forensic specialist, the victims suffered long delays before getting treatment because their injuries are considered less serious as compared to victims of any other type of trauma.<sup>10</sup> Pediatric cases are another issue of concern and the lack of protocol for treating pediatric sexual assault victims is the likely reason for this disparity.<sup>19</sup>

## How Forensic Nurses Make a Difference?

In India, male doctors are more frequently available to conduct forensic exams than female doctors, though victims generally prefer female examiners. The Ministry of Health and Family Welfare (2014) also recommends female doctors for sexual assault examinations.<sup>9</sup> While India has a higher proportion of female nurses than female doctors, these nurses, who are well-integrated and positively viewed by patients, often lack formal forensic training.<sup>20</sup> Despite this, nurses could play a crucial role in the examination and evidence collection for sexual assault victims, given their position as the first point of care. However, without proper training, nurses may unintentionally miss critical evidence that can impact overall prosecutions.<sup>21</sup>

## Sexual Assault Response Team (SART)

Sexual Assault Response Teams (SARTs) are community-based programs aimed at providing immediate care to victims and improving collaboration among professionals who respond to sexual assault cases. SARTs focus on enhancing both the community's support for victims and the management of sexual assault cases within the criminal justice system.<sup>22</sup> The composition of SART members varies depending on local guidelines, needs, and the availability of experts. SART team members may include a Sexual Assault Nurse Examiner (SANE), a doctor, a police officer, a sexual assault counselor, and an advocate. Because the team represents both law enforcement and healthcare, victims only need to recount their story once. It is considered appropriate for law enforcement, media, or other individuals to be present during the forensic examination.<sup>23</sup>

## SANE Functions

SANE programs aim to deliver services to sexual assault victims around the clock. SANE acts as a liaison between the patient and various law enforcement agencies.<sup>1</sup> SANE performs prompt sexual assault examinations along with the collection and recording of vital evidence. They provide

STD prophylaxis, examine for STDs, and provide emergency contraception to the victim's and when indicated.<sup>10</sup>

No country is spared from raising concerns of sexual assault cases. The US Department of Justice published a report in 2019 stating that a sexual assault occurs every 73 seconds in the US and every 9 minutes for pediatric victims. The estimated lifetime rate of sexual assault for women was reported to be 17 to 18% and for men, it was 3%.<sup>24</sup> Initial evidence from the studies of the SANE Program demonstrates improved outcomes in the psychological recovery of the victim, their treatment, the process of evidence collection, and prosecution of the perpetrator.<sup>25</sup>

## Prerequisites for SANE to initiate Sexual Assault Examination

### Safe Environment Setting with All Resources

All articles of the Sexual Assault Forensic Examination (SAFE) kit should be made available. The examination should be started only after all the supplies and resources have been procured, to prevent the omission of any vital evidence and last-minute confusion. This can be facilitated by preparing a list of requirements with the checklist.

- SAFE kits are available to be procured from several government portals. Several items enlisted in the SAFE kit are readily available in hospital supplies including syringes, and gloves. Hospitals are allowed to add items to the SAFE kit as per their local requirement.
- Items like syringes, pricklers, nail clippers, nail files, and cotton swabs should not be reused and then discarded properly as reuse may lead to contamination of samples and cross-infection.

The examination room should be safe, private, and comfortable.<sup>8</sup> Complete confidentiality must be maintained. In the case of children, the examination room should be child-friendly, by incorporating toys, art, and play material.<sup>19</sup>

## History Taking

The examiner should conduct the history collection procedure with a fundamental approach 'to believe the sexual assault victim'. Professionals like lawyers or police may not be required to believe the victim. Despite that, the healthcare provider should believe when the victim says that the sexual assault has occurred. Furthermore, phrases like 'alleged sexual assault' indicate disbelief, which can hamper the history collection process by closing off the already traumatized patient. Conveying the message 'we believe you' helps the victim to relax and trust the examiner<sup>16</sup>

## Victim's Reaction

The victim's reaction can range from crying and sitting in the corner of the room to showing anger.<sup>21</sup> Repeatedly explaining the event multiple times to law enforcement agencies can render the victim exhausted. Victims may have extreme fear due to the horrifying incident and may have a fear of the perpetrator. Victims can be hyper-vigilant and even be under the influence of the substance. The examiner's expectation and the victim's reaction can be strikingly contrasting.<sup>26</sup>

## Consent

It is important to get consent for examination from victims who are over 18 years of age. A valid informed written consent should be taken from the victim or legally accepted guardian in a prescribed manner before any medical examination.

- The consent should be read/ written in a language understandable to the victim and/ or the guardian, and the treatment should be clearly explained. All queries should be clarified in advance.
- Victims aged 18 years and above are entitled to sign consent for SAFE and further management.

## The Process of history-taking

A thorough and precise medical history is essential for the legal examination of a sexual assault victim.

A comprehensive history-taking procedure helps gather relevant information to guide the treatment plan and the forensic evidence collection.<sup>27</sup> There is a consensus advising that the history should contain detailed accounts of previous anogenital surgery, menstrual and gynecological, history of any drug-facilitated sexual assault, and the details of the incident.<sup>30</sup> All this information should be well documented.

The interview should be conducted with a highly sensitive and empathetic approach. Questions should be asked from least invasive to the most invasive. Re-telling of events causes re-traumatization to the victim.<sup>28</sup> The language consistent with the experience should be used. For example, instead of asking 'Did you perform sex?' Ask 'did he force you for sexual contact.' The answers provided by the victim will guide the evidence collection. The narrative of the events of the assault should include the date, time, locations, and the description of the assault in the victims' own words using quotations if any slang term is used to describe the events or the body parts, it should be clarified by the examiner in detail.<sup>29</sup>

## Sexual Assault Forensic Examination (SAFE)

The SANE has three major forensic responsibilities during each examination. The initial responsibility is to furnish evidence confirming whether the sexual activity occurred or not. This is achieved through the collection of samples, selection of appropriate forensic tests, and running these tests within a relevant time frame.<sup>16</sup> The second task is the gathering of the evidence that may lead to the identification of the culprit. The final task centers on meticulously documenting the observed injuries and interpreting them for the court. This is accomplished through the expert certificate, a professional statement, and/or expert witness testimony.

Each jurisdiction has a specific sexual assault evidence kit with directions for the collection of specimens as requested by the crime lab.<sup>29</sup> The examiner must adhere to the lab specifications to ensure proper evidence collection, preservation, packaging, and transportation. Many forensic labs request smears on microscopic slides from suspicious

sites that may include oral, cervical, vaginal, anal, rectal, penile, and the areas of positive wood lamp illumination.

- All the objective and subjective findings reported by the victim as well as witnessed by the examiner and the hospital staff should be documented.
- The documented signs and symptoms include vital signs, pupillary dilation, accommodation, reaction with light, presence of vertical or horizontal nystagmus, nausea, vomiting.

### **SAFE Procedure**

The quality of forensic reports entirely depends on the quality of samples provided by examining officers for testing. The aim of the SAFE examination should be proper visualization of injuries, genital examination, and documentation of exact findings in scientific language.

#### **The Following Steps Need to be Followed for Safe**

- Ensure that the room is comfortable and well-lit.
- The examiner should mention two identification marks to ensure the identity of the victim during prosecution. Identity markers can include moles, and scars on visible areas of the body, such as the face, upper limbs, and lower limbs.
- The victim is required to stand on a white paper sheet and undress to collect any falling debris as evidence.
- Innerwear and clothes should be separately preserved. A clean pair of new clothing is provided to the victim.
- Preferably the examination is done in the standing position.
- The knee and elbow position are used to examine the anal region and the lithotomy position is used to assess the vaginal orifice.

### **Sexual Assault Evidence Collection**

SANE performs SAFE and obtains evidence samples and records history with the help of government-prescribed proforma for SAFE. The majority of the time SAFE is performed in the designated examination room in the emergency area by the SANE.<sup>18</sup>

Another forensic nurse can aid in the evidence collection process as per the need of the case. Evidence may include swabs from bite marks, nail clippings and scrapings, oral and vaginal swabs and their smear swabs, hair samples from the scalp, pubic area, innerwear, blood, and semen samples.<sup>20</sup> During and immediately following a SAFE exam, the evidence must be safely packed and sealed by the examiner who maintains the chain of custody.<sup>25</sup> The examiner remains with the evidence at all times until it is secured in a designated locked area or retrieved by the law enforcement officer.

Available research evidence confirms the importance of having trained nurses in forensic specialty over nonspecialized manpower.

### **Preservation of Trace Evidence**

If the crime is reported within 72 hours all the samples need to be preserved before the victim takes a bath.<sup>35</sup> Spermatozoa can be detected within 72 hours from the vagina. Vaginal discharge can suggest a sexually transmitted infection as a result of the sexual assault which can give a clue for the duration of the incident.<sup>5</sup>

### **Packing and Sealing of Samples**

The examiner must ensure the proper sealing and packaging of evidence samples.<sup>29</sup> The label should include details of the case number, date, time of sample collection, signature, and stamp of the concerned person.

### **The Protocol for Evidence Collection**

- Need to complete consent and documentation formalities before starting evidence collection.

- A detailed history should be recorded as per SAFE proforma
- Control swabs, blood, semen, and saliva samples for DNA examinations. If the crime has a history of oral penetration within 24 hours, an oral swab/smear sample is to be collected
- Samples of fingernails should be collected in case the patient has scratched the perpetrator.
- Debris material samples should be collected during physical examination, wreckage particles that fall during undressing on a white sheet, or those from the ambulance.
- While preserving victims' clothes, attention should be given to preserving holes in clothes, stains of blood, and semen that can be crucial to establish sexual assault.
- A thorough full body examination should be carried out, for micro laceration wounds, secretions, and lesions. Photography of bite marks and injuries must be done as per protocol.
- Lithotomy position should be used to examine female victims.
- Swab samples from the vaginal and anal area. In the case of male victim's swab samples from penile, urethral, and anal injuries.
- Investigations to be conducted for Urine sample for pregnancy, HIV, HBsAg, and VDRL to find the possibility of serology transfer. CBC, LFT, Routine RBS

### **Court Summons to The Witnesses**

Honorable courts issue summons to the medical professionals who have conducted the sexual assault examination and prepared the medico-legal reports to give their testimonies in court. They are required to attend court summons to support the prosecution and victims' side.

### **Safety Assessment of the Victim**

The Sexual Assault Victim Protection Program was introduced by the Ministry of Health and Family

Welfare in 2014, where the victims are provided temporary shelter inside a hospital or protection home to ensure safety.<sup>30</sup> If the victim wants to go home a safety plan should be provided to him/her, keeping in mind the minors' protection policy and other specific precautions. In case the perpetrator is the legal guardian or the parent, it is compulsory to involve social workers child counselors, and different government agencies working for children's rights to secure the safety of the child.<sup>31</sup>

The police officers should not be allowed to stay in the room during the forensic examination and evidence collection. However, if the victim requests it, their family may be allowed to stay.

### **Ensuring Confidentiality of Records**

Confidentiality is the minimum requirement to protect a victim in legal battles, and expecting privacy is the right of every client visiting a hospital. Sexual assault examiners should take all necessary precautions to safeguard the identity of sexual assault victims. According to government guidelines, the identity of a sexual assault victim cannot be disclosed.<sup>9</sup> Maintaining confidentiality will instill confidence in the victim, foster her/his trust in the system, and influence the decision-making regarding pursuing action against the accused. Javorka and Campbell mentioned in their paper in 2019, that any information regarding the victim's health should not be discussed with staff members who are not part of the examination team. The victim's medical file should not contain any personal notes or pictures of the victim to avoid any chance of a privacy breach. Digital records should be encrypted and secured.<sup>32</sup>

### **Follow-up Care**

Foremost, the physical ailments of the sexual assault victim are to be treated. Once the victim is medically stable, a plan of proper monitoring and follow-up is formulated. During the initial encounter in the emergency department, the major focus is on pain control, STD prophylaxis, adequate nutrition, and improvement of sleep.<sup>30</sup> Follow-up psychiatric counseling can help the victim to cope with the psychological, and behavioral issues especially since

the immediate post-assault period may experience a condition known as Sexual Assault Trauma Syndrome that can present among about 16% of sexual assault victims in the immediate post-assault period.<sup>33</sup>

### **Post-traumatic Disorder in Sexual Assault Victims**

The impact of sexual assault on a victim's mental health can be profound, and PTSD is one of the most severe outcomes. Victims may relive the sexual assault through distressing memories, flashbacks, or nightmares. These intrusive thoughts can be triggered by reminders of the event or can occur unexpectedly.

#### **The Acute Phase**

The acute phase of this condition, also known as the disorganized phase manifests as generalized physical pain, mood disorders, eating, and sleep disturbances. A sense of disorganization looms over the victim's life with fear and self-blame as predominating thoughts.

#### **The Delayed Phase**

The delayed phase of the condition manifests as nightmares, flashbacks, and phobias, consistent with PTSD. The victim may experience sexual dysfunction which may render intimacy challenging. In the long run, the victim's overall quality of life may be diminished due to problems like depression and chronic pelvic pain.<sup>34</sup> To combat this issue, it should be ensured that the victim regularly follows up with a psychologist.

#### **Follow up Treatment**

The victim should be called for follow-up after two weeks of initial contact to check for sexually transmitted infections. At a gap of 6 weeks, an appointment for follow-up of HIV, Hepatitis C, and repeat screening for pregnancy. By the end of the 12th week and 24th weeks, follow up for serology investigations. In case the victim was pregnant at the time of sexual assault, gynecology consultation should be conducted for fetus viability and pregnancy outcome.<sup>35</sup> The victim should be offered

legal counseling again, even if they denied it initially. Several victims do not initiate legal proceedings still, there should be a well-documented account of the assault which can aid in a strong prosecution if required.

### **Discussion**

The rising crime rates against women highlight the urgent need to strengthen forensic capabilities in India for timely and effective justice. With witnesses often turning hostile, the criminal justice system increasingly depends on forensic evidence. Recovery from this unfortunate experience can be very complex and lengthy as the victim must not only recover from the physical ordeal but also the emotional and psychological trauma. Sexual assault nurse examiners play a very important role in ensuring that the victim has a positive experience with the medical-legal system during the entire process.

### **Conclusion**

Forensic nursing is a growing specialty with the potential to revitalize the handling of medico-legal cases providing care to victims, supporting families, and offering expert court testimony. Further, victims including women, neglected elders, and children may feel less intimidated and more at ease in explaining the incident and getting examined by the female forensic-trained nurses as compared to their male counterparts. It can be concluded that Forensic nurses and designated Sexual Assault Nurse Examiners (SANE) can help in the service of justice for the victims by supporting law enforcement agencies in their tasks. Most importantly, SANE can prove to be a boon for assault victims, ensuring expert forensic services amalgamated with the compassion and sensitivity that is the hallmark of the nursing profession.

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# From Trial by Ordeal to Trial by Science: A Forensic Commentary on Bharatiya Nyaya Pranali

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## Abstract

This forensic commentary examines the evolution of forensic science in the Bharatiya Nyaya Pranali, tracing the transition from traditional trial by ordeal to modern evidence-based scientific investigation. It critically analysis the contributions of the Indian Knowledge System (IKS), colonial forensic reforms, and contemporary legal frameworks to the development of forensic practices in India. A qualitative doctrinal research design was adopted using secondary sources, including ancient legal texts, historical records, statutory provisions, government reports, judicial decisions, and peer-reviewed literature. The analysis demonstrates that early Indian jurisprudence incorporated principles of observation, logical reasoning, and medico-legal practices that contributed to the foundations of forensic thought. British colonial administration subsequently institutionalized forensic science through fingerprint identification, medico-legal procedures, and forensic laboratories, establishing scientific evidence within criminal investigations. The study further examines the expanding role of forensic technologies under the Bharatiya Nyaya Sanhita, Bharatiya Nagarik Suraksha Sanhita, and Bharatiya Sakshya Adhiniyam, highlighting advancements in DNA profiling, digital forensics, artificial intelligence, and next-generation sequencing. The study concludes that integrating scientifically validated traditional knowledge with contemporary forensic innovations, supported by adequate infrastructure, skilled professionals, and ethical governance, can enhance the reliability, transparency, and effectiveness of India's criminal justice system while preserving its indigenous intellectual heritage.

**Key words:** Bharatiya Nyaya Pranali, Indian Knowledge System (IKS), Bharatiya Sakshya Adhiniyam (BSA), Bharatiya Nagarik Suraksha Sanhita (BNSS), Digital Forensics.

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## Introduction

The role of forensic science in the Bharatiya Nyaya Pranali (Indian legal system) reflects a profound transformation spanning ancient to modern times. In ancient India, legal procedures were grounded largely in trials by ordeal and traditional knowledge systems, drawing on texts such as Manu-smriti and Dharma-shastra. These early judicial mechanisms prioritized oral testimonies, communal arbitration, and divinely guided ordeals, where physical endurance or supernatural judgment served as evidence of guilt or innocence. Scientific inquiry was nascent, and forensic methods, as presently understood, were largely absent in these proceedings.<sup>1-5</sup>

During the medieval period, Indian legal systems witnessed growing formalization influenced by Islamic jurisprudence and Mughal administration, alongside classical Hindu law. This era marked a more structured approach to justice, incorporating witness testimonies, oaths, and the examination of administrative records such as pargana documents. While there was movement toward methodical inquiry, forensic science remained rudimentary, relying on traditional and observational techniques rather than laboratory-based or scientific evidence.<sup>3-5</sup>

The advent of British colonial rule introduced modern forensic practices into the Indian judicial framework. This period witnessed foundational developments such as fingerprint identification systems, medico-legal investigations, and scientific policing, bridging the gap between traditional judicial practices and empirical investigation. The pioneering contributions of William James Herschel, Edward Henry, and later Indian scholars Azizul Haque and Hem Chandra Bose established the scientific basis of fingerprint identification and institutionalized forensic science within criminal investigations.<sup>6-10,17</sup> Post-independence, India accelerated the integration of advanced forensic disciplines, including DNA analysis, forensic toxicology, ballistics, and questioned document examination. Central agencies like the Central Bureau of Investigation and specialized forensic laboratories became instrumental in

aligning legal processes to internationally recognized scientific standards.<sup>13-16</sup>

In contemporary Bharatiya Nyaya Pranali, forensic science is characterized by sophisticated technologies such as PCR-based DNA profiling, Massively Parallel Sequencing, digital and cyber forensics, and emerging Artificial Intelligence (AI) applications in image and audio analysis. Government initiatives, including the National Forensic Science University and policy support from bodies like the Science Technology and Innovation (STI) sector and the National Crime Records Bureau, have bolstered infrastructural capacity and standardization. Yet challenges persist—limitations in resources, uneven protocol standardization, Infrastructural inadequacies, and ethical dilemmas regarding data privacy and forensic errors remain critical issues to address.<sup>10-16,18</sup>

Recent technological advancements promise transformative improvements in forensic investigations. Artificial intelligence facilitates rapid analysis of complex forensic datasets, while next-generation sequencing, advanced DNA technologies, and machine-learning-based digital forensic tools have enhanced the identification, examination, and interpretation of biological and electronic evidence. Contemporary forensic research further emphasizes international cooperation, standardized reporting practices, and statistically robust interpretation of forensic evidence to improve scientific reliability and judicial confidence.<sup>13-1,19</sup>

Critically, while forensic science has fundamentally modernized the Indian legal system, the full realization of its potential requires confronting systemic challenges. These include resource constraints, the need for standardized quality assurance, bridging the divide between forensic capabilities and legal understanding, and safeguarding ethical conduct in forensic practice. Strengthening institutional frameworks, promoting continuous professional development, and fostering international collaboration stand as vital recommendations to enhance forensic contributions within Bharatiya Nyaya Pranali.<sup>10-19</sup>

In sum, the evolution from trials by ordeal to a science-based legal system illustrates Indian jurisprudence's gradual but decisive embrace of forensic science. This progress manifests not only in technological sophistication but also in the commitment to objective, empirically grounded evidence to uphold justice. Continued modernization, ethical vigilance, and integration with global forensic standards are imperative for forensic science to sustain its crucial role in fair and effective legal adjudication in India.

### Objectives of The Research

1. To examine the contribution of ancient Indian jurisprudence, ethical philosophy, and empirical investigative practices to the development of early forensic thought.
2. To analyze the role of colonial forensic reforms in institutionalizing scientific investigation and their continuing relevance in the contemporary Bharatiya Nyaya Pranali.
3. To evaluate the integration of modern forensic technologies and scientific evidence under the Bharatiya Nyaya Pranali in strengthening the Indian criminal justice system.

### Review of Literature

1. **To examine the contribution of ancient Indian jurisprudence, ethical philosophy, and empirical investigative practices to the development of early forensic thought.**

**Kautilya's Arthashastra** provides one of the earliest systematic accounts of criminal investigation and governance in ancient India. Kautilya emphasized the importance of observation, interrogation, corroboration of evidence, witness examination, document verification, and medico-legal inquiry in the administration of justice. Rather than relying solely on divine intervention, the text advocates rational investigation and verification of facts before imposing punishment. Scholars have interpreted these principles as reflecting an early form of forensic reasoning based on empirical observation and

logical analysis. Although the methods differed from modern forensic science, the Arthashastra established foundational concepts of evidence assessment, crime detection, and judicial accountability that continue to hold historical significance in understanding the evolution of forensic science in India.<sup>1-2</sup>

**Manu-smriti and other Dharma-shastra** texts significantly influenced the ethical and legal foundations of ancient Indian jurisprudence. These texts emphasize truthfulness, judicial impartiality, witness credibility, and procedural fairness while acknowledging ordeals (*divya*) as exceptional methods of resolving disputes when conventional evidence was unavailable. Modern scholars argue that these legal traditions combined moral philosophy with practical judicial procedures, thereby shaping early concepts of evidence evaluation and legal accountability. Although trial by ordeal does not conform to contemporary scientific standards, the broader jurisprudential framework promoted systematic inquiry, ethical governance, and reasoned decision-making, representing an important precursor to the later development of evidence-based criminal justice and forensic practices in India.<sup>3-4</sup>

2. **To analyze the role of colonial forensic reforms in institutionalizing scientific investigation and their continuing relevance in the contemporary Bharatiya Nyaya Pranali.**

**Sir William James Herschel** is widely recognized for introducing the systematic use of fingerprints for personal identification while serving as a magistrate in Bengal during the nineteenth century. His pioneering work demonstrated the permanence and individuality of fingerprints, providing an effective means of preventing impersonation and fraud in official transactions. This innovation was subsequently refined through the scientific studies of Francis Galton and institutionalized by Edward Henry, forming the basis of modern fingerprint identification. The adoption of fingerprinting by the colonial administration marked a significant transition from subjective methods of criminal identification to standardized scientific techniques, laying the foundation for forensic identification

practices that continue to influence contemporary criminal investigations in India.<sup>17,6</sup>

Sir **Edward Richard Henry** played a pivotal role in institutionalizing forensic science within British India through the introduction of the Henry Classification System and the establishment of the world's first Fingerprint Bureau in Calcutta in 1897. These reforms standardized criminal identification, replaced less reliable anthropometric methods, and strengthened the evidentiary value of fingerprints in judicial proceedings. Sir further acknowledge the substantial contributions of Indian officers Khan Bahadur Azizul Haque and Rai Bahadur Hem Chandra Bose in developing the mathematical framework underlying the classification system. The colonial forensic infrastructure established during this period became the cornerstone of modern forensic practice in India and continues to influence biometric identification and criminal investigation under the Bharatiya Nyaya Pranali.<sup>6,9</sup>

### **3. To evaluate the integration of modern forensic technologies and scientific evidence under the Bharatiya Nyaya Pranali in strengthening the Indian criminal justice system.**

Recent scholarship on India's new criminal laws highlights a paradigm shift from witness-centred investigations to scientifically driven criminal investigations. The Bharatiya Nagarik Suraksha Sanhita (BNSS), 2023 mandates the involvement of forensic experts in the investigation of offences punishable with imprisonment of seven years or more, while the Bharatiya Sakshya Adhiniyam (BSA), 2023 strengthens the admissibility and integrity of electronic evidence. These reforms institutionalize forensic science as an essential component of criminal investigation by emphasizing crime scene management, DNA analysis, digital evidence, videography, and expert testimony. **Scholars argue** that these statutory changes improve evidentiary reliability, reduce investigative errors, and promote transparency and efficiency in criminal justice, although their effectiveness depends upon adequate forensic infrastructure, trained personnel, and uniform implementation across states.<sup>10-18</sup>

Advances in forensic science have significantly enhanced the accuracy and reliability of criminal investigations through technologies such as DNA profiling, artificial intelligence, digital forensics, and next-generation sequencing (NGS). DNA analysis remains the benchmark for human identification, while AI-assisted image analysis, pattern recognition, and digital forensic tools facilitate the examination of electronic devices and cyber evidence. Systematic reviews further demonstrate that machine learning has improved the efficiency of digital evidence analysis, particularly in image forensics and large-scale data processing. **Contemporary researchers** conclude that the successful integration of these technologies within the Bharatiya Nyaya Pranali can strengthen evidence-based justice, provided that ethical safeguards, standardized protocols, and continuous capacity building are maintained.<sup>18-19</sup>

### **Research Gap**

Although several scholars have examined the historical development of forensic science, Indian criminal jurisprudence, and the implementation of the Bharatiya Nyaya Sanhita (BNS), Bharatiya Nagarik Suraksha Sanhita (BNSS), and Bharatiya Sakshya Adhiniyam (BSA), existing studies generally address these themes independently. Research on ancient Indian jurisprudence primarily focuses on legal philosophy and historical judicial practices, while contemporary studies emphasize forensic technologies, digital evidence, and legislative reforms. Limited scholarly attention has been given to integrating these historical and modern perspectives within a unified forensic framework.

Furthermore, few studies critically examine the transition from traditional trial by ordeal to evidence-based scientific investigation by linking indigenous investigative practices, colonial forensic institutionalization, and contemporary forensic innovations under the Bharatiya Nyaya Pranali. This gap highlights the need for a comprehensive forensic commentary that evaluates the continuity and evolution of forensic science while exploring how scientifically validated traditional knowledge can complement modern forensic technologies to strengthen India's criminal justice system.

## Research Methodology

### Aim of the study

To critically examine the evolution of forensic science in the Bharatiya Nyaya Pranali from traditional trial by ordeal to modern scientific investigation. The study also aims to evaluate the contributions of ancient Indian jurisprudence, colonial forensic reforms, and contemporary forensic technologies to the Indian criminal justice system.

### Research Design

The present study adopted a **qualitative descriptive doctrinal research design** to critically examine the evolution of forensic science in the Bharatiya Nyaya Pranali (BNP), tracing its development from traditional trial by ordeal to modern scientific investigation. The design was selected because it facilitates the systematic analysis of legal developments, historical evolution, forensic practices, and scholarly perspectives through documentary sources.

### Research Approach

The study employed a **qualitative research approach**. This approach enabled an in-depth examination of legal principles, historical developments, forensic science literature, and contemporary legislative reforms relating to the Bharatiya Nyaya Pranali.

### Nature of the Study

The study is a **forensic commentary** based on doctrinal legal research and historical analysis. It critically evaluates the contribution of ancient Indian jurisprudence, colonial forensic reforms, and modern scientific advancements to the evolution of forensic science within India's criminal justice system.

### Sources of Data

#### Secondary Data

The study is based entirely on secondary sources of information. Data were collected from:

- Ancient Indian legal texts such as the *Arthashastra*, *Manusmriti*, and other *Dharmaśāstras*.
- The Bharatiya Nyaya Sanhita, 2023 (BNS).
- The Bharatiya Nagarik Suraksha Sanhita, 2023 (BNSS).
- The Bharatiya Sakshya Adhiniyam, 2023 (BSA).
- Judicial decisions relating to forensic evidence.
- Government reports and publications.
- Books on forensic science, criminal law, and legal history.
- Peer-reviewed national and international journal articles.
- Reports published by forensic institutions and governmental agencies.

### Method of Data Collection

Data were collected through an extensive review of published literature, statutory provisions, judicial pronouncements, government documents, historical records, and scholarly publications. Relevant information was systematically identified, classified, and organized according to the objectives of the study.

### Inclusion Criteria

The study included:

- Peer-reviewed journal articles related to forensic science and criminal justice.
- Ancient Indian legal and philosophical texts relevant to judicial practices.
- Books and scholarly publications on forensic science and legal history.
- Indian statutes relating to the Bharatiya Nyaya Pranali.
- Published literature discussing modern forensic technologies, including DNA profiling, digital forensics, artificial intelligence, and next-generation sequencing.

## Exclusion Criteria

The study excluded:

- Unauthenticated online sources and non-scholarly publications.
- Newspaper articles lacking academic relevance.
- Opinion pieces without supporting legal or scientific evidence.
- Duplicate publications.
- Sources unrelated to forensic science, criminal investigation, or the Bharatiya Nyaya Pranalī.

## Research Tool

The principal research tool comprised **document analysis** and **content analysis**. Statutory provisions, historical documents, judicial decisions, forensic literature, and scholarly publications were critically examined to identify major developments in forensic science across different historical periods.

## Data Analysis

The collected information was analysed using **doctrinal legal analysis**, **historical analysis**, and **thematic content analysis**. The findings were interpreted under three major themes:

- Ancient Indian jurisprudence and early forensic thought.
- Colonial institutionalization of forensic science.
- Modern forensic technologies and their integration within the Bharatiya Nyaya Pranalī.

## Ethical Considerations

As the study is based exclusively on secondary sources, no human participants were involved and ethical approval was not required. Appropriate academic standards were maintained by accurately citing all primary and secondary sources, ensuring originality, avoiding plagiarism, and presenting information objectively.

## Results and Discussion

### 1. To examine the contribution of ancient Indian jurisprudence, ethical philosophy, and empirical investigative practices to the development of early forensic thought.

The analysis demonstrates that ancient Indian jurisprudence incorporated several principles that closely resemble the conceptual foundations of modern forensic investigation. Rather than relying exclusively on supernatural beliefs, legal texts such as the Arthashastra advocated systematic observation, verification of facts, witness examination, interrogation, document scrutiny, and corroboration of evidence before the administration of justice.<sup>1,2</sup> These principles indicate that rational inquiry formed an important component of judicial decision-making in ancient India. Similarly, the Manusmṛiti and other Dharmaśāstras emphasized ethical governance, judicial impartiality, truthfulness, and the credibility of witnesses.<sup>3,4</sup> Although trial by ordeal (*divya*) was recognised within these legal traditions, it was generally prescribed only when material evidence or reliable testimony was unavailable, suggesting that it functioned as an exceptional judicial mechanism rather than the primary method of determining guilt.<sup>3</sup> This finding challenges the common perception that ancient Indian justice relied predominantly on supernatural practices. The findings further indicate that early Indian legal philosophy integrated moral principles with empirical observation, thereby establishing a judicial framework that valued fairness, accountability, and logical reasoning. While these practices cannot be equated with contemporary laboratory-based forensic science, they represent the intellectual origins of evidence evaluation and investigative reasoning that later evolved into scientific forensic methodologies.<sup>2,5</sup>

From a forensic science perspective, these findings demonstrate that the transition from trial by ordeal to scientific investigation was not an abrupt historical shift but rather a gradual evolution in which indigenous legal traditions contributed foundational investigative concepts. The emphasis on observation, verification, and procedural fairness remains

consistent with modern forensic principles that prioritize objective evidence over subjective belief. Consequently, the study supports the view that scientifically valid aspects of the Indian Knowledge System should be recognised as part of the historical evolution of forensic science and may continue to inform ethical and evidence-based criminal justice practices within the Bharatiya Nyaya Pranali.

## **2. To analyze the role of colonial forensic reforms in institutionalizing scientific investigation and their continuing relevance in the contemporary Bharatiya Nyaya Pranali.**

The findings indicate that British colonial rule marked a significant turning point in the evolution of forensic science in India by transforming criminal investigations from predominantly subjective practices to standardized scientific procedures. The introduction of fingerprint identification, the Henry Classification System, medico-legal services, and organized forensic institutions established a structured framework for criminal identification and evidence examination.<sup>6-9</sup> These reforms enhanced the reliability, consistency, and admissibility of scientific evidence within judicial proceedings and laid the institutional foundation for modern forensic practice. The analysis also highlights that the development of fingerprint science was not solely a colonial achievement, as the pioneering contributions of Indian scholars such as Azizul Haque and Hem Chandra Bose played a crucial role in refining the classification system, demonstrating India's significant yet often under-recognized contribution to forensic science.<sup>9</sup>

The continued relevance of these colonial forensic reforms is evident in the contemporary Bharatiya Nyaya Pranali, where fingerprint identification, medico-legal investigations, expert testimony, and scientific documentation remain integral components of criminal investigation. Rather than replacing colonial forensic principles, the new criminal laws have strengthened and modernized them by integrating advanced technologies such as DNA profiling, digital forensics, and artificial intelligence into the existing forensic framework. This demonstrates that India's forensic system has evolved through continuity rather than discontinuity,

combining historically validated investigative methods with modern scientific innovations to improve the accuracy, transparency, and credibility of the criminal justice system.<sup>7-10</sup>

## **3. To evaluate the integration of modern forensic technologies and scientific evidence under the Bharatiya Nyaya Pranali in strengthening the Indian criminal justice system.**

The findings demonstrate that the Bharatiya Nyaya Pranali has significantly strengthened the role of forensic science by promoting scientific evidence as a central component of criminal investigation and adjudication. The enactment of the Bharatiya Nagarik Suraksha Sanhita (BNSS), 2023 and the Bharatiya Sakshya Adhinyam (BSA), 2023 reflects a legislative shift towards evidence-based justice by emphasizing mandatory forensic investigation in serious offences, recognizing the admissibility of electronic and digital evidence, and reinforcing the importance of expert opinion in judicial proceedings.<sup>11,12</sup> Furthermore, advances in DNA profiling, digital forensics, artificial intelligence, biometric identification, and next-generation sequencing have substantially enhanced the accuracy, efficiency, and reliability of criminal investigations by facilitating crime scene reconstruction, human identification, cybercrime investigation, and scientific analysis of complex physical and digital evidence.<sup>13-15</sup> These developments collectively indicate that contemporary forensic technologies have become indispensable for ensuring objective and scientifically validated criminal investigations.

However, the findings also suggest that legislative reforms alone cannot ensure effective implementation of forensic science within the criminal justice system. Persistent challenges, including inadequate forensic infrastructure, shortage of trained forensic experts, delays in laboratory examination, uneven technological accessibility, and the absence of uniform quality assurance protocols, continue to affect the timely delivery of justice.<sup>16</sup> Therefore, the successful realization of the objectives of the Bharatiya Nyaya Pranali depends on sustained investment in forensic laboratories, continuous professional training, adoption of internationally accepted quality

standards, interdisciplinary collaboration, and ethical governance of emerging technologies such as artificial intelligence. Strengthening these institutional and technological capacities will not only improve the credibility and transparency of

forensic evidence but also facilitate India's continued transition towards a modern criminal justice system founded on scientific accuracy, procedural fairness, and evidence-based decision-making.<sup>13,16</sup>

**Table 1. Comparative Overview of Traditional Detection Methods and Their Modern Forensic Technological Applications.**

### Use of Electrochemical Sensors Inspired by Traditional Detection

| Traditional Method                                   | Modern Integration Technology                                                   | Benefits and Potential                                                   |
|------------------------------------------------------|---------------------------------------------------------------------------------|--------------------------------------------------------------------------|
| Neikuri (urine analysis, Siddha medicine)            | AI-powered image processing with deep learning (CNNs) (Srinivasan et al., 2025) | Standardised, objective diagnosis enhances traditional practice accuracy |
| Biological markers in traditional forensic practices | Multi-omics (genomics, proteomics, metabolomics) (Procopio and Bonicelli, 2024) | Molecular-level identification enriching classical methods               |
| Physical micro particle/traditional trace analysis   | AI-assisted microscopy and spectroscopy (Javadova, 2024)                        | Automated, precise identification and matching of evidence               |
| Indigenous detection of substances                   | Electrochemical sensor technology (Mohamed and Mousavi, 2024)                   | Improved sensitivity and specificity in detecting illicit compounds      |

While not traditionally forensic, the broad discipline of electrochemistry offers technologies that can be applied to evidence detection, such as tracing illicit substances or detecting biological materials. These sensor technologies could be tailored and validated using insights from traditional detection methods based on natural indicators or material properties, allowing indigenous wisdom in detecting certain compounds to inform the design of electrochemical forensic sensors.

### Conclusion

The evolution of forensic science in the Bharatiya Nyaya Pranali reflects India's progressive transition from traditional trial by ordeal to a criminal justice system founded on scientific evidence, objectivity, and

technological innovation. The review demonstrates that ancient Indian jurisprudence, particularly the Arthashastra and Dharmaśāstras, incorporated principles of observation, ethical reasoning, witness examination, and procedural fairness that contributed to the foundations of early forensic thought. British colonial reforms subsequently institutionalized forensic science through fingerprint identification, medico-legal services, forensic laboratories, and standardized investigative procedures, establishing the framework for evidence-based criminal investigation. Contemporary legislative reforms under the Bharatiya Nyaya Sanhita, Bharatiya Nagarik Suraksha Sanhita, and Bharatiya Sakshya Adhiniyam further reinforce the central role of forensic science by recognizing digital

evidence, strengthening forensic investigations, and encouraging the adoption of advanced technologies such as DNA profiling, artificial intelligence, digital forensics, and next-generation sequencing. Nevertheless, challenges relating to forensic infrastructure, skilled manpower, standardization, and timely forensic analysis continue to affect effective implementation. The study concludes that the successful realization of the Bharatiya Nyaya Pranalī requires sustained investment in forensic capacity, interdisciplinary collaboration, and ethical governance while preserving scientifically validated principles of India's indigenous legal heritage to ensure a transparent, reliable, and evidence-based criminal justice system.

### Declaration

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# Identification from Salivary Trace Evidence using STR Profiling: Forensic Case-Based Analysis

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## Abstract

Saliva as well as objects with saliva traces is good and valuable sources of DNA for genotyping in forensic cases. DNA profiling of these trace evidence helps in identifying the persons involved in the crime as well the presence of the individual at the crime scene. The present study gives insight into understanding the possibility of obtaining STR (Short Tandem Repeat) profile from the traces of salivary stains from objects which may be collected from crime scene. Saliva trace evidences collected from crime scenes were received at forensic science laboratory for DNA profiling (STR). The present study is a report on forensic cases received for STR profiling from uncommon trace evidences and moreover, there are very few reports on forensic case based analysis. The objects with saliva traces collected as evidences from 3 different forensic cases, sent for analysis were case I: garment (saliva from biting), case II: garment (saliva from gutkha spitting) and gutkha swab from floor collected at crime scene and case III: green leafy vegetable (saliva from spitting). Presence of saliva was confirmed from above evidence materials and enough DNA was recovered from saliva traces by automated extraction method. STR profiles were generated using 0.5ng-1ng of DNA. In Case I and II, 23 autosomal STR markers were analyzed. In case III, 27 Y-chromosomal STR markers were analyzed. The alleles in the STR profile obtained from the saliva samples were identical and matching completely with the alleles at each locus (23 autosomal STR loci/27 Y-STR loci) in the STR profile obtained from reference blood of the suspect thus proving the presence and probable involvement of suspect in crime. The forensic STR profiling analysis plays a major role in all three cases, determining the individual's involvement with the crime scene which is necessary to provide evidence in court.

**Keywords:** Saliva, forensic science, Short Tandem Repeat, STR, Gutkha,

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## Introduction

Biological evidences (DNA materials) from crime scenes are collected routinely and are used as a key element in solving not only serious major crimes such as sexual assault and murder but also property crime (burglary and autocrime)<sup>1</sup>. Saliva stains collected at the crime scenes play an important role as biological evidence in solving the crime. Saliva is a composite fluid (Water along with epithelial cells, enzymes, electrolytes and inorganic components) and the forensic analysis of salivary DNA on evidential material, can provide important information to the forensic scientist<sup>2</sup>. Human saliva is a good source of DNA, as the mean number of epithelial cells/1ml of saliva is about  $4.3 \times 10^5$  with mean quantity of  $48.4 \pm 8.2 \mu\text{g/ml}$  of DNA obtained from saliva<sup>3</sup>. The surface layer of epithelial cells in mouth are replaced on an average every 2.7h and saliva may prove to be useful source of DNA for forensic purpose<sup>3,4</sup>. DNA in saliva primarily originates from epithelial cells from the inner cheek lining as well as white blood cells and therefore saliva could contain enough DNA to be extracted and amplified resulting in a DNA profile<sup>5,6</sup>.

DNA recovered from saliva on objects collected as evidence can be of help in determining the accused as well the victim and their involvement with the crime scene. Forensic studies have demonstrated the possibility and use of saliva on trace evidences collected from crime scene such as cigarette butts, tooth brushes, marks and prints left on stamps, documents, envelope, bite marks, dental appliances, chewing gums and gutkha spittings to name few as source of DNA<sup>3, 7, 8, 9, 10, 11, 12</sup>. If the sample is in trace form, the best way to go is for DNA profiling which gives the exact identity of the perpetrator. Short tandem repeat (STR) analysis is regularly used technique to generate DNA profiles from various biological samples collected as evidence to link the suspect to the crime<sup>9, 13, 14</sup>. The trace evidences can be used as DNA sources for STR profiling, which can play an important role in identifying individuals.

Stains of dried saliva are invisible, making its recognition and collection difficult. The conventional method to locate the saliva stains on evidence surfaces

is by testing for the presence of saliva by doing amylase test and a positive amylase test result thus may also indicate the presence of probable DNA which can be isolated<sup>15, 16</sup>. The presence of saliva can be confirmed by an amylase assay or by immunochromatographic tests<sup>17</sup>. Our forensic laboratory had received different cases pertaining to DNA-STR profiling of salivary stains found on various types of evidence objects. The current objective is to compile the STR profile data obtained from above mentioned different samples containing suspected salivary stains.

The current study reports the possibility of obtaining STR-profiles from salivary trace evidences collected from crime scenes. There are no reported forensic case studies that have demonstrated the possibility and use of saliva on trace evidences such as spitting on leafy vegetables and on garments. Such evidences collected from forensic cases are few to rare and our study proves the possibility of obtaining results from invisible salivary trace evidences. India being one of the largest consumers of gutkha, there has been observed an increasing trend in collection of gutkha stains from crime scenes in Karnataka (Indian State). The current study is the second only report on possibility of obtaining STR-profiles from gutkha stains. Getting a STR profile from biological traces on forensic trace evidences is often a serious challenge in solving forensic cases and it is one of the major limitations. This study will give insight into understanding the possibility of obtaining STR profile from the traces of salivary stains from objects which can be collected from crime scene and adds information to the existing knowledge of choice of crime scene sample collections.

## Materials and Methodology

### Source of DNA (Sample)

#### Case I: Garment (saliva from biting)

Police uniform was sent to forensic science laboratory (FSL) for detection of saliva and to generate STR-profile. Sampling was done by collection of garment cuttings and subjected to detection of possible human saliva presence on the uniform before extraction of DNA.

### Case II: Saliva in gutkha spitting on garment and gutkha swab

One pant with gutkha stain and gutkha swab collected from flour at the crime scene was sent to FSL for detection of saliva and to generate STR-profile. Sampling was done by collection of garment cuttings (pant) and from swab and subjected to detection of possible human saliva presence before extraction of DNA.

### Case III: Green leafy vegetable (saliva from spitting)

Dried leafy vegetable bundle was sent to FSL for detection of saliva and to generate STR-profile. Using nuclease free water, swabs were collected from the vegetable surface and subjected to detection of possible human saliva presence before extraction of DNA.

### Detection of Saliva

Presence of human saliva was detected by using commercially available SERATEC® Amylase Test kit. The saliva swabs/garments with stains were extracted in sufficient amount of buffer and detection was done as per the manufacturer's protocol<sup>18,19</sup>.

### Extraction of DNA

For extraction of DNA from positive saliva samples, the EZ1&2 DNA Investigator Kit (QIAGEN) was used on automated EZ1 advanced XL instrument

as per the machine guidelines and recommended machine protocol<sup>20</sup>. It enables the isolation of DNA with high efficiency yield from trace samples with removal of inhibitors. Kit provides optimized chemistries for automated DNA purification with more efficient yields. After completion of the program, elution tubes containing extracted DNA were stored at 4°C till the next PCR amplification process.

### Quantification of DNA

BioSpectrometer was used to quantify DNA concentration and purity via A260/280 and A260/230 ratios. The blank control was also analyzed using the BioSpectrometer to verify the absence of measurable DNA and assess background contamination. Quantified DNA was taken for downstream application.

### Multiplex PCR Amplification

In case I and II, 0.5ng of DNA was used for amplification by the polymerase chain reaction (PCR) using VeriFiler™ Plus PCR Amplification kit containing primers for 23 autosomal STR loci, Yindel and Amelogenin. PCR was performed as per manufacturer's protocol as shown in figure 1a. In case III, 1ng of DNA was used for amplification by the polymerase chain reaction (PCR) using Yfiler™ Plus Kit containing primers for 27 STR loci. PCR was performed as per manufacturer's protocol as shown in figure 1b. PCR performed without DNA template was used as negative control.

Figure 1.

| Temp °C | Time (Min) | Cycles |
|---------|------------|--------|
| 95.0    | 1.00       |        |
| 96.0    | 0.10       | 2      |
| 62.0    | 1.30       |        |
| 96.0    | 0.10       | 27     |
| 59.0    | 1.30       |        |
| 60.0    | 5.00       |        |
| 4.0     | ∞          |        |

Figure 1a: Cycling conditions for VeriFiler Plus amplification kit

**Figure 2.**

| Temp °C | Time (Min) | Cycles |
|---------|------------|--------|
| 95.0    | 1.00       | 29     |
| 94.0    | 0.04       |        |
| 61.5    | 1.00       |        |
| 60.0    | 22.00      |        |
| 4.0     | ∞          |        |

**Figure 1b: Cycling conditions for Yfiler™ Plus PCR Amplification Kit**

### STR Profiling (Genotyping)

Amplified PCR products were separated on Genetic Analyzer (Applied Biosystems Inc.) SeqStudio 24 Flex and analyzed using GeneMapper™ ID-X Software v1.7 to generate allele profile. In case I and II, the following loci were examined D3S1358, vWA, D16S539, CSF1PO, D6S1043, D8S1179, D21S11, D18S51, D5S818, D2S441, D19S433, FGA, D10S1248, D22S1045, D1S1656, D13S317, D7S820, Penta E, Penta D, TH01, D12S391, D2S1338, TPOX, Yindel and Amelogenin. In case III, the following loci were examined DYS576, DYS389I, DYS635, DYS389II, DYS627, DYS460, DYS458, DYS19, YGATAH4, DYS448, DYS391, DYS456, DYS390, DYS438, DYS392, DYS518, DYS570, DYS437, DYS385, DYS449, DYS393, DYS439, DYS481, DYF387S1 and DYS533. Inclusion and Exclusion criteria: For each locus, if crime scene saliva sample profile alleles are identical and match completely with the reference blood sample allele, then the individual is “included” as donor. If even 1 locus is “exclude”, individual is excluded as donor.

### Results

The enzyme  $\alpha$ -amylase present in high concentration in human saliva makes it a suitable marker in forensic investigation. The presence of saliva stains on trace evidences were confirmed by human  $\alpha$ -amylase based immunochromatographic tests. All the samples which gave positive results

for immunochromatographic tests were subjected for DNA extraction. The extracted DNA from all the positive saliva samples (for case I & II) were typed by using VeriFiler™ Plus PCR Amplification kit containing primers for 23 autosomal STR loci, Yindel and Amelogenin using the PCR amplification technique. For case III, the extracted DNA from the positive saliva samples were typed by using Yfiler™ Plus PCR Amplification kit. The following tables show STR loci of the individual whose saliva was identified on evidence object. Tables shows the DNA profiles with the comparative study between the alleles obtained at every locus of evidence samples along with the alleles obtained from reference blood sample of the suspect (case I-Table 1), (case II-Table 2), (case III-Table 3).

In case I, the DNA profile obtained from saliva on garment (biting) found to be of female origin having XX alleles at Amelogenin locus and matched at every locus with DNA profile obtained from reference blood sample of the female individual as shown in table 1. Since, saliva sample profile alleles at each locus are identical and matching completely with the reference blood sample allele the female individual is “included” as donor of saliva on garment. In case II, the DNA profile obtained from saliva in gutkha spitting on garment collected at crime scene was found to be of male origin having XY alleles at Amelogenin locus and matched at every locus with DNA profile obtained from reference blood sample of the male

suspect A-2 and gutkha swab collected at crime scene was found to be of male origin having XY alleles at Amelogenin locus and matched at every locus with DNA profile obtained from reference blood sample of the male suspect A-3 as shown in table 2. Since, saliva sample profile alleles at each loci are identical and matching completely with the reference blood sample allele the suspect A-2 is "included" as donor of saliva in gutkha spitting and since, saliva sample profile alleles at each loci are identical and matching completely with the reference blood sample allele

the suspect A-3 is "included" as donor of saliva in gutkha swab. In case III, the Y-chromosomal DNA profile obtained from saliva in spitting on a green leafy vegetable was found to be of male origin and matched at every locus with Y-chromosome specific DNA profile obtained from reference blood sample of the male suspect as shown in table 3. Since, saliva sample Y-chromosomal profile allele/s at each loci are identical and matching completely with the reference blood sample allele the male suspect is "included" as possible donor of saliva on green leafy vegetable.

**Table 1. DNA profiles obtained from saliva stain detected on uniform and reference blood of the suspected accused.**

| STR Locus | Genotype                         |                              |
|-----------|----------------------------------|------------------------------|
|           | Saliva stain detected on uniform | Blood<br>(suspected accused) |
| D3S1358   | 15, 16                           | 15, 16                       |
| vWA       | 14, 17                           | 14, 17                       |
| D16S539   | 13, 13                           | 13, 13                       |
| CSF1PO    | 11, 12                           | 11, 12                       |
| D6S1043   | 11, 17                           | 11, 17                       |
| D8S1179   | 12, 15                           | 12, 15                       |
| D21S11    | 28, 31.2                         | 28, 31.2                     |
| D18S51    | 14, 17                           | 14, 17                       |
| D5S818    | 11, 12                           | 11, 12                       |
| D2S441    | 10, 11                           | 10, 11                       |
| D19S433   | 13, 13                           | 13, 13                       |
| FGA       | 23, 23                           | 23, 23                       |
| D10S1248  | 13, 14                           | 13, 14                       |
| D22S1045  | 11, 16                           | 11, 16                       |
| D1S1656   | 11, 15                           | 11, 15                       |
| D13S317   | 9, 10                            | 9, 10                        |
| D7S820    | 8, 8                             | 8, 8                         |
| Penta E   | 5, 12                            | 5, 12                        |

Continue....

|            |        |        |
|------------|--------|--------|
| Penta D    | 10, 10 | 10, 10 |
| TH01       | 9, 9.3 | 9, 9.3 |
| D12S391    | 20, 21 | 20, 21 |
| D2S1338    | 19, 23 | 19, 23 |
| TPOX       | 11, 11 | 11, 11 |
| Yindel     | -      | -      |
| Amelogenin | X, X   | X, X   |

**Table 2. DNA profiles obtained from saliva in gutkha spitting on garment and suspected gutkha stain swab collected from crime scene and reference blood of the suspected accused.**

| STR Locus | Genotype                   |                              |                                              |                              |
|-----------|----------------------------|------------------------------|----------------------------------------------|------------------------------|
|           | Gutkha spitting on garment | Blood (suspected accused A2) | Gutkha stain swab collected from crime scene | Blood (suspected accused A3) |
| D3S1358   | 15, 18                     | 15, 18                       | 15, 18                                       | 15, 18                       |
| vWA       | 17, 19                     | 17, 19                       | 14, 18                                       | 14, 18                       |
| D16S539   | 11, 11                     | 11, 11                       | 11, 13                                       | 11, 13                       |
| CSF1PO    | 11, 12                     | 11, 12                       | 10, 12                                       | 10, 12                       |
| D6S1043   | 11, 12                     | 11, 12                       | 12, 12                                       | 12, 12                       |
| D8S1179   | 14, 16                     | 14, 16                       | 16, 16                                       | 16, 16                       |
| D21S11    | 29, 32.2                   | 29, 32.2                     | 30, 32                                       | 30, 32                       |
| D18S51    | 15, 21                     | 15, 21                       | 12, 16                                       | 12, 16                       |
| D5S818    | 12, 13                     | 12, 13                       | 11, 12                                       | 11, 12                       |
| D2S441    | 10, 10                     | 10, 10                       | 10, 11                                       | 10, 11                       |
| D19S433   | 13, 13                     | 13, 13                       | 13, 13                                       | 13, 13                       |
| FGA       | 23, 25                     | 23, 25                       | 23, 23                                       | 23, 23                       |
| D10S1248  | 15, 16                     | 15, 16                       | 14, 16                                       | 14, 16                       |
| D22S1045  | 14, 16                     | 14, 16                       | 14, 15                                       | 14, 15                       |
| D1S1656   | 8, 15                      | 8, 15                        | 17, 17.3                                     | 17, 17.3                     |
| D13S317   | 8, 12                      | 8, 12                        | 8, 11                                        | 8, 11                        |
| D7S820    | 8, 12                      | 8, 12                        | 9, 11                                        | 9, 11                        |

Continue....

|            |        |        |        |        |
|------------|--------|--------|--------|--------|
| Penta E    | 5, 11  | 5, 11  | 11, 15 | 11, 15 |
| Penta D    | 10, 11 | 10, 11 | 9, 12  | 9, 12  |
| TH01       | 9, 9   | 9, 9   | 7, 9   | 7, 9   |
| D12S391    | 17, 20 | 17, 20 | 18, 18 | 18, 18 |
| D2S1338    | 17, 24 | 17, 24 | 20, 22 | 20, 22 |
| TPOX       | 9, 11  | 9, 11  | 9, 11  | 9, 11  |
| Yindel     | 2      | 2      | 2      | 2      |
| Amelogenin | X, Y   | X, Y   | X, Y   | X, Y   |

**Table 3. DNA profiles obtained from saliva stain detected on suspected spitting on green leafy vegetables and reference blood of the suspected accused.**

| STR Locus | Genotype                                        |                              |
|-----------|-------------------------------------------------|------------------------------|
|           | Saliva stain detected on green leafy vegetables | Blood<br>(suspected accused) |
| DYS576    | 18                                              | 18                           |
| DYS389I   | 13                                              | 13                           |
| DYS635    | 20                                              | 20                           |
| DYS389II  | 29                                              | 29                           |
| DYS627    | 18                                              | 18                           |
| DYS460    | 11                                              | 11                           |
| DYS458    | 16                                              | 16                           |
| DYS19     | 15                                              | 15                           |
| YGATAH4   | 12                                              | 12                           |
| DYS448    | 19                                              | 19                           |
| DYS391    | 10                                              | 10                           |
| DYS456    | 16                                              | 16                           |
| DYS390    | 23                                              | 23                           |
| DYS438    | 9                                               | 9                            |
| DYS392    | 11                                              | 11                           |
| DYS518    | 39                                              | 39                           |
| DYS570    | 17                                              | 17                           |
| DYS437    | 14                                              | 14                           |

Continue....

|          |        |        |
|----------|--------|--------|
| DYS385   | 16     | 16     |
| DYS449   | 31     | 31     |
| DYS393   | 12     | 12     |
| DYS439   | 12     | 12     |
| DYS481   | 21     | 21     |
| DYF387S1 | 38, 40 | 38, 40 |
| DYS533   | 12     | 12     |

### Discussion

The SERATEC® Amylase Test is a chromatographic immunoassay for rapid detection of human  $\alpha$ -amylase to identify saliva in forensic samples. The SERATEC® Amylase Test kit specifies high sensitivity and positive results with dilutions of 1:2000 – 1:5000 of human saliva. Testing for saliva using traditional biological methods can result in loss of sample in testing. In case of chromatographic immunoassay tests, extracted samples can be stored for further analyses for DNA profiling. Extracted sample is compatible with DNA analyses and it is also possible to extract DNA from the sample pad (from chromatographic immunoassay kit) for further analysis<sup>18, 19</sup>.

In case I, the women officers of law enforcement were assigned to arrest few female individuals. Accordingly the women officers arrested all the individuals in spite of resistance from them and their family, but except one female individual. The female individual resisted arrest and denied to get into the vehicle (van). Despite of repeated instructions from the women officers she continued to obstruct the arrest and later the women officers used lawful force to put her inside the vehicle. Inside the vehicle the female individual stripped herself and assaulted by biting a women law enforcement officer. The female individual verbally abused and injured the women law enforcement officer biting onto her abdomen causing bleeding. The female individual also physically assaulted others in the vehicle and obstructed the female officers (public servants)

on duty from performing their lawful duties. The uniform of the women law enforcement officer was sent to forensic laboratory for the detection of presence of saliva, deposited due to biting on the uniform, which caused the injury on abdomen. The area around the injury on the uniform was examined and subjected for saliva detection. The presence of saliva was confirmed by immunochromatographic tests. The samples which confirmed the presence of saliva was subjected to DNA extraction. DNA profiling of the saliva stains proved to be strong evidence against the female individual.

In case II, thieves broke into the complainant's house and stole valuable gold and silver items. During the crime scene investigation, gutkha stains were observed on floor and garment. Crime scene investigating officer collects the suspected gutkha swab and garment with suspected gutkha stain for presence of saliva and DNA profiling. The presence of saliva was confirmed by immunochromatographic tests and the samples which confirmed the presence of saliva was subjected to DNA extraction. Two male DNA profiles were obtained suggesting the presence of two male individuals at the crime scene. Blood of 7 suspected male individuals were sent as reference blood for identification. Out of the 7 accused, two male individual profiles matched with suspect no. A2 and suspect no. A3. In the absence of eye-witness, DNA profiling of the saliva stains proves to be strong evidence against both the suspect which is necessary to provide evidence in court. DNA profiling from gutkha spitting proved the presence of both the suspect A2 and A3 at the crime scene.

In case III, the complainant while buying vegetables observed that the male accused, deliberately spat on the green leafy vegetable (*Amaranthus*). The accused, who is a vegetable vendor, did this in public. On the grounds of unlawful act performed by the accused, the case was registered against him under sections for street offences and public nuisance, specifically regarding hygiene and annoyance in public areas, provocation with the intent to cause a riot, unlawful act to spread a dangerous disease, malignant acts to spread infection of disease dangerous to life and street nuisances and offences section designed to maintain public hygiene and order. The case was registered and the green leafy vegetable (*Amaranthus*) were sent to forensic laboratory for the detection of presence of saliva, deposited due to spitting on them. The green leafy vegetable (*Amaranthus*) was dried plant material with leaves and stem part. A proper swab was collected from the evidence and presence of saliva was confirmed by immunochromatographic test. The samples which confirmed the presence of saliva was subjected to DNA extraction. Y-chromosomal DNA profiling of the saliva stains proved to be strong evidence against the accused. This is one of the important pieces of evidences against the accused to strengthen the case in court. The forensic analysis corroborates the complaint statement with the forensic science laboratory report in the court of law.

The present report has some limitations, there are very few such reports on forensic case based analysis. The present study is a report on forensic cases received at forensic science laboratory for STR profiling from uncommon trace evidences collected from crime scenes. Since there are no similar reports available, the limitation of the study is lack of comparative interpretation of the findings with other studies. Recently, Vaishali et al., (2021) reported a similar forensic report based on two cases received at laboratory at Nashik (India), in which DNA profiling from traces of gutkha was used to solve the crime<sup>9</sup>. DNA evidence material is regularly collected from crime scenes in most of the cases and its timely processing is acknowledged as a key element to its success in solving crime<sup>1</sup>. Unavailability or controlled

use of actual crime scene evidence material for research purpose is also limitation in this area, as most of the published data are derived from simulated/ designed objects for study. The amount of research on saliva as a DNA source seems to be limited, compared to the wealth of data on forensic studies<sup>21</sup>.

## Conclusion

Saliva and saliva-stained material are good and valuable sources of DNA for genotyping in certain forensic cases as it can play a pivotal role in personal identification. The present study clearly indicates the possibility of using saliva/saliva stains from crime scene materials as a source of evidence for person identification. However, it is important to observe that DNA recovery in forensic samples is crucial and thus collected materials from crime scene and its timely processing are key element in solving crime. Our data indicates that the DNA profile of the crime scene samples (saliva/saliva stains) proves the involvement of one or more than one accused in crime and also presence of the suspected accused person in crime scene.

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**Conflicts of Interest:** None to declare

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# The Frequency of Myocardial Infarction in different Age Groups: A Histopathological Study at The Medico-Legal Directorate, Baghdad, Iraq

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## Abstract

**Background:** Myocardial infarction (MI) is one of the primary causes of cardiovascular death globally and represents the most critical form of coronary artery disease (CAD). At the same time that Iraq is lacking comprehensive surveillance data on MI frequency across age groups, there is an escalating burden of cardiovascular disease (CVD) in low and middle-income countries. The present study represents the first histopathology-based assessment of age-specific and gender-specific MI frequency among medico-legal autopsy cases in Baghdad.

**Methods:** In Baghdad, a retrospective study utilized the archived records from the Histopathological Department of the Medico-Legal Directorate. Data collection was made between May 2025 and September 2025. The collection included cases that were verified MI autopsies, which were recorded from January 2022 to December 2024. An exclusion was made, including only cases with myocarditis or cardiomyopathy. The total cases manually extracted were 4,344 MI cases. They were categorized by gender, age group, and year of occurrence. SPSS (v28), GraphPad Prism (v10.4.1), and Microsoft Excel 2021 were utilized to conduct descriptive statistics and Chi-square tests with statistical significance set at  $p < 0.05$ .

**Results:** A noticeable elevation in MI cases was spotted over three years period: 2022 (1,089 cases), 2023 (1,541 cases), and 2024 (1,714 cases). The majority of cases were consistently represented by individuals aged  $>50$  years, composing (58.1%), (68.2%), and (67.9%) of MI deaths in 2022, 2023, and 2024, respectively ( $p < 0.001$ ). The second most affected age group was those between 41 and 50 years old. Nevertheless, fewer than 3% cases of patients  $\leq 30$  years were recorded. Across all years, a significant male preponderance was noticed, with men constituting 73.8% during 2022, 71.5% during 2023, and 70.3% during 2024 of MI cases ( $p = 0.001$ ). Although the ratio of MI proportional to overall autopsy cases exhibits minimal variation, the constant increase indicates a rising cardiovascular burden.

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**Conclusions:** The analysis of this forensic histopathological study demonstrates an upward trend in MI frequency in the capital of Iraq (Baghdad), preponderantly harming male individuals over 50 years across all age groups. Although the current study did not assess individuals' risk factors, previously reported cardiovascular risk factors such as diabetes, hypertension, overweight, and smoking may contribute to this burden and upward MI frequency, which warrants further investigation. Furthermore, the progressive rise in female MI cases suggests different lifestyles and intense health hardship. The study highlights the necessity for enhanced national surveillance and preventive measures targeting CVD.

**Keywords:** Age group, Cardiovascular disease, Frequency, Histopathology, Myocardial infarction.

## Background

Myocardial infarction (MI) represents a critical and potentially fatal event, manifesting as the most severe clinical presentation of coronary artery disease (CAD) and a frequent cause of sudden cardiac death (SCD)<sup>[1]</sup>. Globally, cardiovascular disease (CVD) represents the primary cause of mortality, where ischemic heart disease was responsible for nearly 49.2% of all recorded deaths in 2019<sup>[2]</sup>. According to the World Health Organization (WHO) estimation, 19.8 million individuals died from CVD in 2022, of which 85% of these deaths were due to heart attacks and strokes<sup>[3]</sup>. Similar to many high-income countries during the 20th century, low- and middle-income countries are seeing an alarming increase in the rates of CVD, and this change is accelerating, which is responsible for 80% of global deaths<sup>[4]</sup>. For instance, in Iraq, which is considered a middle-income country<sup>[5]</sup>, the early-onset MI comprises up to one third of all MI cases, which surpasses the 5–10% reported in high-income countries widely<sup>[6]</sup>. In recent years, interest in SCD among young people has intensified, owing to its unpredictable nature, substantial societal consequences, and common links to undetected or hereditary cardiac disorders<sup>[7]</sup>. Between 1990 and 2010, the rates of Ischemic heart disease in the Middle East and North Africa (MENA) Region grew tremendously, where in the Arabs world it replaced lower respiratory infection, contributing to 14.3% of deaths, while it accounted for nearly 2.55 million (95% UI 2.29–2.83) incidence cases in the MENA Region in 2019<sup>[8]</sup>.

Age-related patterns of myocardial infarction have also received attention, which also considered age as a predictive factor for acute coronary syndrome, in which the age group older than 45 years had the highest prevalence rate<sup>[23]</sup>.

An acute decrease in myocardial blood supply occurring from a thrombotic occlusion within an artery or bypass graft, eventually leading to heart failure and death, is what typically leads to acute MI<sup>[2]</sup>. It is also characterized by a notable increase in cardiac biomarkers in blood, indicating myocardial injury or necrosis in regional wall motion<sup>[9]</sup>. Extensive evidence links hypertension to CVD worldwide, with hypertension exhibiting particular prevalence in Iraq, where it constitutes a primary cause of it<sup>[10]</sup>.

A significant limitation is the lack and scarcity of evidence-based national guidelines for CVD management and comprehensive surveillance studies in Iraq, especially when compared to other nations in the Eastern Mediterranean region. Epidemiological data concerning the frequency and prevalence of CAD are confined<sup>[4]</sup>. Therefore, this study aims to determine the frequency and age-specific distribution of MI among autopsy cases in Baghdad as a histopathological analysis. To our knowledge, this is the first forensic histopathological-based study that has an in-depth analysis of forensic pathological and histopathological features of ischemic heart disease in Baghdad, including both genders (male and female) and across different age groups.

## Methodology

The current study, a retrospective analysis, was conducted at the archives of the histopathological department of the medico-legal directorate in Baghdad/Iraq, during the period between (May 2025 till September 2025), with the researcher making an average of three weekly visits, each lasting approximately two hours, to access archival records, which is the main source of information including histopathological reports diagnosed with ischemic heart disease and acute MI, which already had been investigated and examined conducted by senior pathologists according to their standards operating procedures. The authors did not perform any independent histopathological assessment, review tissue slides, or reclassify cases as acute or chronic MI. procedures such as inter-observer variability, pathologist standardization, and quality-control were not available within this method. However, myocarditis and cardiomyopathy reports were excluded. The period of the collected data spanned from January 2022 to December 2024. From a total of 16,127 death cases, only 4,344 MI cases were meticulously compiled. The author manually reviewed the archived data, extracting relevant information and organizing it into tabulated datasets. This information was subsequently categorized based on variables such as age, sex, and the year of data archiving <sup>[11]</sup>.

## Data Analysis

For a better understanding of the distribution of the MI cases each year, we divided the extracted archived data into three studied years: 2022, 2023, and 2024. Then, a descriptive analysis of the collected data was conducted, calculating the relative frequencies utilizing the statistical software packages SPSS (Version 28) by IBM and GraphPad Prism (Version 10.4.1), and Microsoft Excel 2021. The statistical analyses involved employing a Chi-square test. A p-value cut-off of  $<0.05$  was considered significant for differences.

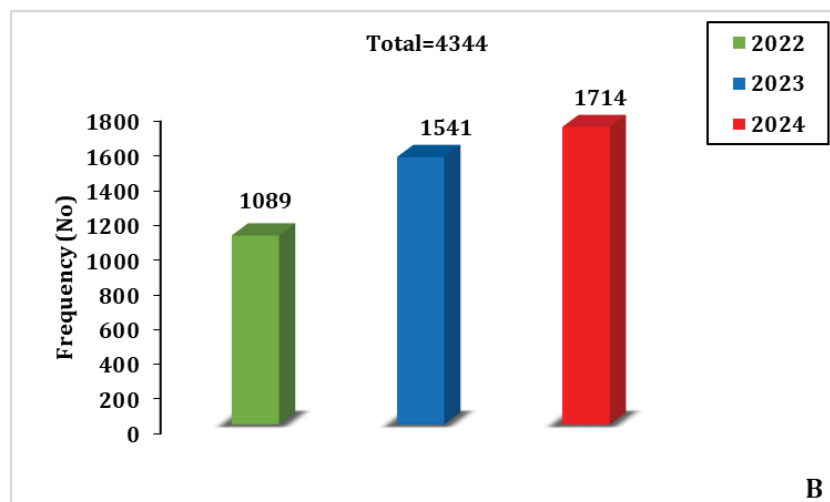
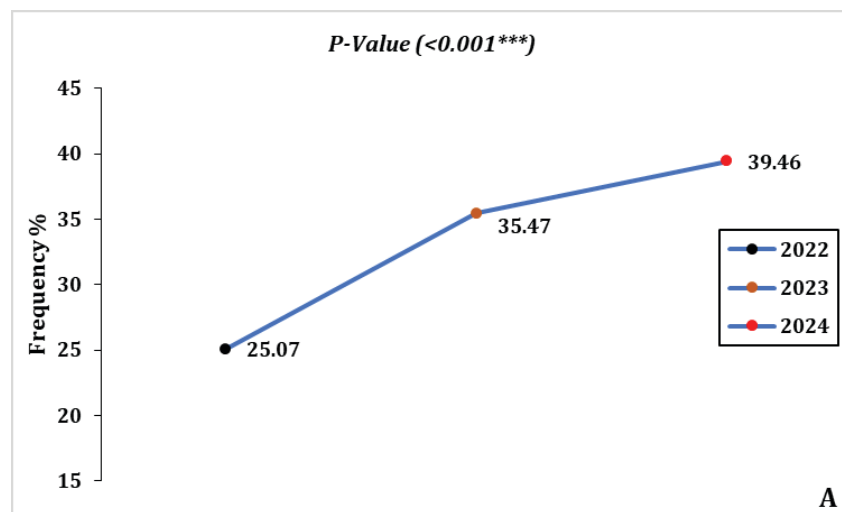
The limitation of the bias was depending on the fact that in Baghdad, most of the death cases that do not have a hospital record and die naturally outside the hospital, whether males, females, old aged, or even children, are automatically referred to the medico-legal directorate. Also, any death outside the hospital and in clinics with suspicious circumstances will be transferred to the medico-legal directorate. However, patients with frequent visits to the hospital with a medical record and a witnessed death inside the hospital, which are small in number, are excluded and not referred to the medico-legal directorate. Lately, most deaths have been referred to the medico-legal directorate.

## Results

As shown in Table 1, a statistically significant gender disparity was observed, indicating a higher distribution of MI among males: 3112 (71.64%; 95% CI: 70.30-72.98%) relative to females: 1232 (28.36%; 95% CI: 27.02- 29.70%) throughout the three years. Nevertheless, both sexes demonstrated a comparable age-dependent increase in MI frequency, with the majority of cases presenting in individuals over 50 years of age. The whole eligible MI cases (manually compiled) recorded at the medico-legal directorate in Baghdad, during the period of this research (2022, 2023, and 2024) were 4,344 cases, in which, the annual distribution showed 1,089 cases in 2022, 1,541 cases in 2023, and 1,714 cases in 2024, revealing an upward direction over the three-year period ( $p > 0.001$ ) (Figure 1). While the ratio of MI proportional to overall autopsy cases exhibits minimal variation, these differences were not statistically significant ( $p > 0.05$ ). However, the results demonstrate a consistent increase in the definite number of MI-related deaths over time, indicating a possible increase in cardiovascular deaths within the studied population.

**Table 1. Profile of the cases, demonstrating with numbers the gender, age, and years of the cases collected.**

| year       | 2022  |        |       | 2023  |        |       | 2024  |        |       |
|------------|-------|--------|-------|-------|--------|-------|-------|--------|-------|
| Age groups | Male  | Female | Total | Male  | Female | Total | Male  | Female | Total |
| 20         | 6     | 1      | 7     | 3     | 3      | 6     | 7     | 4      | 11    |
| 21-30      | 22    | 9      | 31    | 25    | 17     | 42    | 29    | 13     | 42    |
| 31-40      | 104   | 40     | 144   | 107   | 20     | 127   | 112   | 28     | 140   |
| 41-50      | 207   | 71     | 278   | 238   | 77     | 315   | 256   | 100    | 356   |
| > 50       | 465   | 164    | 629   | 730   | 321    | 1,051 | 801   | 364    | 1,165 |
| Total      | 1,089 |        |       | 1,541 |        |       | 1,714 |        |       |



**Figure 1: Comparison of total fatal MI cases across 2022, 2023, and 2024, revealing an increasing trend in both the number and frequency of cases over the last three years; A, B.**

### 1. Distribution of Myocardial Infarction Cases by Age Group

The total cases of MI recorded were 1,089 in 2022, 1,541 in 2023, and 1,714 in 2024. Based on age categorization of the collected data, results reveal the highest proportion of fatal MI cases was consistently observed in patients with > 50 years old, accounting for 58.1% (95% CI: 54.8 - 60.7%) in 2022 (Fig 2), 68.2% (95% CI: 65.8 - 70.5%) in 2023 (Fig 3), and 67.9% (95% CI: 65.8 - 70.2%) in 2024 (Fig 4). The prevalence of the 41-50 age group represented the second-highest proportion, constituting 25.5% (95% CI: 22.9 - 28.1%), 20.4% (95% CI: 18.3 - 22.4%), and 20.8% (95% CI: 18.9 - 22.7%) in 2022, 2023,

and 2024, respectively. This was followed by the 31-40 age group category with 13.2% (95% CI: 11.2 - 15.2%), 8.24% (95% CI: 6.8 - 9.5%), and 8.2% (95% CI: 6.9 - 9.5%) of cases. Patients in the 21-30 age group category were substantially lower with 2.6% (95% CI: 1.9 - 3.8%), 2.7% (95% CI: 1.9 - 3.5%), and 2.5% (95% CI: 1.8 - 3.2%) of cases, whereas patients aged ≤ 20 years accounted 0.6% (95% CI: 0.2 - 1.1%), 0.4% (95% CI: 0.1 - 0.7%), and 0.7% (95% CI: 0.3 - 1.0%) in 2022, 2023, and 2024, respectively. The analysis reveals a statistically significant correlation formed between MI frequency and older age groups, referring to an increase in MI frequency with older age ( $p < 0.001$ ).

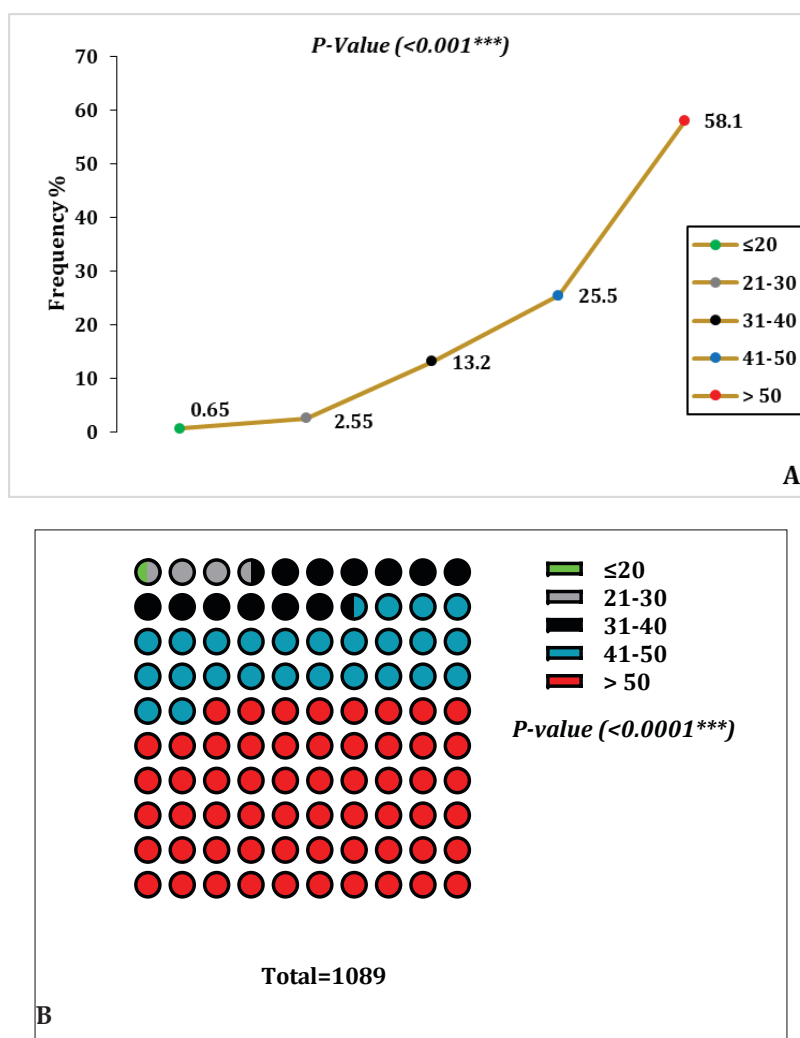


Figure 2: Age classification of MI cases recorded in 2022; A, B.

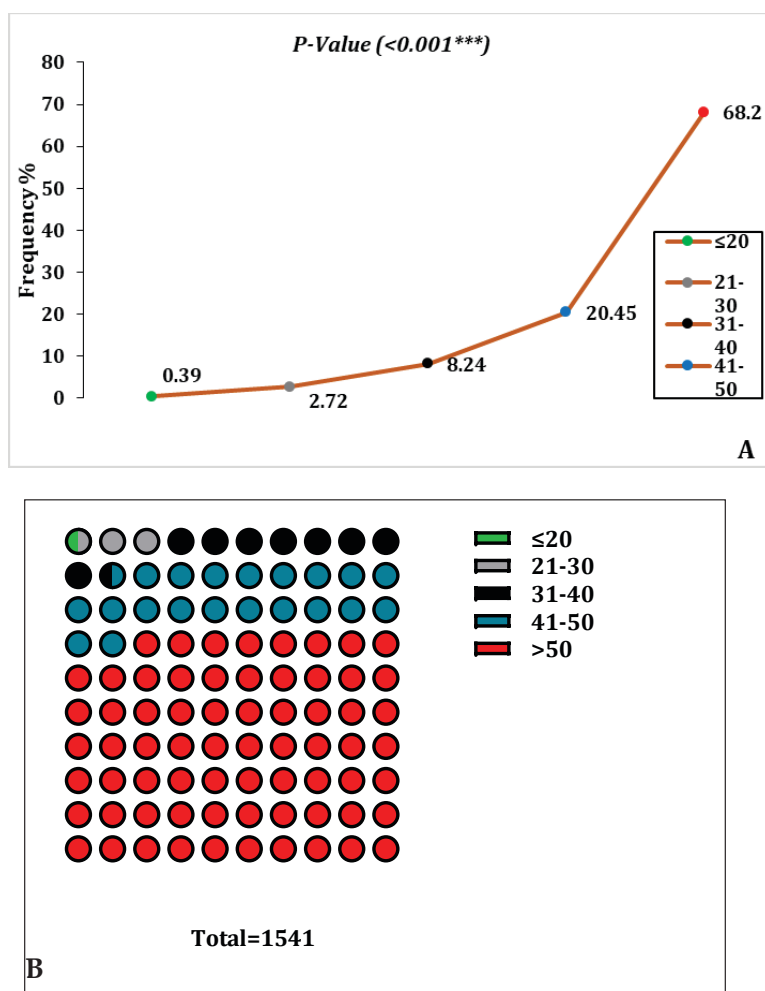
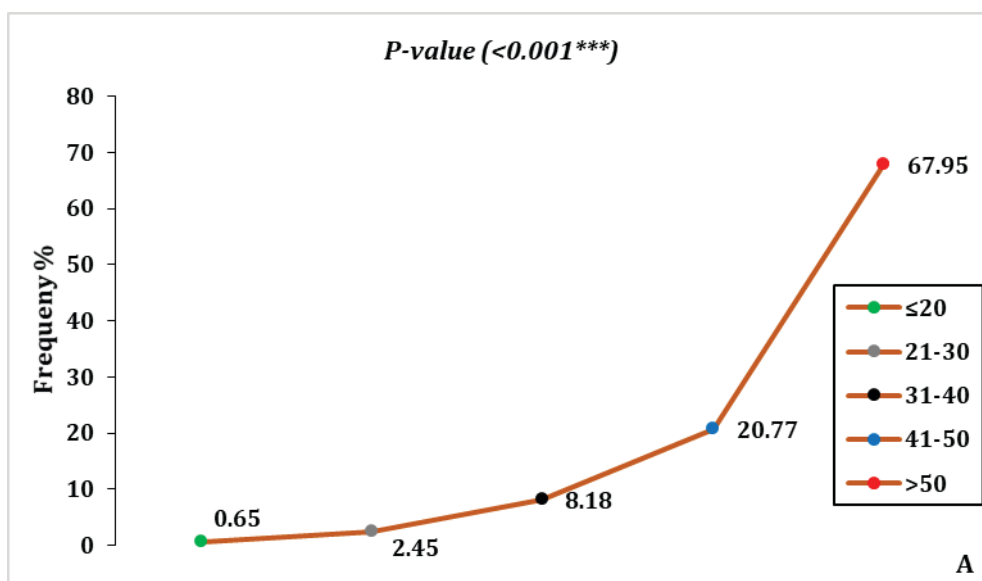


Figure: 3 Age classification of MI cases recorded in 2023; A, B.



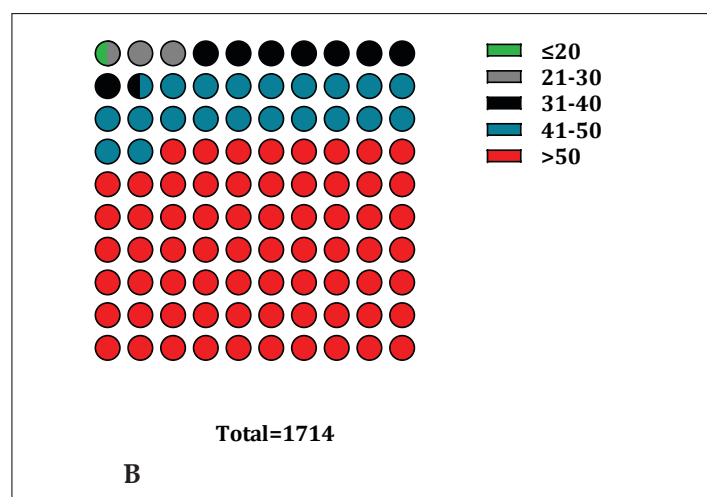
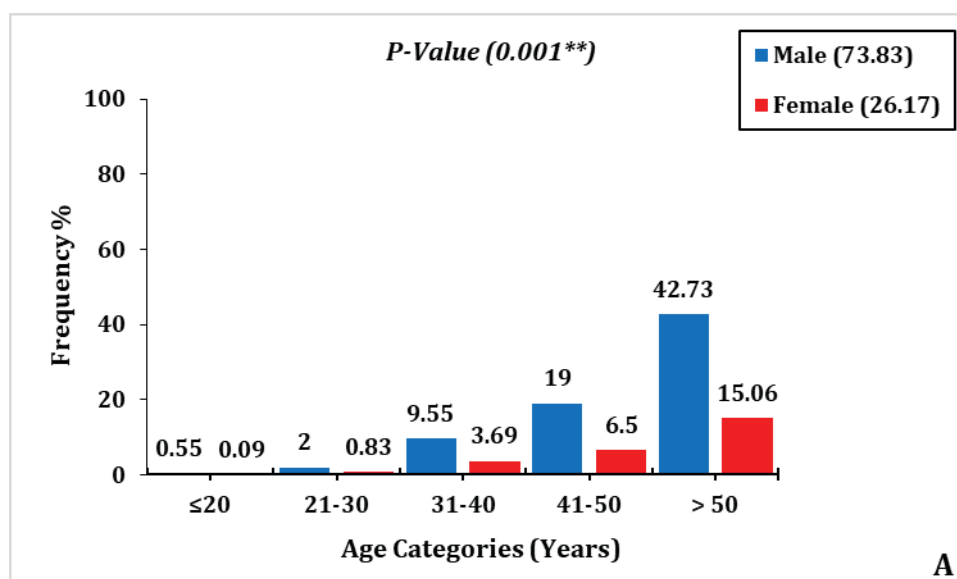


Figure: 4 Age classification of MI cases recorded in 2024; A, B.

Throughout the reviewed years, patients over 50 predominantly present the highest proportion of fatal MI Cases. Meanwhile, patients aged 41-50 years acquire the second-largest proportion. On the contrary, younger age groups showed the lowest frequency of MI, participating in less than 1% totally. Statistical analyses asserted a significant correlation between age group and MI burden, proving that MI frequency increases significantly with older ages, where  $p < 0.001$ .

## 2. Gender Distribution of Myocardial Infarction Cases

As Figure 5 shows, the result of the statistical analysis of gender distribution indicates a significant difference, with male cases ( $n = 804$ ; 73.83%; 95% CI: 71.22- 76.44% ) outnumbering female cases ( $n = 285$ ; 26.17%; 95% CI: 23.56- 28.78%) in 2022, where  $p = 0.001$ . This result reveals a significant proportion, with regard to gender, which occurred in males over 50 years old, indicating a consistent gender-specific risk profile across age groups.



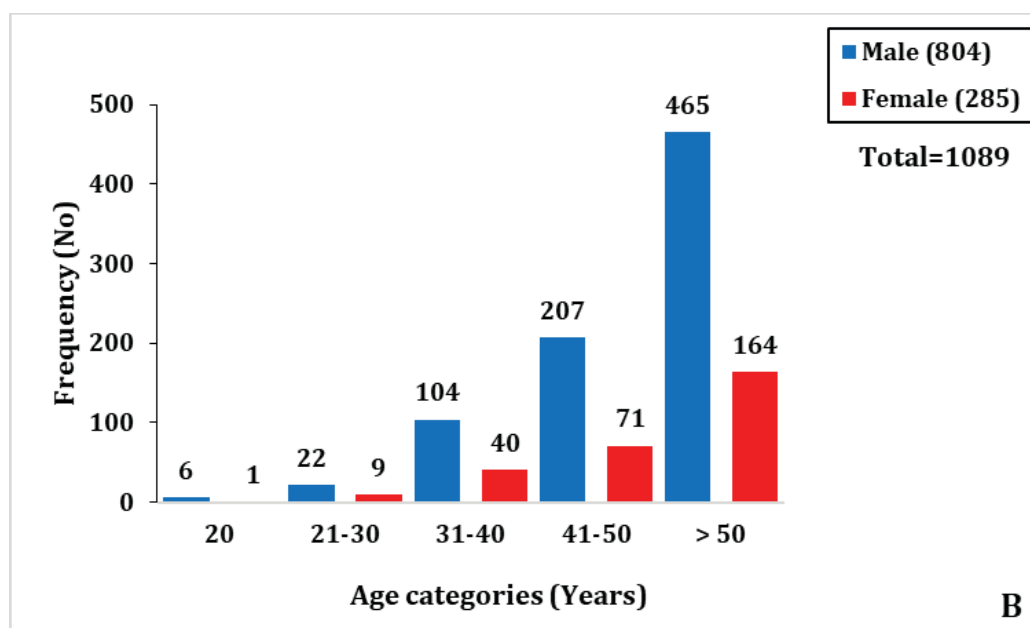
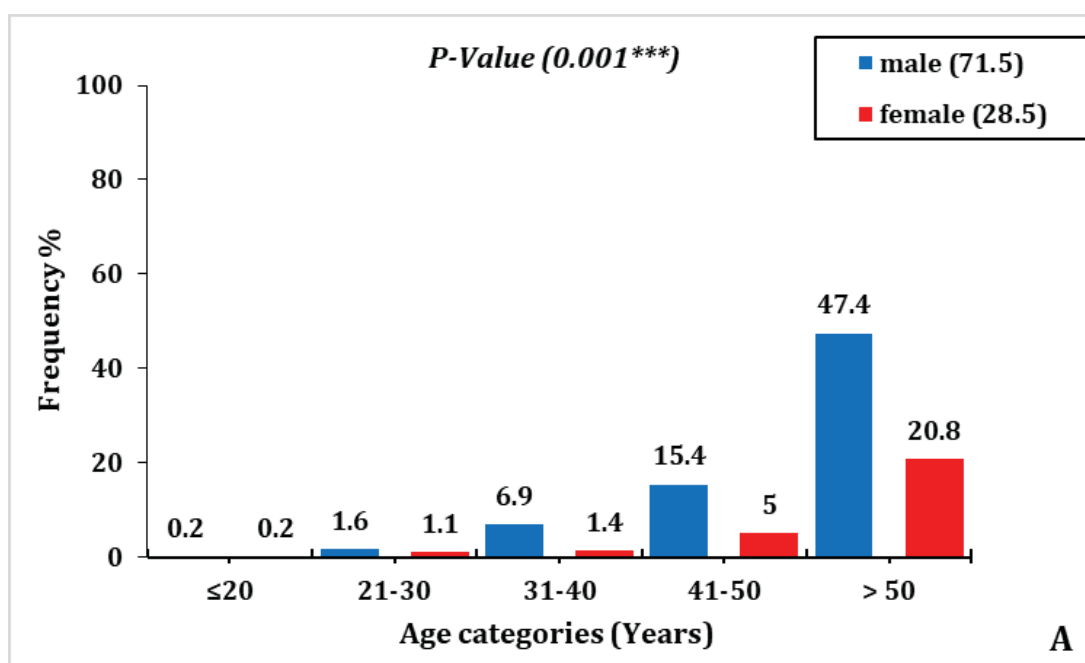


Figure: 5 Gender classification of MI cases recorded in 2022; A, B.

For 2023, the reported data indicated 1,103 male cases (71.5%; 95% CI: 69.33- 73.83%) and 438 female cases (28.5%; 95% CI: 26.17- 30.67%), with this male predominance confirmed as statistically significant

( $p = 0.001$ ). Consistent with 2022, the highest proportion of fatal MI Cases in both genders was recorded among males over 50 years of age, followed by the 41–50 age group (Figure 6).



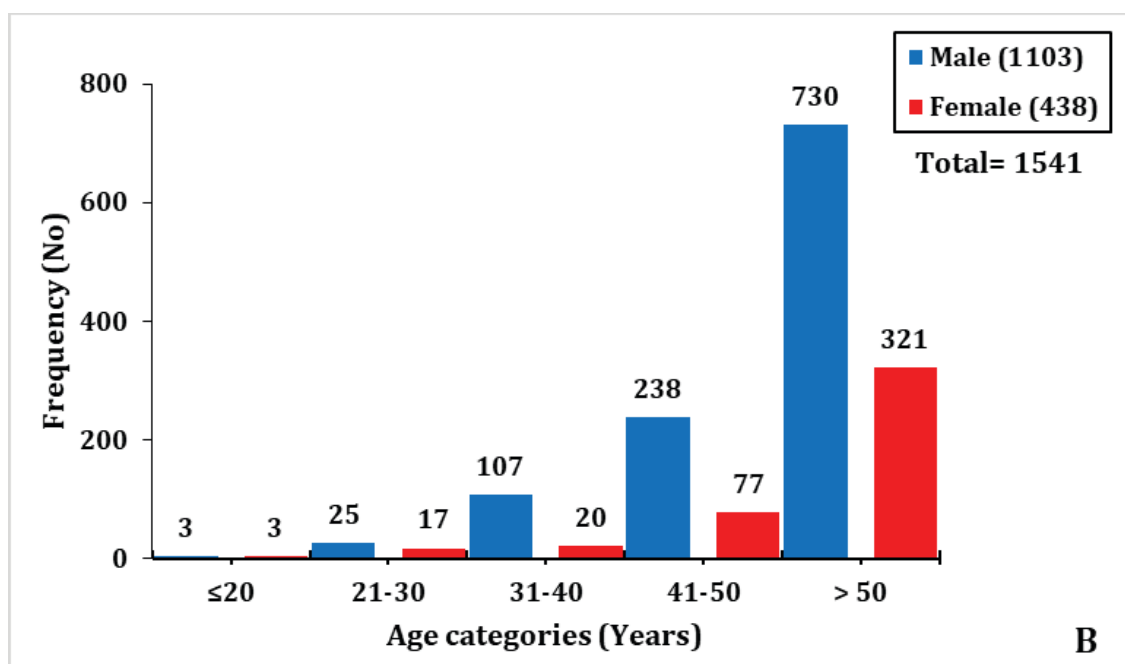
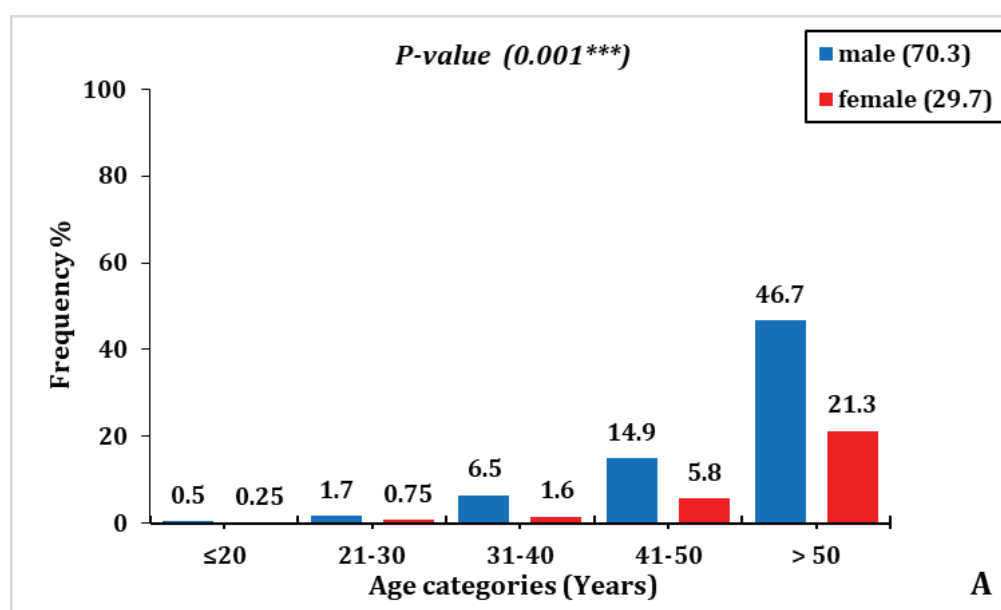


Figure 6: Gender classification of MI cases recorded in 2023; A, B.

As shown in Figure 7, the results of gender analysis for 2024 revealed a consistent male dominance, with 1,205 cases (70.3%; 95% CI: 68.14- 72.46%) of males compared with 509 cases (29.7%; 95% CI: 27.54-

31.86%) of females, where  $p = 0.001$ . Similar to the previous year's trend, the burden of MI confirmed a relative increase in males compared to females. The major diagnosed male patients were > 50 years old.



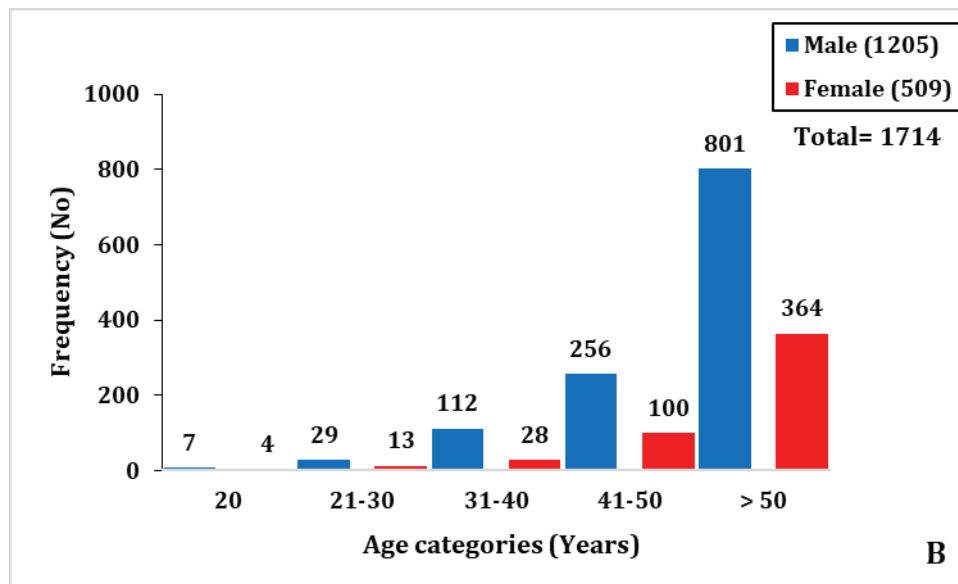


Figure: 7 Gender classification of MI cases recorded in 2024; A, B.

### Discussion1

The current retrospective study demonstrates a clear and progressive increase in the frequency of MI cases recorded at the histopathological department at the Medico-Legal Directorate in Baghdad from 2022 to 2024. The annual rise from 1,089 cases in 2022 to 1,541 in 2023 and 1,714 in 2024 indicates a growing burden of MI-related mortality in the studied population. Although the relative proportion of MI among total autopsy cases showed minimal variation, the absolute numbers reflect a genuine upward trend. Despite the lack and scarcity of data concerning the frequency of MI in Iraq, this pattern aligns with global reports showing increases in CVD burden in low and middle-income countries, potentially driven by lifestyle changes, population aging, and inadequate preventive healthcare infrastructure [12][13]. Nevertheless, in Saudi Arabia, a nation with a strong and rapidly growing economy, a cross-sectional study reveals a considerable frequency of MI with a range from 47.8% to 72.8%, which indicates a severe problem [28]. In Iran, a similar trend of increased MI frequency was observed in a systematic review study [29]. In Iraq, the observed trends may reflect increasing prevalence of established cardiovascular risk factors, with recent studies documenting increasing

prevalence of risk factors including hypertension, diabetes, obesity, and tobacco consumption, especially after 2010, which in turn, these risk factors will also be considered as the main causes for increasing CVDs [4][14].

Across all three years, MI occurred disproportionately in individuals above 50 years of age, who consistently represented the majority of cases (58.1%; 95% CI: 54.8- 60.7% (2022), 68.2%; 95% CI: 65.8- 70.5% (2023), and 67.9%; 95% CI: 65.8- 70.2% (2024). The statistically significant association between increasing age and MI frequency is consistent with the well-established role of age as one of the strongest cardiovascular risk factors, as the rising mortality rates highlight the significant burden of CVDs in older populations [15][16][17]. Age-related endothelial dysfunction, increased atherosclerotic burden, and higher prevalence of comorbidities such as hypertension and diabetes may explain this pattern [18][19]. The very low rates of MI among individuals aged 20 years align with recent Iraqi epidemiological data showing that MI remains exceptionally low in the paediatric population [4]. Notably, the 41-50 age group was the second most affected category, accounting for around 20-25% of cases yearly, which nearly resembles the result of a

study performed in one of the developing countries, which shows a 25.3% for the same age group<sup>[20]</sup>. This finding suggests that MI is not limited to elderly individuals but also affects middle-aged adults, reflecting growing exposure to risk factors such as smoking, metabolic syndrome, chronic stress, and sedentary lifestyles<sup>[21]</sup>. The distribution of MI in the age group  $\leq 40$  years was lower than in the older age group, as they exhibit fewer traditional risk factors. However, a higher rate of smoking, obesity, and non-atherosclerotic causes like spontaneous coronary artery dissection or coronary spasm in this age group may lead to an increase in the burden of SCD<sup>[22]</sup>.

The study revealed a persistent and statistically significant male predominance in MI proportion, representing 73.83%; 95% CI: 71.22- 76.44% in (2022), 71.5%; 95% CI: 69.33- 73.83% in (2023), and 70.3%; 95% CI: 68.14- 72.46% in (2024), across all age groups. This aligns with country literature, as shown in Sulaimani city, in which higher MI rates are found in men, particularly the age group 45-65, compared to women<sup>[23]</sup>. Also, male sex has consistently been identified as a major risk factor due to the increased risk of hypertension<sup>[10][24]</sup>.

The prevalence of smoking and stress-related behaviors among men in Iraq was much higher than that among females<sup>[25]</sup>. Also, protective hormonal effects in premenopausal women lower the frequency of CVD due to a decrease in the frequency of hypertension, atherosclerosis, heart failure, and myocardial ischemia compared to men at the same age<sup>[27]</sup>. Despite the fact that male sex is predominant, the results of this study show an increase in the rate of MI burden among Iraqi women, with a gradual rise in cases that may reflect increasing cardiovascular risk due to lifestyle shifts and rising metabolic disease<sup>[26]</sup>.

### Limitations of the study

The difficulties and time-consuming nature of extracting the data. Also, a lack of personal information about the patients, including their age or gender. Not to forget that many of the cases did not have previous medical records or documented risk factors.

### Conclusion

This three-year retrospective histopathological analysis demonstrates a clear and consistent rise in myocardial infarction (MI) frequency among medico-legal autopsy cases in Baghdad, underscoring a growing cardiovascular burden in Iraq. Individuals aged  $>50$  years constituted the majority of MI-related deaths, reaffirming age as a dominant risk factor. Middle-aged adults (41-50 years) represented the second most affected group, reflecting increasing exposure to lifestyle and metabolic risks. The persistent male predominance across all age categories aligns with established epidemiological patterns and is likely influenced by higher smoking rates, greater occupational and psychosocial stress, and the absence of hormonal protection seen in premenopausal women. Although females represented a smaller proportion of MI cases, the gradual rise observed over the study period suggests evolving lifestyle patterns and increasing metabolic disease among Iraqi women. The overall upward trajectory in MI mortality highlights the pressing need to strengthen cardiovascular disease surveillance, prevention, and risk-factor modification strategies at the national level.

### Abbreviations

|     |                           |
|-----|---------------------------|
| CAD | Coronary Artery Disease   |
| CVD | Cardiovascular Disease    |
| MI  | Myocardial infarction     |
| SCD | Sudden Cardiac Death      |
| WHO | World Health Organization |

### Declarations

**Conflicts of Interest:** The author declares no conflicts of interest

**Statement on the Incorporation of Artificial Intelligence (AI) in Scientific Writing:** Not applicable.

### Human Ethics and Consent to Participation

**Declaration:** This study was approved by the scientific and ethical council in Iraq medical legal directorate / Ministry of Health. This research was conducted based on Article (3) of the Iraqi Forensic Medicine Law of 2013.

**Consent of Human Research Subjects:** Not Applicable.

**Funding:** Not Applicable.

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# The Influence of Individual Characteristics on Life Activity and work Ability during Injuries from A Forensic-Medical Aspect

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## Abstract

This research examines the impact of individual characteristic, such as digestive conditions and pregnancy, on life activity and work ability after injuries, with a focus on forensic medical aspects. The study analyzed the relationship between gastrointestinal disease, pregnancy, and individual functionality, where severe bodily injuries often lead to permanent disability. The aim of the research is to contribute to a more precise assessment of injury consequences in a forensic medical context, which can improve the classification of injuries and the evaluation of work ability in forensic practice.

**Key words:** forensic medicine, injuries, work ability, pregnancy, injury classification.

## Introduction

Injuries in many life situations affect the remaining life activity and work capacity, and depend on numerous factors, including specific individual characteristics (digestive status and pregnancy), personal characteristics (constitution), as well as the specific circumstances of the injury case. These factors can affect the recovery process, and in the forensic context, we analyze how specific individual characteristics affect the course, outcome of treatment and prognosis of different forms and types of injuries, recovery time and especially life activity and work capacity.<sup>1,2</sup>

The severity of the injury is assessed based on the potential impact on the health, life and functional abilities of the individual. Minor bodily injuries do not cause serious damage to health, do not endanger life and functionality, unlike severe bodily injuries, which cause permanent consequences, disability, and endanger life (fractures, hematomas, contusions, excoriations, lacerations, cuts, complications of injuries to internal organs of the chest and abdominal cavity).<sup>3,4,5</sup>

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## Research Objective

The objective of this research is to determine the influence of specific individual characteristics (digestive status and pregnancy) in the context of the forensic aspect of the classification and qualification of bodily injuries in the assessment of the reduction of remaining life activity and work capacity.

## Research Hypothesis

Pregnancy can significantly prolong the healing period and recovery from physical injuries (mild or severe) due to the extent and severity of the injuries at the time of injury, physiological and psychological changes, and the special and specific circumstances of their occurrence that pregnant women face.

There is a significant correlation between the state of digestion (digestive system disorders) after injuries due to the conditions, mechanism, and dynamics of the occurrence of physical injuries and their impact on remaining life activity and work capacity.

## Methodology

### Sample

The research sample includes 100 patients who suffered physical injuries of varying extent and severity at the time of injury. Of this number, 30 are pregnant women, and 40 patients have certain chronic

digestive disorders of the digestive system (irritable bowel syndrome, colitis, celiac disease, malignancy, bloating or fullness in the stomach). The patient sample was obtained at the Clinical Hospital Center Kosovska Mitrovica during a routine examination. Informed consent was obtained from the patient in advance. All necessary approvals were obtained in accordance with ethical protocols.

## Variables

### Independent variables

Pregnancy – yes or no;

Digestive status – without digestive system disorders versus patients with chronic digestive disorders;

### Dependent variables

Life activity – minimal, reduced, complete loss;

Work ability – able, limited able, unable.

### Statistical analysis

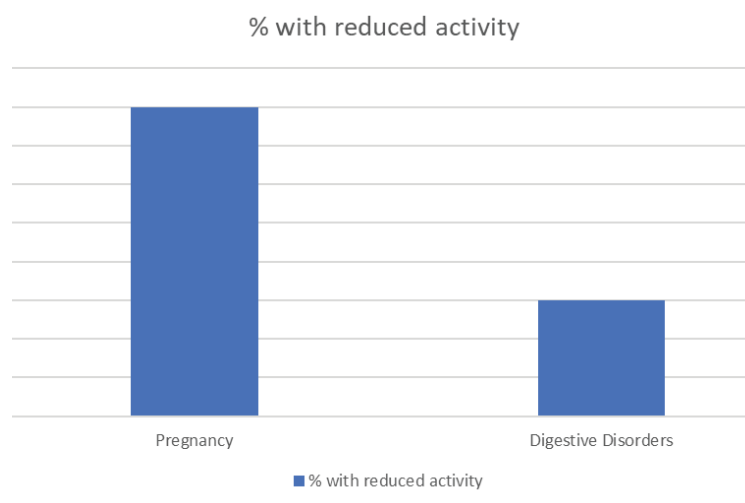
Descriptive statistics

T test

## Results

Descriptive statistics of samples

**Table 1. Impact on Life Activity by Group**



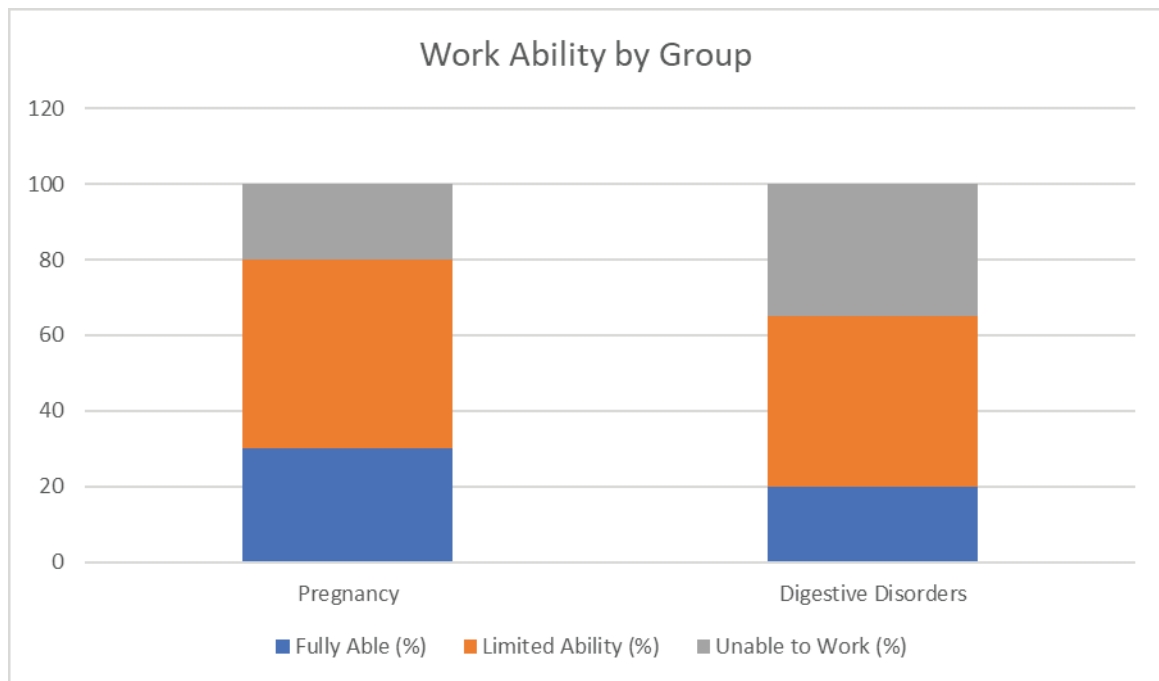
This table shows the **percentage of patients** who experienced a **significant reduction in daily life activity** due to **physical injuries** or **digestive disorders**.

- **Pregnant women:** 60% of pregnant women reported a significant reduction in daily activities following injuries. This can include limitations in performing everyday tasks or needing more rest.

- **Digestive disorders:** 55% of patients with chronic digestive disorders reported a reduction in life activity. This could include difficulties in physical activities, managing symptoms, and a need for more rest due to digestive issues.

These data demonstrate how specific health issues, such as pregnancy or chronic digestive disorders, can significantly affect a person's ability to carry out daily tasks. This highlights the need for targeted medical care and support for these groups to mitigate the impact on their daily lives.

**Table 2. Work Ability by Group and Health Condition**



This table presents the **percentage of work ability** in patients with pregnancy or chronic digestive disorders.

- **Pregnant women:**
  - **30% fully able:** 30% of pregnant women were fully able to work without significant limitations.
  - **50% limited ability:** 50% of pregnant women reported a reduced ability to work due to physical limitations, fatigue, or other health-related factors during pregnancy.
  - **20% unable to work:** 20% of pregnant women were unable to continue working due to the severity of their condition.
- **Digestive disorders:**
  - **20% fully able:** 20% of patients with chronic digestive disorders were fully capable of working without any limitations.
  - **45% limited ability:** 45% of patients reported having reduced work capacity, possibly due to ongoing symptoms affecting their productivity.

- **35% unable to work:** 35% of patients with chronic digestive disorders were unable to work due to the severity of their symptoms.

This table highlights how **health conditions** like pregnancy and chronic digestive disorders can **limit work ability** and **affect productivity**. Employers and healthcare providers need to be aware of these challenges and offer appropriate support to help individuals maintain their work capabilities.

## Discussion

Forensic medical analysis of disability following bodily injuries requires a detailed consideration of functional consequences, which are largely modified by the presence of physiological and digestive disorders. In the research sample, 60% of the pregnant women and 55% of patients with chronic gastrointestinal disorders showed a significant decrease in life activity, while 20% of pregnant women and 35% of chronic patients were found to be completely unable to work (table 1, table 2). These results indicate that injuries, although classified by severity, have different functional outcomes depending on the individual health condition of the patient. Therefore, in forensic medical assessment of disability, it is essential to objectively evaluate the influence of these factors on quality of life and work capacity, as they represent a key element in the assessment of damage compensation, work incapacity and the evaluation of permanent functional impairment.

Research shows that pregnancy and digestive status significantly affect the remaining life activity and work ability of the patient after injury.

Pregnant women often have greater difficulties during recovery, healing, length of time for wound healing due to physiological changes, while the digestive status further complicates recovery, prognosis and activities of daily living due to impaired digestive system function due to the circumstances of the occurrence of the injury, its extent, severity at the time of injury.

There may be complex mechanisms surrounding wound healing during pregnancy. Wound healing

may differ from one pregnant woman to another depending on factors such as genetics, comorbidities, nutritional status, exercise, alcohol and drug use.

Pregnancy is associated with elevated estrogen levels, and estrogen has been implicated in negatively affecting wound healing. Estrogen negatively regulates epithelial wound healing.

Most pregnant women suffer from zinc deficiency, especially in the last two trimesters (WHO). Zinc deficiency also negatively affects wound healing. Zinc is not only an antioxidant that protects healing cells from free radicals, but is also essential for the proper functioning of the immune system.

Research in Germany shows that digestive disorders are one of the factors that can significantly reduce work capacity, especially in patients with chronic diseases (Crohn's disease and irritable bowel syndrome). According to some studies, about 20-30% of people have a decrease in work capacity after physical injuries, including the condition and mechanism of injury.<sup>5</sup>

In the United States, digestive disorders such as irritable bowel syndrome and diseases such as celiac disease can significantly reduce quality of life and work capacity. According to some sources, about 20-25% of people with chronic gastrointestinal diseases report significant impairment in daily activities, including a decrease in work capacity due to pain, fatigue, and other symptoms after minor physical injuries.<sup>5</sup>

In Sweden, research suggests that about 15-20% of people with chronic digestive disorders report decreased productivity at work, while reduced life activity is also often due to symptoms.<sup>5</sup> In the US, according to research data, about 10-20% of pregnant women report decreased work capacity, mainly due to complications of physical injuries and injuries to the organs of the chest cavity.<sup>5</sup>

## Conclusion

Based on the obtained results, the main factors of disability-pregnancy, chronic digestive disorders and the severity of bodily injuries-clearly stand

out as significantly impacting the patients daily functioning and work ability. In the context, forensic analysis plays a crucial role by enabling an accurate assessment of the causal relationship between the injury and the degree of functional impairment. The presence of these factors directly influences the evaluation of disability, compensation claims, and the determination of permanent incapacity, making them key elements in forensic medical expertise.

Digestive status and pregnancy significantly affect the life activity and work ability of patients after the occurrence of physical injuries. These factors should be taken into account in forensic medical analyses of the classification and qualification of physical injuries (conditions, mechanisms, dynamics of the occurrence of physical injuries) in order to accurately determine the consequences and long-term complications on the health of patients and liability in legal cases.

Forensic aspects of injuries play a key role in the justice system, as they provide the necessary medical evidence for resolving disputes, making judgments and awarding monetary damages.

Modern forensic methods- including digital monitoring of functionality, the use of artificial intelligence in capacity assessment and the integration of the forensic quality of life concept offer a solid foundation for more precise, objective and equitable damage evaluation. Further development of these approaches holds strong potential to enhance both forensic theory and practice, particularly in the assessment of vulnerable and individually specific cases.<sup>6,7</sup>

## Declaration

**Source of Funding:** None

**Conflict of Interest:** None

**The Ethical Approval** (1519/December 15.2022) covers a larger study framework, from which the current analysis is derived.

**Consent to Publication:** Authors consent to publication.

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## Prognostic Utility of Serum Lactate in Acute Poisoning Cases: A Retrospective Observational Study

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### Abstract

**Background and Aims:** Poisoning remains a major public health issue in low- and middle-income countries, contributing significantly to morbidity and mortality. Early risk stratification is crucial in managing poisoning. To assess the prognostic utility of initial serum lactate levels in poisoning, determine an optimal cut-off for predicting mortality, and evaluate its association with mortality, ICU admission, mechanical ventilation, and hospital stay.

**Methods and Patients:** A retrospective observational study was conducted in the Emergency Department of a tertiary care centre in Karnataka, India. Adults (>18 years) presenting within 24 hours of toxin ingestion were included. Exclusions were prescription drug overdoses, sepsis, trauma, and hypoperfusion unrelated to poisoning. Initial serum lactate levels were correlated with clinical outcomes.

**Results:** Among 99 patients (62.6% male, mean age 32.8±11.9 years), the mean lactate level was 2.97±2.49 mmol/L. ROC analysis identified a cut-off of 3.25 mmol/L for mortality prediction (AUC: 0.691; sensitivity: 71.4%; specificity: 73.8%). Mortality was significantly higher in patients with lactate >3.25 mmol/L (31.3%) compared to ≤3.25 mmol/L (6.1%) (OR: 7.05; 95% CI: 2.00–24.77; p=0.002). Elevated lactate was associated with increased ICU admission (OR: 9.4) and mechanical ventilation (OR: 7.8). Rodenticide poisoning had the highest lactate levels and mortality.

**Conclusion:** An initial serum lactate >3.25 mmol/L is significantly associated with mortality and critical care needs in poisoning cases, making it a valuable early risk stratification tool in emergency settings.

**Key words:** Slactate, Poisoning, Prognostic marker, Mortality prediction, Emergency department, Risk stratification, Toxin ingestion, ICU, Intubation.

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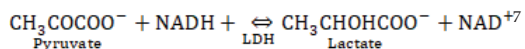
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## Introduction

The World Health Organization estimated that poisoning causes approximately 640,000 deaths each year, with the majority of these fatalities occurring in low- and middle-income countries<sup>1</sup>. National data in India estimated 70 thousand people die annually due to poisoning with a rate of 2.4 per 1,00,000 population<sup>2</sup>. Risk stratification and early intervention are essential for improving the prognosis and reducing the mortality and morbidity of these patients. In a study conducted by Anthony L<sup>3</sup>, it was found that 67% of poisoning patients required admission to the Intensive Care Unit (ICU). Among these ICU patients, 21.9% needed mechanical ventilation. Notably, the need for ventilation was significantly higher in patients poisoned with organophosphates compared to other types of poisoning. These findings highlight the importance of early prognostication in poisoning cases. Multiple scoring systems using vitals, Glasgow Coma Scale (GCS), and presenting signs and symptoms have been used for the same.

Serum lactate, a by product of anaerobic respiration, has been used as a good criterion for the prognosis of many systemic illnesses like sepsis and trauma<sup>4, 5, 6</sup>. Recent studies have started identifying lactate as a multifunctional signalling molecule<sup>7</sup>. Lactate is generated when pyruvate is reduced by lactate dehydrogenase (LDH) during glycolysis, occurring under both anaerobic and aerobic conditions<sup>7</sup>.



Some studies have found elevated initial lactate levels in systemic poisoning to be correlated with poor short-term outcomes, including complications and death<sup>8,9</sup>. A survey conducted in 2017 by Erfantalab et al<sup>10</sup> showed that elevated lactate levels were associated with higher mortality rates in aluminum phosphide poisoning. A meta-analysis conducted in 2018 showed significantly raised mortality and morbidity between paraquat consumption and elevated lactate levels<sup>11</sup>. Manini et al, found a correlation between elevated lactate and acute drug overdose for significant mortality and ICU stay<sup>12</sup>.

A 2018 meta-analysis demonstrated a significant association between raised lactate levels and higher mortality and morbidity following paraquat ingestion<sup>10</sup>. Furthermore, Cheung et al<sup>13</sup> established a correlation between elevated lactate levels, increased mortality, and extended ICU stays in patients with acute drug overdose. There have been limited studies on the use of serum lactate as a prognostic criterion, irrespective of the type of poisoning. The precise role of lactate as a prognostic tool in all types of poisoning requires further investigation. This study seeks to evaluate the predictive value of lactate for adverse outcomes and its potential to inform clinical decision-making.

## Materials and Methods

### Aims & Objectives

To determine the prognostic utility of serum lactate levels in poisoning cases by identifying an optimal cut-off value for predicting mortality.

To assess the association between elevated lactate levels and outcomes such as mortality, hospital stay duration, need for hemodialysis, ICU admission, and mechanical ventilation.

To examine the association between mortality rates and different types of poisoning.

### Method

This retrospective cohort study was carried out in the Emergency Department of SSIMS & RC, a tertiary care hospital in Davangere. Data were retrieved from electronic medical records. Serum lactate levels were assessed from the initial blood sample obtained in the Emergency Department and were correlated with patient outcomes. Blood samples were drawn within 24 hours of ingestion of poison. Arterial blood lactate concentrations were measured at the bedside for all control patients using the Radiometer ABL800 FLEX analyser, which utilizes amperometric electrodes with enzymatic membranes. The decision to obtain lactate values was made independently by the treating physician as part of routine clinical care, and results were immediately available to the clinical team. Only the initial arterial lactate obtained

in the emergency department was included in the analysis for both cases and controls; any subsequent measurements were excluded regardless of changes in value. Samples were analysed promptly after collection, following standard pre-analytical protocols. According to manufacturer specifications, the normal reference range for arterial lactate on the ABL800 FLEX is 0.7–2.5 mmol/L.

Using the sensitivity of serum lactate in predicting prognosis in acute drug overdose reported by Manini et al<sup>11</sup>, and applying a 95% confidence interval with a 10% relative allowable error, the calculated minimum required sample size was 95.

Data were entered in MS Excel and analysed using SPSS Version 25. Numerical variables were presented as mean  $\pm$  SD or median (IQR), and categorical variables as frequency and percentage. Associations with mortality were assessed using Chi-square test for categorical variables and Mann-Whitney U test for numerical variables. Relationships between continuous variables were assessed using Pearson or Spearman correlation analyses, depending on data distribution. A p-value  $<0.05$  was considered statistically significant.

### Inclusion and Exclusion Criteria

The study included adult patients ( $>18$  years) who arrived at the Emergency Room within 24 hours of consuming a toxic substance. Patients were excluded if they presented with any prescription drug overdose, were pregnant, or had an alternative diagnosis causing elevated lactate, such as sepsis or

trauma. Patients who had any organ hypoperfusion not secondary to toxin consumption were also excluded.

### Ethical Considerations

This retrospective observational study utilized anonymized patient data without direct patient contact or intervention. The study was submitted to the Institutional Review Board (IRB) of SSIMS & RC under Reference Number: IERB 236/2025. As per institutional policy, retrospective studies using anonymized data do not require formal IRB approval, and therefore, the requirement for approval and informed consent was waived under institutional policy.

Use of AI: LLM was used for grammar and spelling checking.

During the study period, 148 cases were screened, 49 cases were excluded, and a total of 99 patients were included in the study. Among the 99 included patients, 62 (62.6%) were males and 37 (37.4%) were females. In our study, 14 (14.1%) patients expired, 74 (74.8%) were discharged, and 11 (11.1%) were discharged against medical advice (DAMA). Out of 99 patients, 3 (3.0%) required haemodialysis (HD) sessions.

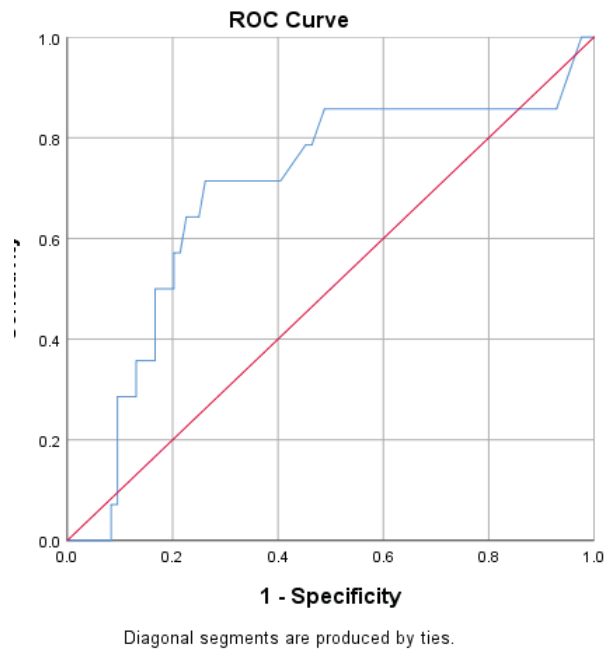
The mean age of the cohort was  $32.81 \pm 11.88$  years, with a mean serum lactate value of  $2.97 \pm 2.49$  (Table 1). The mean duration of hospital stay was  $4.47 \pm 4.77$  days, ICU stay was  $1.41 \pm 2.89$  days, and mechanical ventilation was  $1.20 \pm 3.20$  days.

**Table 1. Descriptive statistics for patient demographics and clinical variables.**

| Variables                      | N  | Minimum | Maximum | Mean  | SD    |
|--------------------------------|----|---------|---------|-------|-------|
| Age                            | 97 | 18      | 71      | 32.81 | 11.88 |
| S. Lactate Value               | 98 | 0.4     | 12.6    | 2.97  | 2.49  |
| Days of Hospital Stay          | 99 | 0       | 38      | 4.47  | 4.77  |
| Days of ICU stay               | 96 | 0       | 19      | 1.41  | 2.89  |
| Days of Mechanical Ventilation | 96 | 0       | 25      | 1.20  | 3.20  |

ROC analysis identified an optimal cut-off value for serum lactate of 3.25 mmol/L for predicting mortality (Figure 1). This cut-off demonstrated an AUC value of 0.691 with a 95% Confidence Interval of 0.530 to 0.853. The sensitivity was 71.4%, specificity 73.8%, positive predictive value 31.25%, negative predictive value 93.9%, and accuracy 73.5%. Serum lactate levels at admission demonstrated moderate predictive value for mortality in poisoning patients. These findings suggest that while elevated lactate levels may aid in identifying patients at higher risk, their high negative predictive value makes them particularly useful for excluding mortality risk in this cohort.

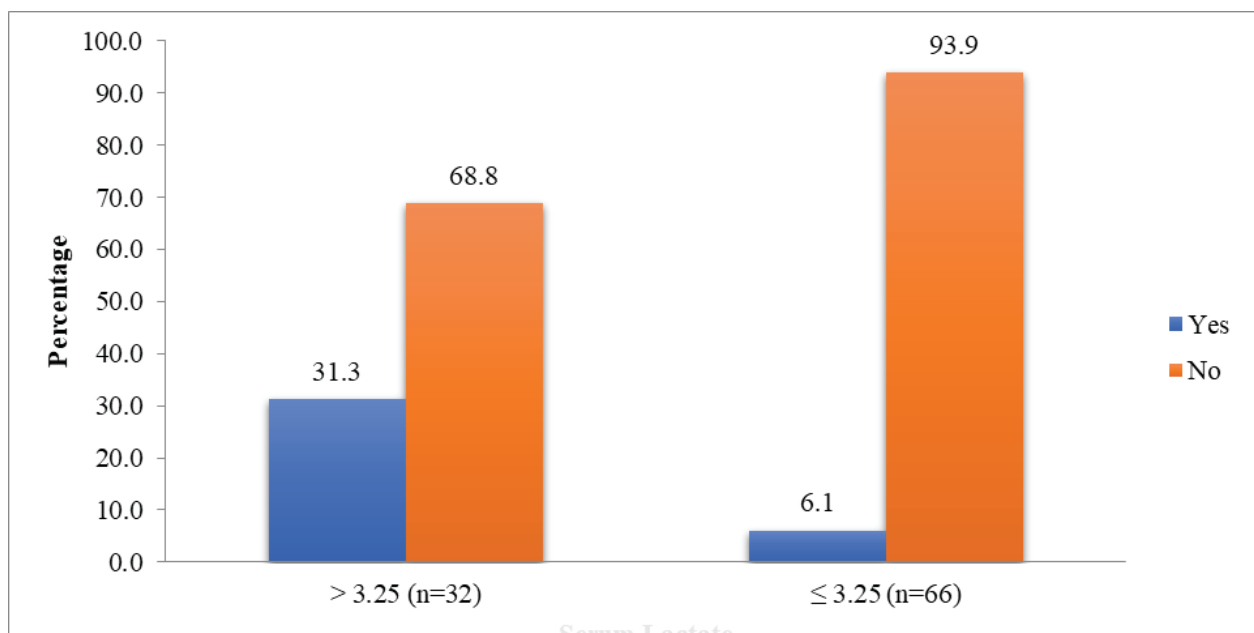
This figure shows the ROC curve for serum lactate in predicting mortality. The area under the curve (AUC) was 0.691. Diagonal segments are produced by ties.



**Figure 1: ROC curve for lactate**

Mortality was significantly higher among patients with serum lactate levels exceeding 3.25 mmol/L (31.3%) compared to those with levels at or below 3.25 mmol/L (6.1%) (Figure 2, Table 2). The association between serum lactate levels and

mortality was assessed using the Odds Ratio (OR), revealing that patients with serum lactate >3.25 mmol/L had 7.05 times higher odds of mortality compared to those with lactate ≤3.25 mmol/L (OR = 7.05, 95% CI: 2.00–24.77, p = 0.002).



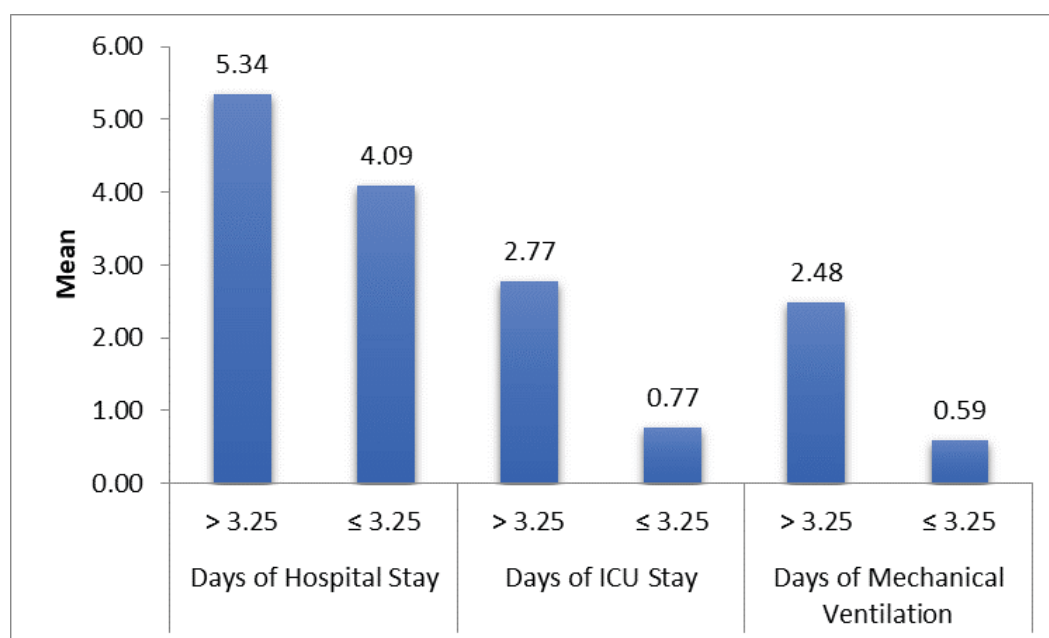
**Figure 2: Association between serum lactate and mortality.**

**Table 2. Association of Serum Lactate with Mortality**

| Serum Lactate | Mortality |      |    |      | $\chi^2$ Value | p Value |
|---------------|-----------|------|----|------|----------------|---------|
|               | Yes       |      | No |      |                |         |
|               | N         | %    | N  | %    |                |         |
| > 3.25 (n=32) | 10        | 31.3 | 22 | 68.8 | 9.205          | 0.002   |
| ≤ 3.25 (n=66) | 4         | 6.1  | 62 | 93.9 |                |         |

The mean duration of ICU stays ( $2.77 \pm 3.71$  days) and mechanical ventilation ( $2.48 \pm 4.60$  days) was significantly higher in patients with a serum lactate value > 3.25 compared to those with a value ≤ 3.25 (ICU stay:  $0.77 \pm 2.15$  days; mechanical ventilation:  $0.59 \pm 2.04$  days) (Figure 3, Table 3). The proportion of patients requiring mechanical ventilation was also significantly higher in the higher lactate group (65.6%) compared to the lower lactate group (19.7%) (Table 4). The odds ratio was approximately 7.78, indicating that patients with a serum lactate level >3.25 mmol/L had about 7.8 times higher odds of requiring mechanical ventilation compared to those with lower lactate

levels. Similarly, ICU admissions were notably higher among patients with serum lactate levels above 3.25 mmol/L (75.0%) compared to those with lower levels (24.2%) (Table 5). The odds of requiring ICU care were about 9.4 times greater in the higher lactate group, reflecting a strong association between elevated lactate and ICU admission in these patients. In contrast, no statistically significant association was found between elevated serum lactate levels and the requirement for haemodialysis sessions ( $p=0.515$ ), despite a higher proportion of patients with lactate >3.25 requiring HD (6.3%) compared to those with ≤3.25 (1.5%).



**Figure 3: Comparison of mean hospital stay, ICU stay, and mechanical ventilation duration in patients with serum lactate >3.25 mmol/L versus ≤3.25 mmol/L.**

**Table 3. Comparison of Hospital stay, ICU stay, and Mechanical Ventilation with Serum Lactate.**

| Serum Lactate                  | N  | Mean | SD   | Median (IQR) | Mann Whitney U Value | p Value |
|--------------------------------|----|------|------|--------------|----------------------|---------|
| Days of Hospital Stay          |    |      |      |              |                      |         |
| > 3.25                         | 32 | 5.34 | 6.93 | 3 (1-7)      | 1032.5               | 0.857   |
| ≤ 3.25                         | 66 | 4.09 | 3.29 | 3 (2-5)      |                      |         |
| Days of ICU Stay               |    |      |      |              |                      |         |
| > 3.25                         | 31 | 2.77 | 3.71 | 1 (1-4)      | 449.5                | <0.001  |
| ≤ 3.25                         | 64 | 0.77 | 2.15 | 0 (0-0.75)   |                      |         |
| Days of Mechanical Ventilation |    |      |      |              |                      |         |
| > 3.25                         | 31 | 2.48 | 4.60 | 1 (0-4)      | 505.5                | <0.001  |
| ≤ 3.25                         | 64 | 0.59 | 2.04 | 0 (0-0)      |                      |         |

**Table 4. Association between Serum Lactate and Mechanical Ventilation**

| Serum Lactate | Mechanical Ventilation |      |    |      | $\chi^2$ Value | p Value |
|---------------|------------------------|------|----|------|----------------|---------|
|               | Yes                    |      | No |      |                |         |
|               | N                      | %    | N  | %    |                |         |
| > 3.25 (n=32) | 21                     | 65.6 | 11 | 34.4 | 20.064         | <0.001  |
| ≤ 3.25 (n=66) | 13                     | 19.7 | 53 | 80.3 |                |         |

**Table-5. Association between Serum Lactate and ICU Stay**

| Serum Lactate | ICU Stay |      |    |      |        | p Value |
|---------------|----------|------|----|------|--------|---------|
|               | Yes      |      | No |      |        |         |
|               | N        | %    | n  | %    |        |         |
| > 3.25 (n=32) | 24       | 75.0 | 8  | 25.0 | 22.984 | <0.001  |
| ≤ 3.25 (n=66) | 16       | 24.2 | 50 | 75.8 |        |         |

The serum lactate value was significantly higher in rodenticide poisoning ( $5.15 \pm 3.68$ ) compared to corrosive ( $1.20 \pm 0.42$ ), herbicide ( $2.87 \pm 2.18$ ), and insecticide ( $2.55 \pm 2.25$ ) poisonings (Figure 4, Table 6). Rodenticide poisoning also had a significantly

higher mortality rate (50.0%) compared to other categories (Figure 5, Table 7), and the highest proportion of patients requiring an ICU stay (87.5%) (Figure 6, Table 8)

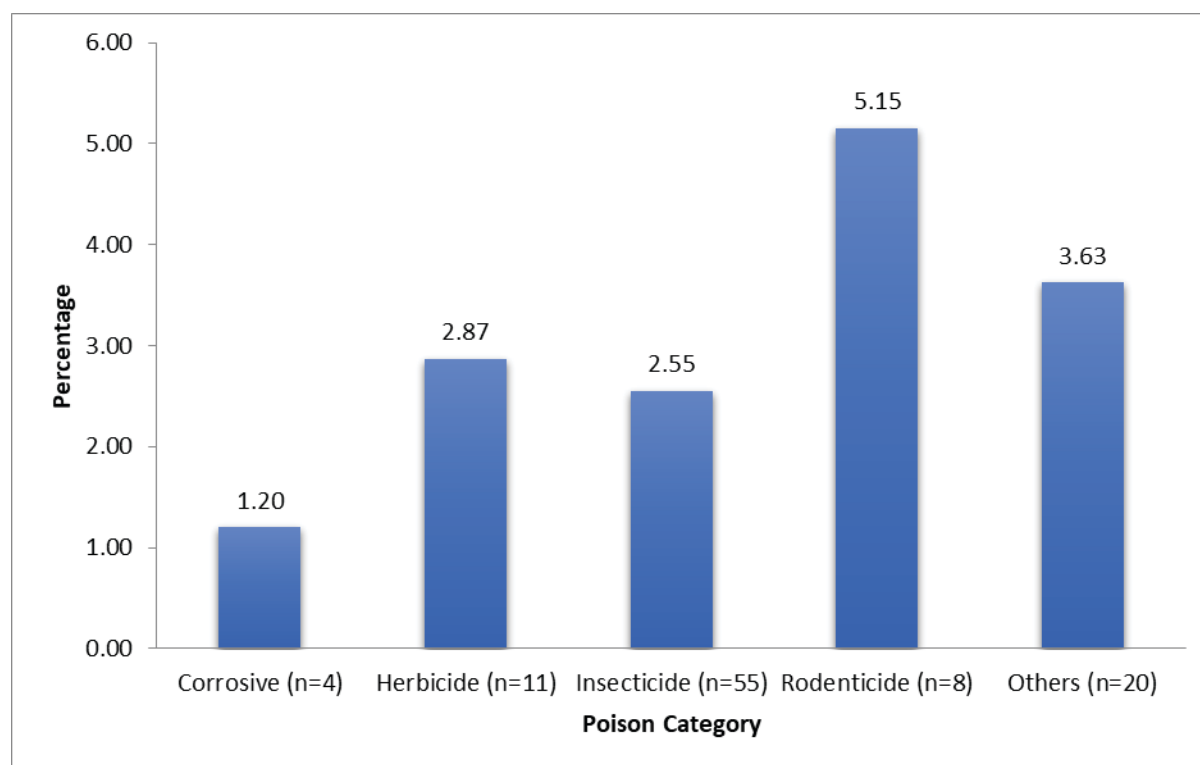


Figure 4: Comparison of mean serum lactate levels across poisoning categories.

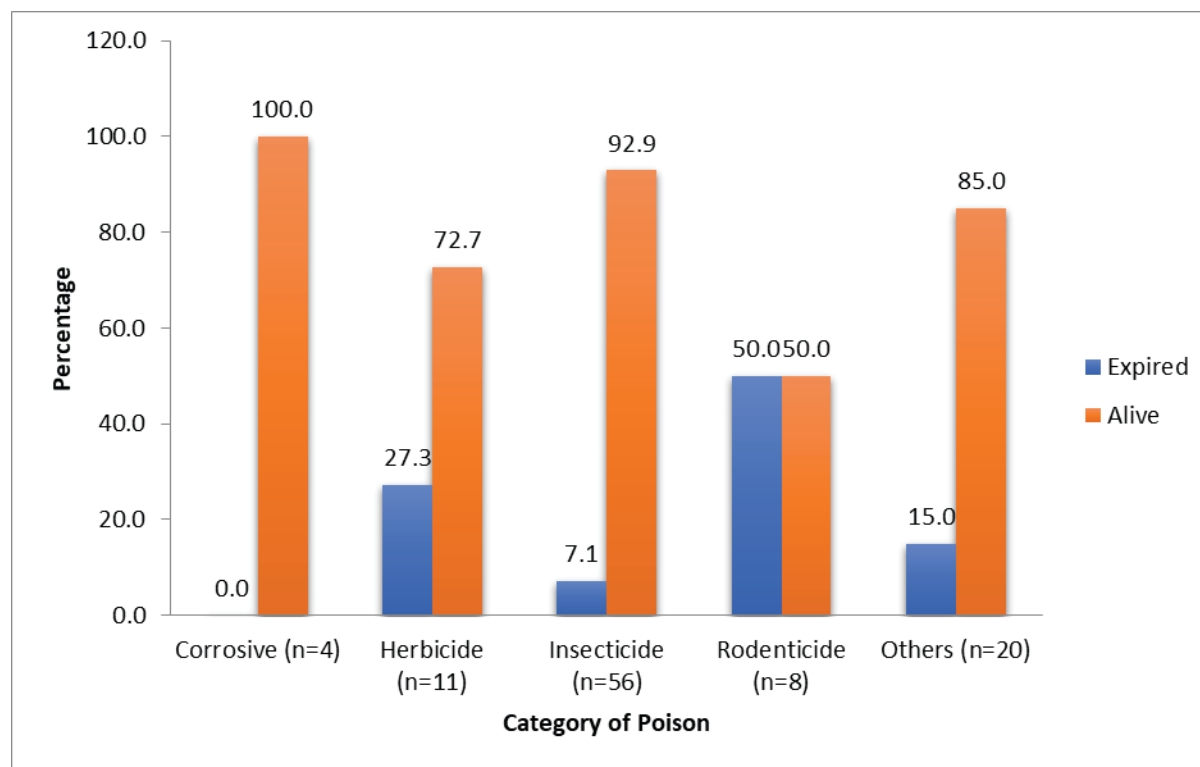


Figure 5: Comparison of mortality rates across poisoning categories.

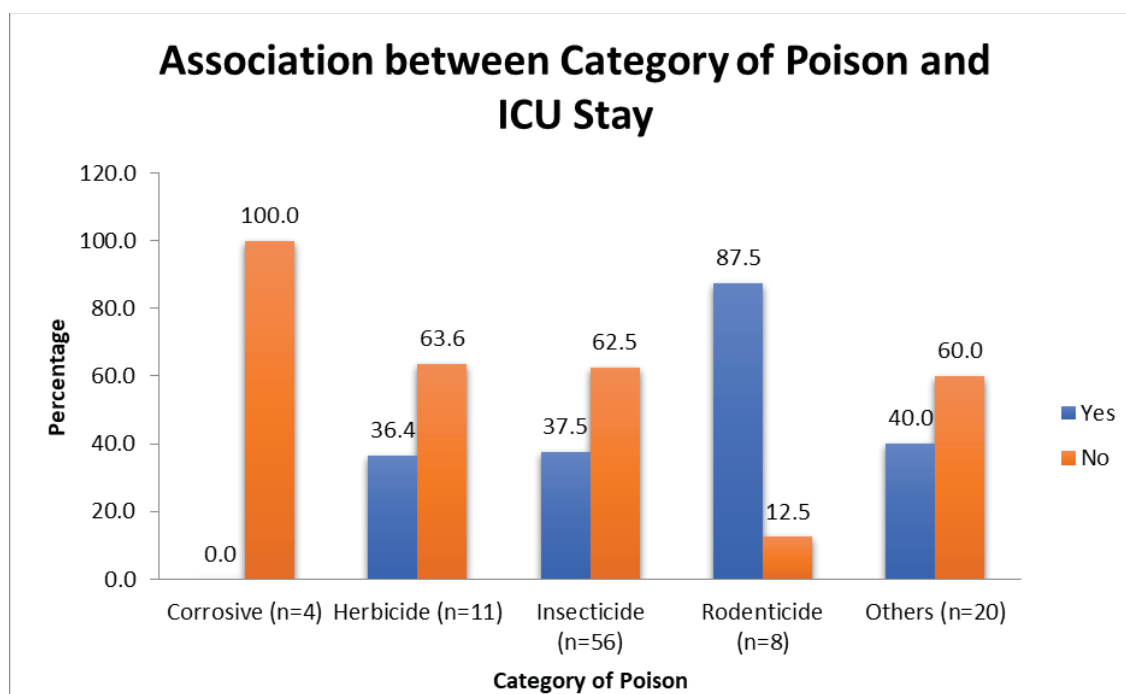


Figure 6: Comparison of ICU stay across poisoning categories.

Table 6. Comparison of mortality rates across poisoning categories

| Category Poison    | Serum Lactate |      | F Value | p Value |
|--------------------|---------------|------|---------|---------|
|                    | Mean          | SD   |         |         |
| Corrosive (n=4)    | 1.20          | 0.42 | 2.993   | 0.023   |
| Herbicide (n=11)   | 2.87          | 2.18 |         |         |
| Insecticide (n=55) | 2.55          | 2.25 |         |         |
| Rodenticide (n=8)  | 5.15          | 3.68 |         |         |
| Others (n=20)      | 3.63          | 2.49 |         |         |

Table 7. Association between Category of Poison and Mortality.

| Category of Poison | Mortality |      |    |       | $\chi^2$ Value | p Value |
|--------------------|-----------|------|----|-------|----------------|---------|
|                    | Yes       |      | No |       |                |         |
|                    | n         | %    | N  | %     |                |         |
| Corrosive (n=4)    | 0         | 0.0  | 4  | 100.0 | 12.964         | 0.011   |
| Herbicide (n=11)   | 3         | 27.3 | 8  | 72.7  |                |         |
| Insecticide (n=56) | 4         | 7.1  | 52 | 92.9  |                |         |
| Rodenticide (n=8)  | 4         | 50.0 | 4  | 50.0  |                |         |
| Others (n=20)      | 3         | 15.0 | 17 | 85.0  |                |         |

**Table 8. Association between Category of Poison and ICU Stay.**

| Category of Poison | ICU Stay |      |    |       | X <sup>2</sup> Value | p Value |
|--------------------|----------|------|----|-------|----------------------|---------|
|                    | Yes      |      | No |       |                      |         |
|                    | N        | %    | N  | %     |                      |         |
| Corrosive (n=4)    | 0        | 0.0  | 4  | 100.0 | 10.353               | 0.035   |
| Herbicide (n=11)   | 4        | 36.4 | 7  | 63.6  |                      |         |
| Insecticide (n=56) | 21       | 37.5 | 35 | 62.5  |                      |         |
| Rodenticide (n=8)  | 7        | 87.5 | 1  | 12.5  |                      |         |
| Others (n=20)      | 8        | 40.0 | 12 | 60.0  |                      |         |

**Table 9. Association between Category of Poison and Mechanical Ventilation.**

| Category of Poison | Mechanical Ventilation |      |    |       | $\chi^2$ Value | p Value |
|--------------------|------------------------|------|----|-------|----------------|---------|
|                    | Yes                    |      | No |       |                |         |
|                    | N                      | %    | N  | %     |                |         |
| Corrosive (n=4)    | 0                      | 0.0  | 4  | 100.0 | 8.375          | 0.079   |
| Herbicide (n=11)   | 4                      | 36.4 | 7  | 63.6  |                |         |
| Insecticide (n=56) | 17                     | 30.4 | 39 | 69.6  |                |         |
| Rodenticide (n=8)  | 6                      | 75.0 | 2  | 25.0  |                |         |
| Others (n=20)      | 7                      | 35.0 | 13 | 65.0  |                |         |

## Discussion

In our study, we found a significant correlation between lactate and the prognosis of poisoning patients. The value of 3.25 was determined through the ROC curve analysis, with values above this threshold being associated with significantly higher mortality, prolonged hospitalization, ICU stay, and need for mechanical ventilation.

Poison-specific lactate values can be used to provide better prognostication. Nevertheless, an initial lactate values at arrival to the ED offers valuable information regarding the prognosis of the patient which can be used regardless of poison and even in case of unknown poisons or mixed overdose.

Our study found a mortality rate of 32% in patients with a lactate value above 3.25 mmol/L, compared to 6% in those with lower initial values,

representing a sevenfold increase in mortality risk. This in comparison to the study done by Manini et al<sup>5</sup> in acute drug overdose, where the optimal lactate cut point was 3.0 mmol, which showed a 15.8-fold increase in mortality.

In comparison to Cheung et al<sup>13</sup>, who reported a lactate cut-off of 5 mmol/L predicting mortality with a 15-fold increase in risk among acute drug overdose patients, our study identified a lower threshold of 3.25 mmol/L associated with a 7-fold increase in mortality across a broader spectrum of poisoning cases. This suggests that while higher lactate thresholds remain significant predictors in drug overdoses, lower lactate levels may still hold prognostic value in mixed or unknown poisonings presenting to the emergency department

In the study conducted by Erfantalab et al<sup>9</sup>, they found that in patients presenting with acute aluminium phosphide toxicity, a lactate level

above 2.75 at 4 hours was associated with a higher fatality rate. This finding aligns with our study, highlighting the utility of lactate levels for early prognostication.

ICU stays were substantially more common in cases where the serum lactate value was higher than 3.25 (75.0%), with the likelihood of needing an ICU stay being almost 9.375 times higher. Patients with serum lactate levels above 3.25 mmol/L (65.6%) were significantly more likely to require mechanical ventilation, with an estimated 7.78-fold increase in the likelihood of needing ventilatory support. A study by Lionte et al<sup>7</sup> showed similar findings where lactate levels of greater than 3.17 showed worse short-term outcomes like the need for ICU care, mechanical ventilation need for inotropes, and increased mortality. A meta-analysis by Li et al<sup>10</sup>, showed elevated lactate correlated with poor prognosis in paraquat poisoning. Lui X et al<sup>12</sup> found that an initial lactate value above 2.5 was associated with significantly higher mortality in paraquat poisoning. These studies support our findings of elevated lactate being associated with higher mortality, ICU stay, and need for mechanical ventilation. Strengths of this study include its real-world, diverse poisoning cohort and practical applicability of lactate thresholds, regardless of the type of poison. Limitations include its retrospective, single-centre design and lack of serial lactate measurements to assess lactate clearance.

However, further studies are necessary to validate these findings across a broader range of toxins and to refine lactate cut-off points for different types of poisoning and lactate-tailored therapy. Serial lactate monitoring with lactate clearance with end-therapy goals may be the future of treatment in poisoning cases.

### Conclusion

Our study underscores the significant prognostic value of serum lactate levels in poisoning cases, with a lactate threshold of 3.25 mmol/L found to be a reliable predictor of adverse outcomes. Specifically, patients with lactate levels >3.25 mmol/L exhibited a significantly higher mortality rate (31.3%) compared

to patients with lower levels (6.1%), representing a more than sevenfold increase in mortality risk. We found a strong association exists between high serum lactate and the need for critical care interventions. Patients with lactate levels exceeding 3.25 showed a significantly higher probability of ICU admission and mechanical ventilation, with odds ratios of approximately 9.4 and 7.8, respectively. These findings support the use of serum lactate as a valuable, non-specific marker for early prognostication in the emergency department, suggesting that lactate levels could effectively guide clinical decision-making regardless of the poison type.

The study also revealed that rodenticide poisoning was significantly associated with higher lactate values ( $5.15 \pm 3.68$ ) and the highest mortality rate (50%). Further studies are necessary to validate these findings across a broader range of toxins and to refine lactate cut-off points for different types of poisoning and lactate-tailored therapy.

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### Declarations

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**Conflict of Interest:** None declared

**Ethical Approval:** IERB 236/2025. As per institutional policy, retrospective studies using anonymized data do not require formal IRB approval, and therefore, the requirement for approval and informed consent was waived under institutional policy.

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# Pattern of Pedestrian Limb Injuries in Road Traffic Accident Cases Autopsied at Victoria Hospital, BMCRI, Bengaluru

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## Abstract

**Background:** Pedestrians represent one of the most exposed and vulnerable categories of road users, bearing a high burden of trauma in urban environments. This study presents a systematic analysis of localized limb injury patterns in fatal pedestrian road traffic accidents (RTAs) and evaluates demographic and situational correlates which include electronic distraction (mobile phone/Earphone usage) and alcohol consumption.

**Materials and Methods:** A retrospective archive-based cross-sectional study examined 189 autopsied cases of fatal pedestrian accidents out of a total population of 1,503 RTA autopsies conducted at the Department of Forensic Medicine and Toxicology in a Tertiary care centre in Bengaluru, from January 2023 to December 2024. Statistical evaluations included Chi-square tests of independence, Odds Ratios (OR), Chi-square goodness-of-fit tests, and two-tailed exact binomial tests.

**Results:** Males, young adults, and people with less education were the most common victims of pedestrian fatalities, which made up 12.57% of road traffic accident autopsies. Many of these accidents took place in peri-urban regions. At the moment of impact, younger pedestrians ( $\leq 40$  years old) were far more likely to be distracted by electronic devices. Light four-wheelers were the most prevalent offending vehicles, and lower limbs were more commonly and severely wounded than higher limbs. The majority of deaths took place in urban and periurban areas.

**Conclusion:** Pedestrian limb trauma shows a strong anatomical preference for the lower extremities, reflecting vehicle impact mechanics. The high association between youth and device-related electronic distraction underscores the need for targeted enforcement and pedestrian infrastructure safeguards along urban outskirts.

**Keywords:** Forensic Autopsy, Pedestrian Trauma, Limb Injuries, Electronic Distraction, Vehicle Mechanics, Biomechanical Pattern.

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## Introduction Or Back Ground

Pedestrian injuries and fatalities resulting from road traffic accidents continue to represent a major global public health concern. According to the World Health Organization (WHO), road traffic injuries remain one of the leading causes of mortality and disability worldwide, with pedestrians contributing a substantial proportion of these deaths.<sup>1</sup> In 2023, road traffic accidents were responsible for approximately 1.19 million deaths globally, with vulnerable road users such as pedestrians, cyclists, and motorized two-wheeler users accounting for a significant share of these fatalities, particularly in low- and middle-income countries.<sup>2,3</sup>

In rapidly expanding cities such as Bengaluru, ongoing infrastructure development, highway expansion, and increasing vehicles are common issues. They have created hazardous environments where pedestrians are exposed to high-speed traffic.<sup>4</sup> Pedestrians are especially vulnerable during collisions as they lack any form of external structural protection. The severity and pattern of injuries depend on many biomechanical and environmental factors, including the force of impact, braking characteristics, vehicle design, bumper height, and the demographic profile of the pedestrian.<sup>5</sup>

Severe cranial, thoracic, and abdominal injuries are often the direct cause of death in pedestrian collisions. Extremity injuries provide important evidence regarding the sequence and nature of impact. The upper and lower limbs frequently serve as the initial contact points or defensive structures during collisions. Careful analysis of extremity trauma may therefore help reconstruct the mechanism of injury, identify the point of primary impact, and evaluate associated behavioral risk factors such as mobile phone distraction. This study presents a detailed topographical assessment of pedestrian limb injuries and examines the demographic, behavioral, and situational variables associated with fatal pedestrian accidents autopsied at a major tertiary care center in South India.

## Aims and Objectives

1. To analyze the distribution and pattern of upper and lower limb injuries sustained by pedestrians in fatal road traffic accidents.
2. To evaluate the influence of demographic variables (age, gender, and educational status) and behavioral risk factors (alcohol consumption and electronic device distraction) on injury patterns and accident characteristics.

## Materials and Methods

**Study Setting:** This retrospective cross-sectional study using autopsy records was conducted in the Department of Forensic Medicine and Toxicology at a Tertiary care centre in Bengaluru.

**Study Period:** January 2023 to December 2024.

**Study Material:** A total of 1,503 consecutive road traffic accident autopsies performed during this period were reviewed. Among these, cases involving pedestrians fatally struck by motorized vehicles were identified, resulting in a final study sample of 189 cases.

## Inclusion and Exclusion Criteria

Inclusion criteria consisted of cases in which the deceased was functioning as a pedestrian at the time of the collision. Confirmation was obtained through police inquest reports, hospital medical records, and post-mortem examination findings.

## Exclusion Criteria

1. All non-pedestrian road traffic fatalities, including drivers, passengers, motorcycle riders, pillion riders, and cyclists, were excluded.
2. Cases with advanced decomposition, severe mutilation, or fragmentation preventing reliable assessment of limb injuries were also excluded.

### Ethical Clearance and Documentation

The study protocol received approval from the Institutional Ethics Committee. As this was a retrospective archive-based study utilizing previously documented autopsy records, no fresh post-mortem photographs were obtained by the investigators. All autopsies followed a departmental standard proforma and same team has handled most of the autopsies. Furthermore, standardized digital photographic records were not uniformly available in historical archives. To ensure transparency and consistency, all injuries were systematically documented and tabulated in Tables 4 and 5, which served as the primary objective data source for injury analysis.

### Data Collection and Statistical Analysis

Data were obtained from autopsy reports, Forensic Science Laboratory (FSL) toxicology reports, hospital records, and police panchnamas. Variables studied included age, gender, educational status, type of offending vehicle, accident location, alcohol consumption, and electronic device use.

Data entry and validation were performed using Microsoft Excel. Statistical analysis was conducted using IBM SPSS Statistics version 30.0 and SciPy libraries.

The following statistical methods were applied:

- Chi-square ( $\chi^2$ ) test of independence and Odds Ratios (OR) were used to assess categorical

associations and estimate risk measures with 95% confidence intervals.

- A two-tailed exact binomial test was applied to compare isolated upper and lower limb involvement.
- Chi-square goodness-of-fit analysis was used to evaluate the distribution of vehicle types and accident locations.
- A p-value of  $<0.05$  was considered statistically significant.

Pedestrian fatalities accounted for 12.57% (189 of 1,503) of all road traffic accident autopsies conducted during the study period. A marked male predominance was observed, with 167 males (88.36%) and 22 females (11.64%), producing a male-to-female ratio of 7.6:1.

The highest number of cases belonged to the 21–30 years age group, accounting for 23.28% ( $n=44$ ), followed by the 41–50 years age group at 20.63% ( $n=39$ ). Individuals aged over 60 years collectively constituted 28.04% ( $n=53$ ) of the study population. Educational analysis revealed that 39.15% had below high school education and 74.7% were below the level of graduate degree. When combined with high number of cases in peri-urban outskirts ( $n=91$ ), there is a strong link indicating that lower educational/socioeconomic cohorts face higher exposure to these hazardous environments. (Table 1; Figures 1, 2, 3).

**Table 1. Socio-Demographic Matrix of Autopsied Pedestrian Fatalities (N = 189)**

| Demographic Parametric Vector | Observed Frequency (n) | Percentage (%) |
|-------------------------------|------------------------|----------------|
| Gender: Male                  | 167                    | 88.36%         |
| Gender: Female                | 22                     | 11.64%         |
| Age Bracket: 11–20 Years      | 9                      | 4.76%          |
| Age Bracket: 21–30 Years      | 44                     | 23.28%         |
| Age Bracket: 31–40 Years      | 29                     | 15.34%         |
| Age Bracket: 41–50 Years      | 39                     | 20.63%         |
| Age Bracket: 51–60 Years      | 15                     | 7.94%          |
| Age Bracket: 61–70 Years      | 29                     | 15.34%         |

Continue....

|                                            |    |        |
|--------------------------------------------|----|--------|
| Age Bracket: 71–80 Years                   | 20 | 10.58% |
| Age Bracket: >80 Years                     | 4  | 2.12%  |
| Educational Level: Illiterate              | 28 | 14.81% |
| Educational Level: Primary School          | 13 | 6.88%  |
| Educational Level: Middle School           | 33 | 17.46% |
| Educational Level: High School             | 34 | 17.99% |
| Educational Level: 12th Standard           | 32 | 16.93% |
| Educational Level: Graduate Degree         | 36 | 19.05% |
| Educational Level: Unknown / Un-documented | 13 | 6.88%  |

### Observations And Results

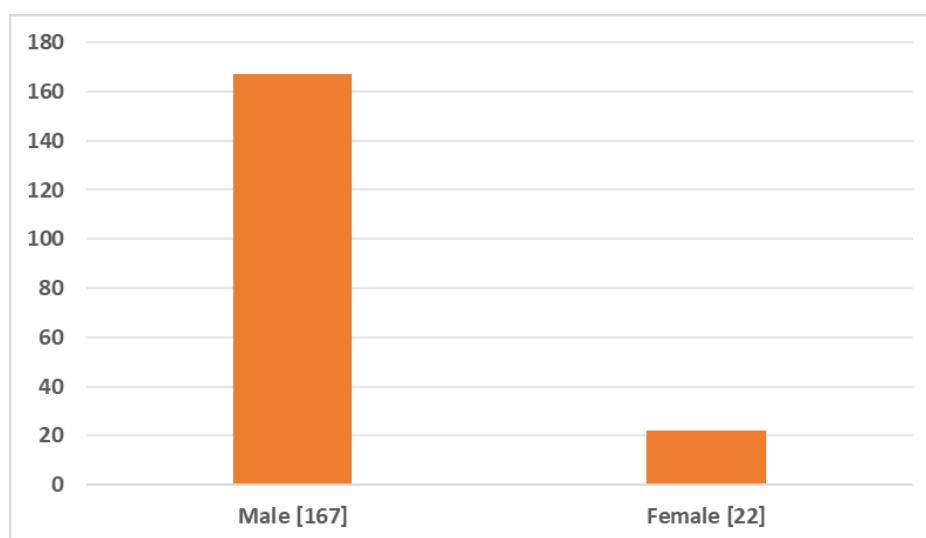


Figure 1: Gender wise distribution of Cases.

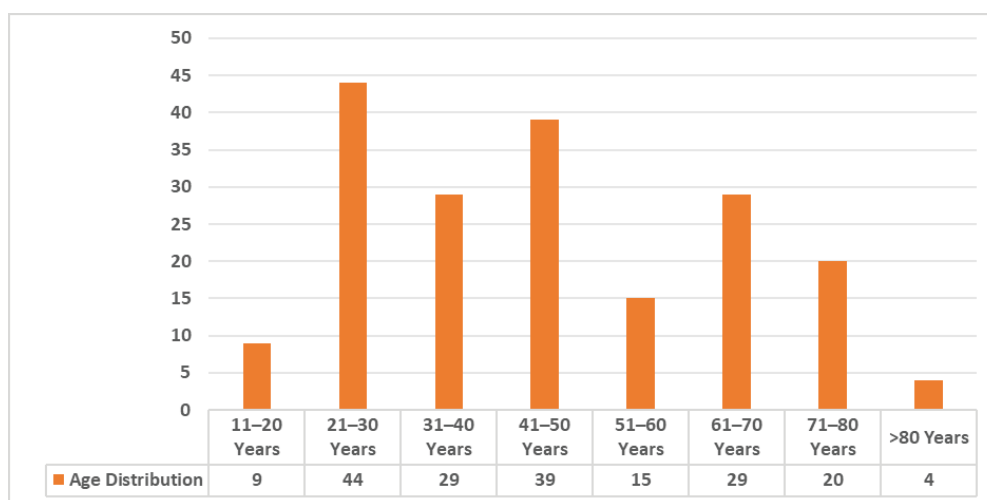


Figure 2: Age wise distribution of Cases.

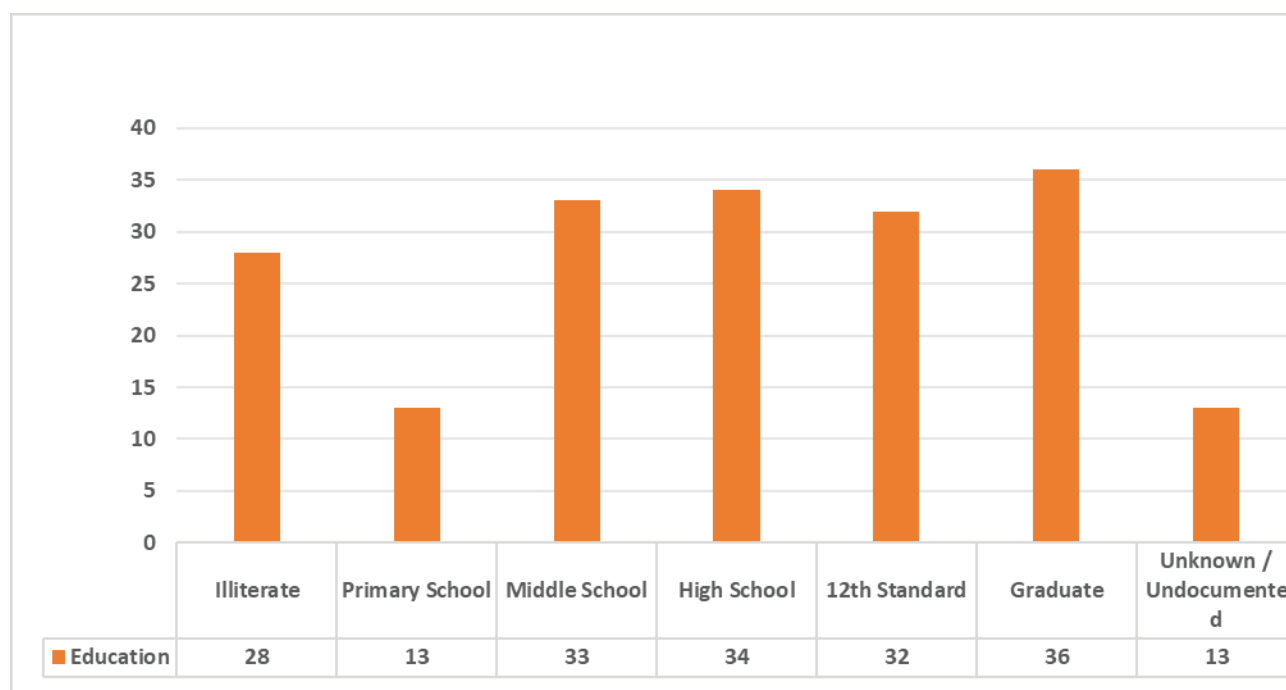


Figure 3: Education Level of Cases.

### Behavioral Co-factors and Risk Association

Acute alcohol consumption was confirmed through FSL toxicological analysis in 12 cases, all of whom were male. Although alcohol positivity was confined to males, Fisher's exact test did not demonstrate statistical significance ( $p = 0.3660$ ), likely because of the relatively small number of positive cases.

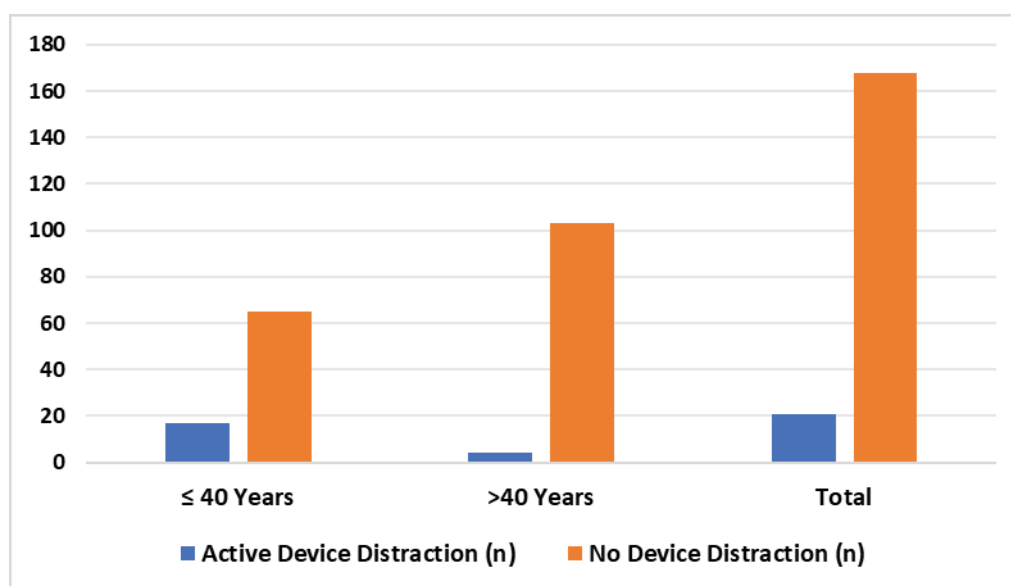
Active electronic device distraction, including mobile phone conversation or earphone use during

the accident, was documented in 21 cases. To evaluate the relationship between age and electronic distraction, the sample was categorized into two groups: individuals aged  $\leq 40$  years and those aged  $> 40$  years.

Among pedestrians aged  $\leq 40$  years, 17 of 82 were actively using electronic devices at the time of impact. In contrast, only 4 of 107 pedestrians aged  $> 40$  years were similarly distracted. (Table 2; Figure 4)

Table 2. Risk Association Matrix for Electronic Distraction Across Age Cohorts.

| Age Category Stratum                    | Active Device Distraction (n) | No Device Distraction (n) | Total Cases (n) | Inferential Metrics Summary                                                                                         |
|-----------------------------------------|-------------------------------|---------------------------|-----------------|---------------------------------------------------------------------------------------------------------------------|
| Youth & Young Adults ( $\leq 40$ Years) | 17                            | 65                        | 82              | Chi-Square ( $\chi^2$ ) = 11.9074<br>$df = 1$ ; $p = 0.000559$<br>Odds Ratio = 6.7346<br>(95% CI: 2.1699 - 20.9023) |
| Older Adults ( $> 40$ Years)            | 4                             | 103                       | 107             |                                                                                                                     |
| Total Population                        | 21                            | 168                       | 189             |                                                                                                                     |



**Figure 4: Distraction by Devices (Mobile/ Earphones).**

Chi-square analysis demonstrated a highly significant association between younger age and electronic distraction ( $\chi^2 = 11.9074$ ,  $df = 1$ ,  $p = 0.000559$ ). The calculated Odds Ratio was 6.7346 (95% CI: 2.1699–20.9023), indicating that pedestrians aged 40 years or younger had significantly greater odds of electronic distraction immediately before the fatal incident.

### **Anatomical Laterality and Injury Distribution**

Combined upper and lower limb injuries were the most common presentation, observed in 119 cases.

Isolated limb injuries accounted for 37.04% of the sample, including isolated lower limb injuries in 49 cases and isolated upper limb injuries in 21 cases.

A two-tailed exact binomial test comparing isolated lower and upper limb injuries revealed a statistically significant predominance of lower limb involvement ( $p = 0.001093$ ), confirming the greater vulnerability of lower extremities in pedestrian collisions. (Table 3; Figure 5)

**Table 3. Distribution of Gross Topographical Extremity Involvement.**

| Gross Topographical Layout | Observed Case Count (n) | Percentage (%) | Exact Directional Inference Metric                                                             |
|----------------------------|-------------------------|----------------|------------------------------------------------------------------------------------------------|
| Isolated Upper Limbs Only  | 21                      | 11.11%         | Dichotomized Paired Discordance:<br>Lower Limb (49) vs. Upper Limb (21)                        |
| Isolated Lower Limbs Only  | 49                      | 25.93%         |                                                                                                |
| Both Upper and Lower Limbs | 119                     | 62.96%         |                                                                                                |
| Total Sample Population    | 189                     | 100.00%        | Two-Tailed Exact Binomial $p = 0.001093$<br><br>Conclusion: Highly significant lower limb bias |

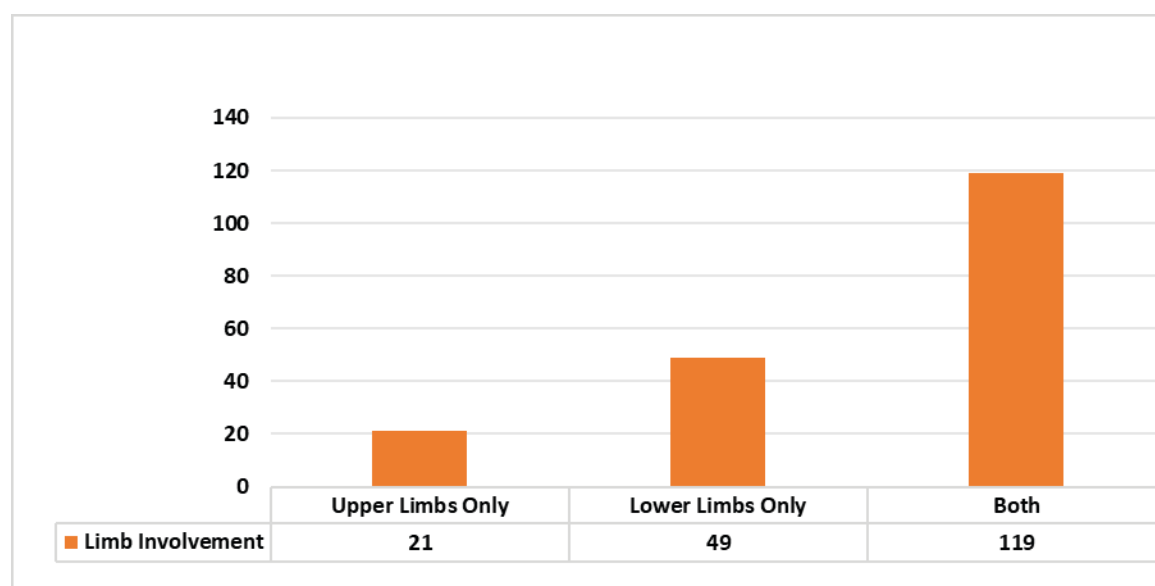


Figure 5: Pattern of Limb Involvement.

### Injury Patterns

Abrasions were the most frequently observed soft-tissue injuries, affecting 86.77% (n=164) of cases, followed by contusions in 70 cases and lacerations in 58. Fractures were identified in 73 cases.

### Upper Extremities

Abrasions were most commonly observed over the hands and digits in 108 cases and forearm region in 57 cases. Lacerations were also predominantly distributed over the hands and fingers. Fractures were relatively less common in the upper limbs and were most frequently noted in the shoulder girdle and upper arm. (Table 4)

Table 4. Detailed Micro-Anatomical Injury Matrix for Upper Extremities (N = 189).

| Anatomical Region | Abrasion (n) | Laceration (n) | Contusion (n) | Fracture (n) | Dislocation (n) | Crush Injury (n) |
|-------------------|--------------|----------------|---------------|--------------|-----------------|------------------|
| Shoulder Girdle   | 49           | 5              | 7             | 9            | 2               | 2                |
| Arm               | 32           | 4              | 11            | 6            | 2               | 1                |
| Elbow Joint       | 55           | 4              | 2             | 0            | 4               | 0                |
| Forearm           | 57           | 7              | 2             | 0            | 3               | 0                |
| Wrist Joint       | 24           | 8              | 2             | 1            | 3               | 0                |
| Hand / Digits     | 108          | 20             | 2             | 2            | 0               | 1                |

### Lower Extremities

Lower limb injuries demonstrated a greater concentration of severe skeletal trauma. Fractures

were predominantly observed in the femoral region, followed by the leg/crural segment and the hip/pelvic region. Abrasions were most commonly present over the knee joint. (Table 5)

**Table 5: Detailed Micro-Anatomical Injury Matrix for Lower Extremities (N = 189).**

| Anatomical Region | Abrasion (n) | Laceration (n) | Contusion (n) | Fracture (n) | Dislocation (n) | Crush Injury (n) |
|-------------------|--------------|----------------|---------------|--------------|-----------------|------------------|
| Hip / Pelvis      | 37           | 2              | 2             | 29           | 2               | 4                |
| Thigh             | 20           | 2              | 4             | 38           | 2               | 9                |
| Knee Joint        | 73           | 7              | 1             | 9            | 11              | 4                |
| Leg               | 40           | 16             | 7             | 31           | 0               | 9                |
| Ankle Joint       | 20           | 5              | 1             | 1            | 9               | 2                |
| Foot / Digits     | 35           | 5              | 1             | 7            | 5               | 9                |

### Vehicle Profile and Accident Location

Light four-wheelers, including passenger cars, SUVs, and light commercial vehicles, were the most common offending vehicles, accounting for 48 cases. This was followed by motorcycles and two-wheelers at 43, heavy commercial vehicles at 39, and hit-and-run cases at 37.

Chi-square goodness-of-fit analysis demonstrated a statistically significant non-uniform

distribution of vehicle involvement ( $\chi^2 = 43.2857$ ,  $df = 5$ ,  $p < 0.000001$ ).

Nearly half of the fatal accidents ( $n=91$ ) occurred in peri-urban or rural transition zones, while 78 cases occurred within urban city limits and 20 cases occurred on highways. Statistical analysis showed a significant concentration of pedestrian fatalities in peri-urban and urban regions compared with highways ( $\chi^2 = 45.3651$ ,  $df = 2$ ,  $p < 0.000001$ ). (Table 6)

**Table 6. Goodness-of-Fit Deviations for Striking Vehicles and Accident Locations.**

| Categorical Factor Vector     | Observed Count (n) | Percentage (%) | Goodness-of-Fit Statistical Metric                                                                                                                           |
|-------------------------------|--------------------|----------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Vehicle: Light 4-Wheeler      | 48                 | 25.40%         | Vehicle Profile Analysis:<br>Chi-Square ( $\chi^2$ ) = 43.2857<br>df = 5<br>p-value < 0.01<br>Highly Non-Uniform Profile<br>Dominance: Light 4-W & 2-W Bikes |
| Vehicle: 2-Wheeler Bike       | 43                 | 22.75%         |                                                                                                                                                              |
| Vehicle: Heavy Commercial     | 39                 | 20.63%         |                                                                                                                                                              |
| Vehicle: Hit-and-Run Case     | 37                 | 19.58%         |                                                                                                                                                              |
| Vehicle: 3-Wheeler Auto       | 15                 | 7.94%          |                                                                                                                                                              |
| Vehicle: 2-Wheeler Scooter    | 7                  | 3.70%          |                                                                                                                                                              |
| Location: Peri-Urban Outskirt | 91                 | 48.15%         | Spatial Location Analysis:<br>Chi-Square ( $\chi^2$ ) = 45.3651; $df = 2$<br>p-value < 0.01; High Outskirt Density                                           |
| Location: Intra-City Urban    | 78                 | 41.27%         |                                                                                                                                                              |
| Location: Main Highway        | 20                 | 10.58%         |                                                                                                                                                              |

## Discussion

Extremity injuries sustained in fatal pedestrian accidents provide valuable information regarding the biomechanics of vehicular impact. The predominance of male victims and the peak incidence among young adults observed in this study are consistent with findings from previous epidemiological studies conducted in developing urban settings.<sup>6</sup> Young adult males are more frequently exposed to traffic environments because of occupational and social mobility patterns.<sup>7</sup>

An important behavioral observation in this study was the strong association between younger age groups and active electronic distraction, including mobile phone use and earphone usage. Pedestrians aged 40 years or younger demonstrated significantly higher odds of distraction at the time of impact when compared with older individuals. Reduced situational awareness caused by electronic device use may impair the ability of pedestrians to identify approaching vehicles and react appropriately in high-risk traffic environments.<sup>8</sup> However we acknowledge small sample, possible under-reporting and lack of data for non-fatal pedestrians.

The injury distribution demonstrated a distinct predominance of lower limb involvement. This finding is biomechanically expected because the bumper and frontal components of moving vehicles usually strike the lower extremities first during pedestrian collisions. The high frequency of fractures involving the femur, leg, and pelvic regions reflects the substantial transfer of kinetic energy during primary impact.<sup>6</sup> The bumper height and frontal design of commonly involved vehicles, particularly light four-wheelers and motorcycles, correspond anatomically to these regions, thereby explaining the observed fracture distribution.

Upper limb injuries were predominantly soft-tissue injuries, particularly abrasions over the hands and digits. These injuries likely represent defensive or protective reflex actions in which pedestrians attempt to break their fall during secondary impact against the vehicle or road surface. The detailed anatomical mapping presented in Tables 4 and 5

provides important insight into the mechanics of pedestrian trauma.

The spatial distribution of accidents revealed that nearly half of the fatalities occurred in peri-urban and transitional road zones.<sup>9</sup> Such regions are commonly associated with mixed traffic movement, inadequate pedestrian infrastructure, poor illumination, and high vehicle speeds.<sup>10</sup> These environmental factors when combined with behavioral risks such as alcohol intake or mobile phone distraction, may contribute to the likelihood of fatal pedestrian injury.

These findings suggest that pedestrian safety strategies should incorporate both behavioral interventions and infrastructural improvements. Public awareness regarding electronic distraction, stricter traffic regulation enforcement, improved street lighting, continuous sidewalks, and designated pedestrian crossings are essential measures, particularly in peri-urban corridors.

## Conclusion

The findings of this study emphasize the substantial risk faced by pedestrians in urban traffic environments and demonstrate a clear predominance of lower limb injuries consistent with vehicle impact biomechanics. Younger pedestrians ( $\leq 40$  years) showed significantly higher odds of electronic device distraction at the time of impact, highlighting the need for targeted awareness programs addressing distracted walking.

The significant clustering of fatal pedestrian accidents in peri-urban areas where low socio-economic/Educational population is abundant further underscores the importance of infrastructural development and pedestrian-focused traffic planning. Effective prevention strategies should combine road safety education, strict traffic law enforcement, and improved pedestrian infrastructure, including adequate lighting, sidewalks, and safe crossing facilities, particularly in high-risk transitional urban zones.

However, few limitations like single-center autopsy-based design, lack of data on non-fatal cases,

probable under reporting of use of electronic devices and alcohol consumption must be acknowledged. Further studies focusing on multi-centre design including non-fatal collisions can help us understand and prevent better.

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# Analysis of Transferred Perfume Fragrance in Different Fabrics: A Forensic Study

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## Abstract

Sexual assault is a major offense, and there has been a rise in the number of reported sexual offenses to the police in India. Transferred trace material can be utilized to ascertain connections between objects, individuals, or locations. Perfumes are imperceptible substances utilized by individuals of both genders. Hence, it is possible for the aromatic constituents of a perfume to be transmitted during interpersonal contact. The purpose of this study is to analyze the transfer of smell from garments to a secondary piece of cloth using UV-Visible Spectroscopy. Cotton swatches were utilized for all perfume transfer trials. The experiments involved the use of direct touch and solvent extraction techniques. This study establishes that perfume aroma is transferable and can be retrieved from various secondary fabric samples, even after the perfume has been matured on distinct primary fabric for a duration of up to 5 hours. This study focuses on analyzing transferred fragrance in secondary fabric using UV-VIS Spectroscopy. The major objective is to provide the forensic area with a reliable and cost-effective analytical method.

**Keywords:** Sexual assault, perfume, fragrance, trace evidence

## Introduction

Sexual violence against women constitutes the most abhorrent act imaginable. It constitutes the pinnacle of cruelty flitted upon femininity, youth, virginity, and motherhood. This constitutes an offense not only against the female victim, but also

against society as a whole.<sup>1</sup> Clothing that is routinely retrieved from crime scenes or gathered during an investigation is frequently analyzed for a variety of trace evidence types.<sup>2</sup> Trace evidence derived from cosmetic products is substantial and useful in forensic investigations due to the fact that it is

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readily transferable and remains imprinted on the transferred object. The identification and establishment of the origin of trace evidence is a critical undertaking in the field of forensic science. Such evidence can establish a connection between the suspect, the victim, and the crime scene, thereby serving as crucial corroborative evidence. In situations where such a connection cannot be established, cosmetic evidence may be utilized to rule out the suspect's exhibits.<sup>3</sup> A cosmetic is any substance or mixture that is applied through spraying, sprinkling, rubbing, or pouring with the intention of cleansing, beautifying, enhancing attractiveness, or modifying the appearance.<sup>4,5</sup> The word cosmetic originates from the Greek word 'kosmtikos', which signifies the ability to organize or the skill of embellishing.<sup>6</sup> "Cosmetates" was the term used to refer to Roman slaves whose duty it was to bathe both men and women.<sup>7</sup>

Researchers in the field of forensics have frequently examined cosmetic items like lipstick, nail polish, kohl, and fragrances. Pungents and fragrances are widely growing trend among both genders to incorporate perfumes into their daily routines.<sup>8</sup> Fragrances, being volatile substances by their very nature, have the ability to be readily absorbed by skin and clothing through direct application, contact with fragranced objects, or exposure to air containing fragrance chemicals.<sup>9</sup> A study conducted by Gniotek in 2003 attempted to quantify the odor of two samples: one cotton fabric that had come into direct contact with an odorant-absorbent fabric, and the other cotton fabric that was in close proximity to the flavored fabric. As a result of the intimate proximity that occurs between the victim and assailant in cases of sexual assault, fragrance analysis may serve as an additional forensic instrument capable of substantiating the occurrence of contact.<sup>10</sup>

In the field of forensic investigation, the extraction process and analytical technique play a crucial and essential role in discovering residues of perfume. A variety of methods have been used to extract volatile organic compounds (VOCs) from various sources, including plants,<sup>11, 12</sup> foods,<sup>13, 14</sup> and soils.<sup>15-17</sup> Nevertheless, there is a scarcity of

published studies into the process of extracting volatile organic compounds (VOCs) from fabrics, despite their potential usefulness. Volatile organic compounds (VOCs) have a significant role in the textile industry, particularly in processes that utilize microencapsulation. This technique involves enclosing small particles or droplets of various substances such as lasting fragrances, skin softeners, disinfectants, and insect repellents within capsules. These capsules enable the substances to be easily released when needed.<sup>18</sup>

Solvent extraction is a commonly employed approach for extracting organic analytes, and it has a long history of use in this field.<sup>19</sup> This method utilizes the solubility characteristics of volatile organic compounds (VOCs) to perform selective extraction. The solubility of analytes in solvents with different polarities is determined by their structure, and so the extraction profile can be influenced by the polarity of the solvent.<sup>20</sup> One of the primary drawbacks of solvent extraction, outside the dilution of the sample, is its generally labor-intensive nature, particularly when compared to the solid phase microextraction (SPME) sampling procedure.

SPME is a solventless technique that combines the processes of sampling, extraction, and concentration of analytes. SPME is a non-exhaustive extraction technique that extracts just a small amount of the target analyte from the sample matrix, in contrast to solvent extraction.<sup>21</sup> This approach offers the advantage of being portable, capable of automation, and having greater sensitivity.<sup>22</sup>

The extraction technique is thereafter accompanied by instrumental analysis to identify and quantify the presence of scent traces. Various analytical instruments has been reported for detecting and analyzing fragrance compounds by using Gas Chromatography and Raman Spectroscopy. Gas chromatography (GC) and liquid chromatography (LC) are analytical techniques that use different detectors to quantify substances. The most commonly used detectors are the mass selective detector (MSD) and the flame ionization detector (FID).<sup>23-25</sup> Gas chromatography coupled to mass spectrometry (GC-MS) is widely utilized in the perfume business,<sup>26</sup> as

well as in forensic laboratories. Gas chromatography-mass spectrometry (GC-MS) is extensively utilized in the field of forensic research. Its applications encompass the study of drugs of abuse,<sup>27</sup> textile fibers,<sup>28</sup> automotive paint traces,<sup>29</sup> and fire debris.<sup>30</sup> These procedures are time-consuming, damaging, and not regarded environmentally friendly due to their high solvent usage.<sup>31</sup> Due to these drawbacks, vibrational spectroscopy techniques are being employed more frequently since they are deemed user-friendly, rapid, and reasonably affordable.<sup>32-34</sup> Several publications have written that discuss the application of Raman spectroscopy for the analyzing and detecting volatile chemicals.<sup>34</sup>

In a previous study, Godinho et.al (2016),<sup>35</sup> introduced a different approach to determine the smell environment in perfumes. They also classified the perfumes based on the guidelines set by high-end perfume manufacturers. Gherghel et.al (2016),<sup>36</sup> conducted an investigation of transmitted fragrance using gas chromatography-mass spectrometry. The researcher examined the relationship between the transmission of scent and both the duration of perfume aging and the duration of perfume contact. Gherghel et.al (2019),<sup>37</sup> described the method of transferring fragrance from one piece of fabric to another, which might happen during an assault. He assessed the transmission of scent by employing solid-phase microextraction gas chromatography-mass spectrometry (SPME GC-MS).

This research presents an examination of the fragrance transferred from several fabric samples that were prior treated with perfume. The perfume transfer method is employed to transmit fragrance between garments. Therefore, the current study has established several durations for the aging of perfumes. In their work on the analysis of transferred scent, Gherghel et.al (2016),<sup>36</sup> employed a comparable perfume ageing time. The analytical technique of UV Spectroscopy has been employed to analyze the transmitted volatile compounds from various fabric kinds. UV spectroscopy analysis of material yields valuable information that can be beneficial for forensic investigations.

## Experimental Section

### Materials

Different types of fabrics were used in these experiments. Each fabric measuring 1m×1m were purchased from the local store in Jhansi. Perfume of Fogg brand name manufactured in India by Helios packaging PVT LTD. was used in this study. Fogg male perfume was purchased from local store in Jhansi. In this study, Rakeem methanol of 2.5-liter purity of 99% was used for solvent extraction method.

### Sample Preparation and Extraction

Cotton swatches of about 8cm×8cm were utilized for all perfume transfer trials. Prior to the experiment, a washing step was conducted to ensure that the fabric was free from any additional volatile organic compounds. The act of hand washing was carried out without the utilization of detergent. Following the drying process, the entire fabric was individually stored in a sealed plastic bag to avoid any contamination. Direct contact experiment was performed for transfer of perfume. For first sample where primary cloth was cotton and secondary cloth was cotton. 10µl male perfume pipetted was aged for 5mins before the contact on a piece primary cloth (cotton) this is known as perfume ageing time (PAT). Following the successful completion of the PAT, a fragrance-free cotton swatch was placed on the surface and allowed to remain in touch for a predetermined duration of 10 minutes, referred to as the contact time. A weight of 450 grams was placed on the secondary object to simulate the transmission of perfume between the clothing. For further experiments, 10µl of the male perfume pipetted was aged for 30mins, 1hrs, 3hrs and 5hrs before contact on the piece of secondary cloth (cotton cloth). For second sample where primary cloth was cotton and secondary cloth was georgette. For third sample where primary cloth was denim and secondary cloth was cotton. All experiment involving transfer of perfume between primary and secondary cloth were carried out in duplicates. After the direct contact experiment, solvent extraction was performed. In a 250ml beaker, 5ml of methanol was taken. After

the contact time of 10mins passed, fabric was kept in beaker having methanol. Beaker was shaken for approximately 8mins for the extraction of perfume components. Thus, total 30 sample extract were analysed under UV Visible Spectroscopy in this approach.

**UV-Visible Spectroscopy** All the samples are analyzed by Shimadzu UV-VIS 2600. Specification of instrument is shown in table. 1

**Table 1. Instrument Specification**

| Sr. No. | Specifications | Details          |
|---------|----------------|------------------|
| 1.      | Model no.      | Shimadzu UV-2600 |
| 2.      | Range          | 200-400nm        |

## Result and Discussion

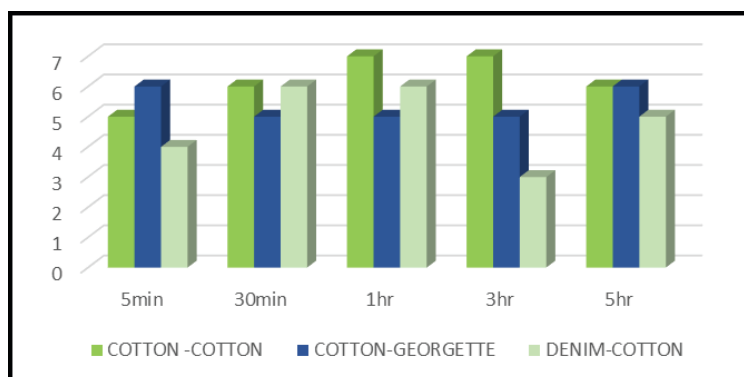
This study has been carried out to determine that the perfume fragrance can be viable form of trace evidence and fragrance can be transferred from one person's clothing to another's clothing where there has a close physical contact. In this study, it is also to determine that perfume fragrance is transferable and can be recovered from different secondary piece of fabric when the perfume was aged on the different primary fabric as long as 5hr ago.

**Standard of Perfume** 2ml of perfume was taken into the cuvette of UV-Vis Spectroscopy and the absorption spectra collected. 8 compounds were identified through the NIST library and rest were not identified. Compounds which were identified through the NIST library are shown below in the given table. 2

**Table 2. List of Compounds Identified from NIST Library found in Std. of Perfume**

| Std. peaks in nm | Compounds         |
|------------------|-------------------|
| 334              | Methyl salicylate |
| 282              | Cyclohexane       |
| 281              | Cinnamaldehyde    |
| 280              | Eugenol           |
| 270              | Coumarin          |
| 257              | Anethole          |
| 250              | Benzaldehyde      |
| 221              | Nonane            |

**Analysis of Transferred Fragrance** In table. 3 and chart. 1. 5, 6, 4 compounds of perfume were detected in combination of cotton-cotton, cotton-georgette and cotton-denim respectively for PAT 5mins. 6,5,4 compounds were detected in combination of cotton-cotton, cotton-georgette, cotton-denim fabric respectively when PAT was 30min. 7,5,6 compounds were detected in combination of cotton-cotton, cotton-georgette, denim-cotton fabric respectively when PAT was 1hr. 7,6,3 compounds were detected in combination of cotton-cotton, cotton-georgette, denim-cotton fabric respectively when PAT was 3hr. 6,6,3 compounds were detected in combination of cotton-cotton, cotton-georgette, denim-cotton fabric respectively when PAT was 5hr. From the above observation, it is observed that the highest number of compounds were recovered from the cotton-cotton combination of fabric and lowest number of compounds of perfume were recovered from the denim-cotton combination of fabric.



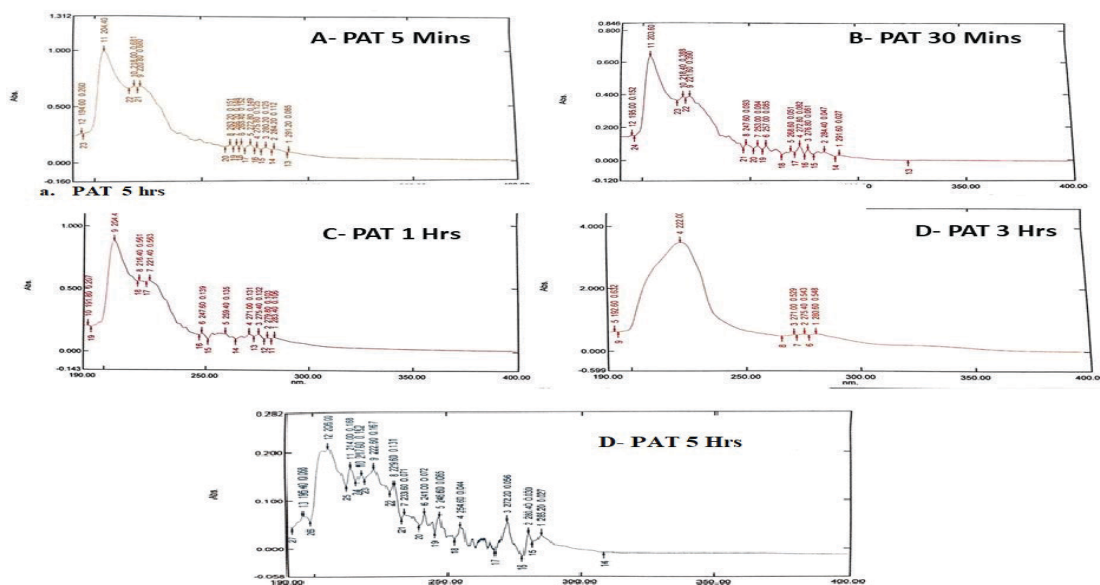
**Chart 1: Comparison of Transferred Fragrance Recovered from Different Fabric**

**Table 3. Comparison of Transferred Fragrance Recovered from Different Fabric**

| PAT   | STD. | COTTON-COTTON | COTTON-GEORGETTE | DENIM-COTTON |
|-------|------|---------------|------------------|--------------|
| 5min  | 8    | 5             | 6                | 4            |
| 30min |      | 6             | 5                | 4            |
| 1hr   |      | 7             | 5                | 6            |
| 3hr   |      | 7             | 6                | 3            |
| 5hr   |      | 6             | 6                | 3            |

**UV-VIS Spectrum Peaks for different combination of fabrics** The UV-Visible spectrum peaks were obtained from the secondary cotton fabric when the contact duration between the primary and secondary fabric was kept constant at 10 minutes. The scent ageing time was varied at different intervals of 5 minutes, 30 minutes, 1 hour, 3 hours, and 5 hours, as shown in Figure 1. The UV-Visible Spectrum Peaks were obtained from the secondary piece of georgette fabric. The contact duration between the

primary and secondary fabric was kept constant at 10 minutes, while the scent ageing period varied at different intervals of 5 minutes, 30 minutes, 1 hour, 3 hours, and 5 hours, as shown in Figure 2. UV-Visible Spectrum Peaks obtained from the secondary piece of cotton fabric when primary piece denim was in contact for 10min and perfume ageing time varies at different time interval of 5min, 30min, 1hr, 3hr and 5hr as shown in Fig. 3

**Figure: 1 UV-Visible Spectrum of Transferred Perfume at Different PAT in Cotton-Cotton fabric combination**

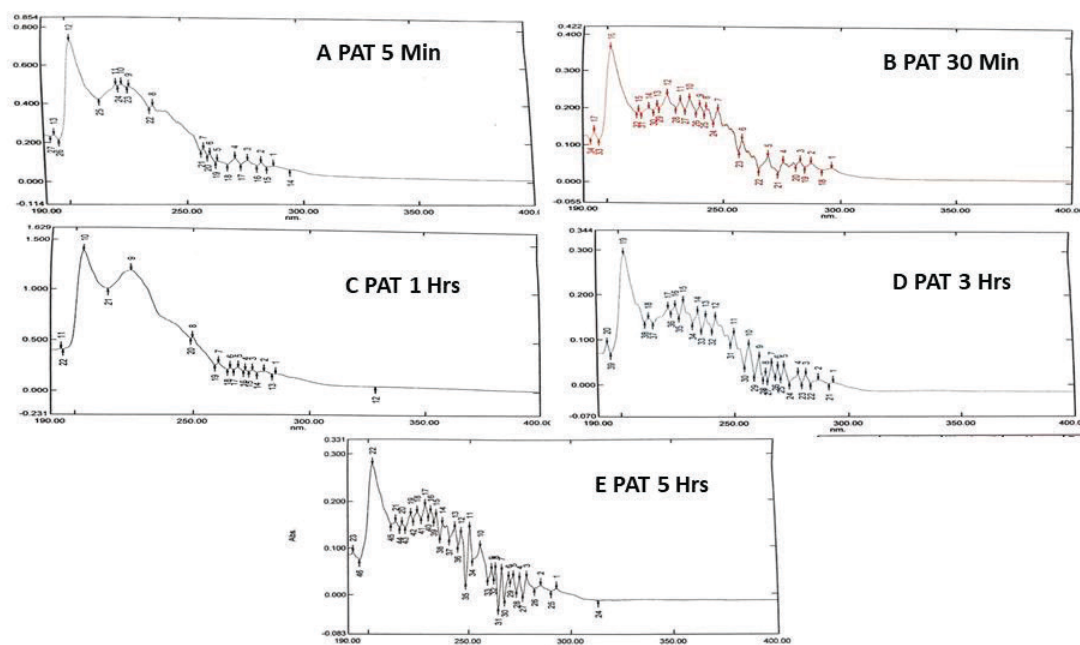


Figure 2: UV-Visible Spectrum Peaks of Transferred Perfume at Different PAT in Cotton-Georgette fabric combination

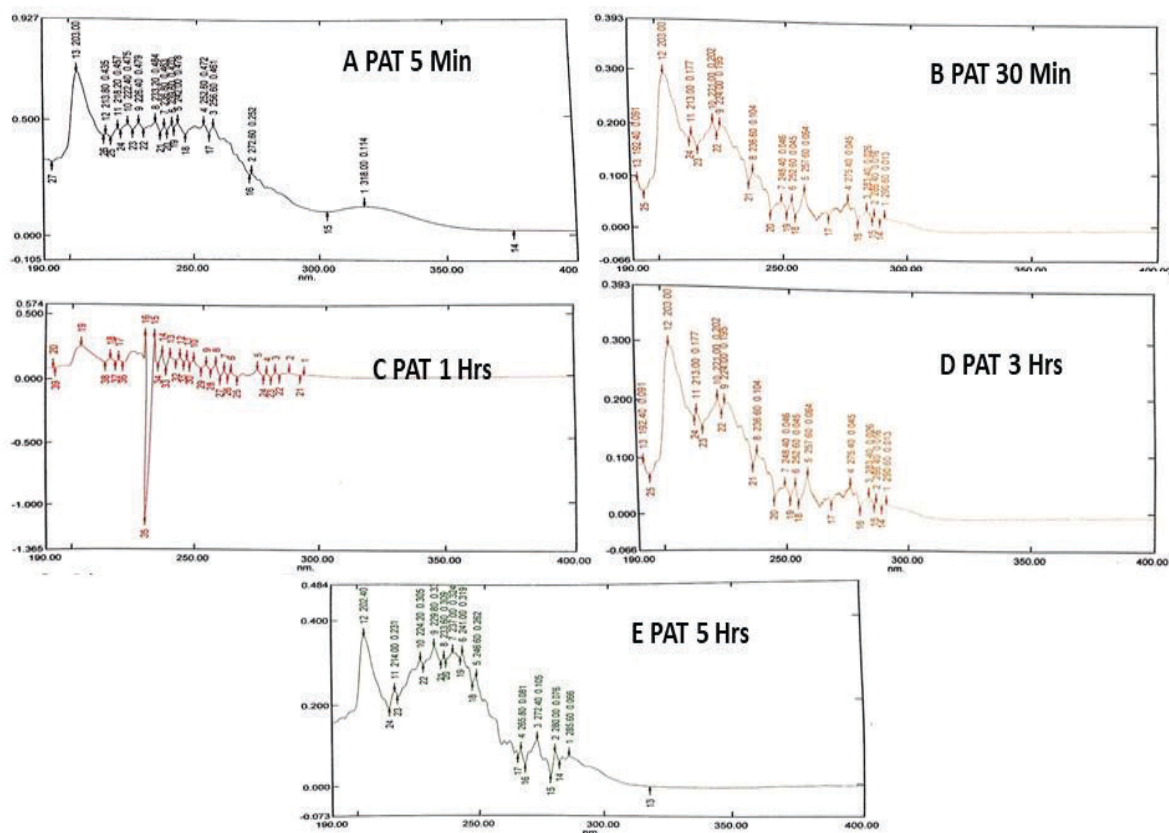


Figure 3: UV-Visible Spectrum Peaks of Transferred Perfume at Different PAT in Cotton-Denim fabric combination

**Statistical Analysis** Standard Deviation of Empirical Data Obtained from Repeated Analysis of Transferred Perfume Fragrance is shown in table. 4

**Table 4. Standard Deviation of Empirical Data Obtained from Repeated Analysis of Transferred Perfume Fragrance**

| Perfume Ageing Time | Cotton-Cotton | Cotton-Georgette | Denim-Cotton |
|---------------------|---------------|------------------|--------------|
| 5mins.              | 0.7071        | 0.7071           | 0.7071       |
| 30mins.             | 0.7071        | 0                | 0            |
| 1hrs.               | 1.4142        | 0                | 0.7071       |
| 3hrs.               | 1.4142        | 1.4142           | 0.7071       |
| 5hrs.               | 0.7071        | 0.7071           | 1.4142       |

### Discussion

Here work on the analysis of transferred fragrance has been carried out. To determine whether fragrance was transferred between the two individual when there was a close contact. Upon analyzing the provided table of findings, it is evident that the perfume compounds were transferred to the secondary fabric. Compounds were observed to be present in the scent even after it had been aged on the primary cloth for up to 5 hours, as determined by UV-Visible Spectroscopy. A different variety of fabrics were used to investigate the transfer of fragrance between primary and secondary pieces of cloth when kept in contact for 10 minutes. From the result it is observed compounds of fragrance persist when the perfume was aged on the primary fabrics as long as for 5hrs. Combination of Cotton-Cotton fabric shows the best result as large amount of compounds were recovered from secondary swatches.

### Conclusion

In this present study, an approach has been made towards the analysis of transferred fragrance in secondary cloth where the main emphasis of

the work is to analyse the fragrance in UV-VIS Spectroscopy instrument to give the forensic field an analytical method which is reliable and cost effective. This study aimed to assess the viability of aroma as a potential kind of trace evidence. The study examines the duration of perfume aging and the type of fabric in relation to the transfer of scent onto a secondary piece of cloth. This study aims to scientifically illustrate the transfer of perfume between clothing items and the possibility of smells to serve as trace evidence in forensic investigations. It was shown that perfume transfers to secondary fabric in a qualitative way. Hence, there is a possibility for the utilization of scent remnants in forensic investigations that entail intimate interactions between individuals. It can be concluded from this study that perfume traces were recovered from the secondary fabric even when the perfume was aged on the primary fabric as long as 5hrs. It can also be concluded that the combination of cotton-cotton shows good result in recovery of perfumes compound in comparison to cotton-georgette and denim-cotton. From the empirical data in this study supports an analytical UV Spectroscopy can be used for perfume analysis. The UV-VIS Spectroscopy method for analysis of perfume from solution and presented here offers means for a quick, accurate analysis of fragrance components.

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