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Original Article

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Comparative Evaluation of Ninhydrin, Iodine Fuming, and Silver Nitrate for Latent Fingerprint Development on Selected Porous and Non-Porous Substrates

Ariza Mehdi[1], Rakhi Rajput[2], Keya Pandey[3], Pradeep Kumar Yadav[4], Rohit Kumar Singh[5]

[1]M.Sc Forensic Science, [2]Subject Expert (Forensic Science), [3]Prof. & Head, Department of Anthropology, University of Lucknow, Lucknow, U.P., India, [4]Assistant Professor, Department of Forensic Medicine & Toxicology, Dr RMLIMS, Lucknow, U.P., India, [5]Scientist C, Department of Public Health, Kalyan Singh Super Speciality Cancer Institute, Lucknow, U.P., India.

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Abstract

Latent fingerprints deposited on surfaces through physical contact are an important source of forensic evidence, and their development can be influenced by the nature of the substrate and the chemical reagent used. This study evaluated three chemical procedures ninhydrin, iodine fuming, and silver nitrate for latent fingerprint development on five porous and five non-porous substrates. A total of 120 samples were processed and assessed using the CAST grading scale (0–4) under the specific immediate and 24-hour storage conditions included in the experimental design. The tested reagent–substrate combinations produced variable degrees of fingermark development. The mean CAST grade was higher for the porous substrates than for the non-porous substrates under the conditions evaluated. Ninhydrin produced good development on selected porous substrates under immediate processing, while iodine fuming produced observable development on the tested non-porous substrates. Silver nitrate also produced identifiable development after 24 hours of storage under the specific conditions in which it was evaluated. Because the experimental design did not include all possible reagent–substrate–storage combinations, the findings were interpreted within the individual conditions tested rather than as a direct comparison of overall reagent performance.

Keywords: Latent fingermark development; ninhydrin; iodine fuming; silver nitrate; porous substrates; non-porous substrates; CAST grading

Introduction

Latent fingerprints are among the most common forms of physical evidence encountered in forensic investigations. Deposits of eccrine and sebaceous secretions consist of amino acids, chloride ions, lipids and water, all of which can be selectively detected using different chemical development reagents.[1–3] On the other hand, apocrine glands are confined to

Corresponding Author: Rakhi Rajput, Subject Expert (Forensic Science), Department of Anthropology, University of Lucknow, Lucknow, U.P, India.

E-mail: rakhirajpoot1@gmail.com

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places such as the armpit and groin region and release thicker secretions with a higher content of proteins.[2] In the field of forensic science, eccrine sweat glands are very important when developing finger-marks since finger-marks contain amino acids and chloride ions. Sebaceous material is another substance that is deposited along with the fingerprints after the contact of the fingers with the face and then with the surface. It is a secretion from sebaceous glands that contains lipids and is the main constituent of iodine fuming. [4,5] The importance of knowing this information is that it determines the choice of the reagent.

The three chemical processes most widely employed in forensic laboratories for developing latent finger-marks include: ninhydrin, iodine fuming, and silver nitrate. Ninhydrin technique has been used since the 1950s, which reacts with free amino acids present in eccrine sweat and produces a purple substance known as Ruhemann's purple. [6–8] This is the standard procedure when it comes to the processing of papers since the amino acids are known to bind with cellulose and prevent them from being evaporated.[6]The iodine fuming process differs from the ninhydrin one in the way that there is no chemical bonding involved, as iodine adsorbs on lipid sweat components, thus creating a yellow-brown fingerprint that disappears when the iodine re-sublimates.[9,10] This feature, while limiting, is also advantageous, since marks will disappear by themselves without any harm done to the substrate, allowing other reagents to be applied successively. [11]Finally, the silver nitrate technique involves a precipitation process, during which the silver chloride is produced through reactions with chloride ions, and darkened when exposed to light.[12,13] Historically, this process was mostly applied to porous materials because the chloride ions were more persistent than organic sweat components.[12]

In this study, the aim was to evaluate latent fingerprint development using ninhydrin, iodine fuming, and silver nitrate on selected porous and non-porous substrates under the experimental conditions used. For selected reagent–substrate combinations, fingerprint development was also assessed following a 24-hour storage period.[14,15]

The effectiveness of any of these reagents, however, will be highly dependent on the substrate. Porous substrates like paper, cardboard, and cloth allow for sweat to penetrate the substrate, thereby concentrating sweat residues and shielding them from evaporation and mechanical disruption.[4,16] Substrates that are non-porous, like glass, plastic, and metal, allow for sweat to remain on the surface alone. [16,17] **Champod et al**. pointed out the porosity of the substrate and its surface energy as two of the most significant factors in determining finger-mark development.[16] And this applies to each of these reagents since ninhydrin works poorly on smooth and non-absorbent substrates since the substrate must bind the amino acids to the surface,[6,7] whereas silver nitrate and fuming of iodine can work on both types of substrates.[9,13] As Magro pointed out, there is no one chemical technique that can effectively develop finger-marks on all types of substrates.[18]

Immediate processing of evidentiary materials is seldom a possibility in practical casework. Sweat deposits will start breaking down once deposited. The breakdown of sweat starts with the more volatile fractions and is followed by the less volatile fractions, which are inorganic salts.[1,19] The speed at which the samples break down will depend on factors such as temperature, humidity, presence of light and the nature of the substrate.[17,20] **Jelly et al.,** who conducted a review on amino acid-based reagents on porous substrates, observed that as time passes, sensitivity in detection reduces.[6] Sodhi and Kaur, on the other hand, showed that silver nitrate can be used on finger-marks on paper that are wet and old.[12] This could imply that the chloride fraction does not break down as easily as the organic fractions.

Although individual reagents have been widely studied, their performance can vary with substrate characteristics and storage conditions. Comparative evaluation across different porous and non-porous substrates remains relevant because the choice of processing method may depend on the nature of the surface and the condition of the deposited fingerprint. The present study therefore examined selected reagent–substrate combinations under controlled laboratory conditions. [7,11] There is knowledge regarding the influence of substrate chemistry and storage conditions on the results;[13,14] A controlled evaluation of selected reagent–substrate combinations can therefore provide useful descriptive

observations regarding fingerprint development under defined laboratory conditions. Specifically, the study sought to:

1. Evaluate latent fingerprint development using ninhydrin, iodine fuming, and silver nitrate on selected porous and non-porous substrates;
2. Describe fingerprint development under immediate and, where applicable, 24-hour storage conditions; and
3. Assess the variation in CAST scores among the selected substrate–reagent combinations.

Materials and Methods

Ten substrates were selected for this study, representing two broad categories of forensically relevant surfaces. The porous substrates comprised cotton cloth, plain paper, denim cloth, brown paper, and cardboard, while the non-porous substrates included a mobile screen protector, glass slide, plastic sheet, aluminium foil, and stainless-steel spoon. Each substrate was standardised to a 5 × 5 cm contact area prior to sweat deposition to ensure consistency across samples.

Sweat was deposited onto each substrate through direct finger contact following a period of physical activity. The same general deposition procedure was followed for all samples. Variation related to individual sweat production and deposition characteristics was not independently controlled. For each substrate, three treatment conditions were evaluated according to the experimental design. For porous substrates, ninhydrin was used for immediate processing, iodine fuming was used for immediate processing, and silver nitrate was applied after 24 hours of storage. For non-porous substrates, silver nitrate was evaluated under immediate and 24-hour storage conditions, while iodine fuming was evaluated under immediate conditions. Each substrate–reagent–condition combination was represented by three observations, while one uncontaminated control was prepared for each substrate. Thus, 30 substrate–reagent–condition combinations generated 90 experimental observations, together with 30 corresponding controls, giving a total of 120 samples. Ninhydrin was excluded from non-porous substrates on account of its dependence on substrate absorption for effective amino acid detection, which renders it largely ineffective on non-absorbent surfaces. [6,7] Therefore, the study does not represent a complete three-reagent comparison across both porous and non-porous substrate categories.

All developed fingerprints were assessed visually using the CAST (Centre for Applied Science and Technology) grading scale. The score was assigned based on the visibility and quality of the developed fingerprint in relation to the substrate background. The CAST scores were assigned by a single observer using visual assessment. Therefore, inter-observer variability could not be assessed in the present study. Mean, standard deviation, minimum, maximum, and range were calculated for the treatment conditions evaluated. Because the experimental design did not include all possible reagent–substrate–storage combinations and did not provide repeated independent observations for every treatment condition, the results were primarily summarized descriptively.

Results

For the porous substrates, the mean CAST score was 3.00 for immediate ninhydrin treatment, 3.20 for immediate iodine fuming, and 3.40 for silver nitrate following 24 hours of storage. These values describe the individual treatment conditions evaluated and should not be interpreted as a direct ranking of the three reagents because the storage conditions differed between treatments.

Table 1: Descriptive Statistics - Reagent Performance on Porous Substrates (CAST 0–4)

Reagent / Treatment	Mean Grade	SD	Min	Max	Range	n (substrates)
Ninhydrin - Immediate	3.00	1.00	2	4	2	5
Iodine Fuming - Immediate	3.20	1.09	2	4	2	5
$AgNO_3$ - Stored 24 hrs	3.40	0.89	2	4	2	5
Overall - All Porous Combinations	3.20	0.86	2	4	2	15

n = number of porous substrates assessed under each treatment condition (n = 5); each grade represents the visual assessment score assigned to the corresponding substrate–reagent–storage combination; SD = standard deviation. [14]

The corresponding values of non-porous substrates are shown in Table 2. Among the non-porous treatment conditions evaluated, iodine fuming showed a mean CAST score of 2.60 ± 1.14, while silver nitrate showed mean scores of 2.20 ± 0.44 under immediate conditions and 2.20 ± 0.84 after 24 hours of storage. These values represent the specific treatment conditions evaluated and should not be interpreted as evidence of overall reagent superiority.

Table 2: Descriptive Statistics - Reagent Performance on Non-Porous Substrates

Reagent / Treatment	n	Mean Grade	SD	Min	Max	Range
$AgNO_3$ - Immediate	5	2.20	0.44	2	3	1
Iodine Fuming - Immediate	5	2.60	1.14	1	4	3
$AgNO_3$ - Stored 24 hrs	5	2.20	0.84	1	3	2
Overall - Non-Porous Substrates	15	2.33	0.82	1	4	3

n = number of non-porous substrates assessed under each treatment condition (n = 5); SD = standard deviation; grades based on the CAST 0–4 scale.

Among the porous substrates, plain paper and cotton cloth showed strong fingerprint development under the treatment conditions evaluated, with CAST scores reaching 4 in at least two conditions. Cardboard showed comparatively lower scores under some of the tested conditions, with each material getting a maximum grade of 4 in at least two conditions. The cardboard proved to be the poorest porous material. Out of the non-porous materials, the mobile screen protector got the highest grade (4, when tested with iodine fume), while aluminium foil showed lower CAST scores under some of the treatment conditions evaluated (grade 1) with immediate iodine test. It is important to note that $AgNO_3$ treatment for glass slides and aluminium foil, the CAST scores observed after 24 hours of storage were higher than those observed under immediate silver nitrate treatment. These observations are specific to the substrates and conditions tested and do not establish a general storage effect.

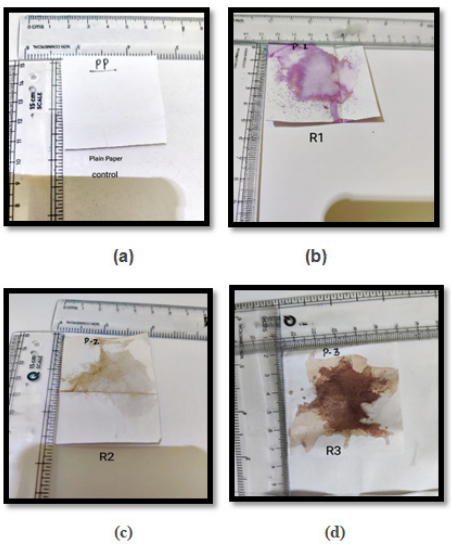


Figure 1: Representative latent fingermark development on plain paper (porous substrate): (a) control; (b) ninhydrin, immediate treatment, dark purple (CAST 4); (c) iodine fuming, immediate treatment, faint brown (CAST 2); (d) silver nitrate, 24-hour storage, dark brown (CAST 4).

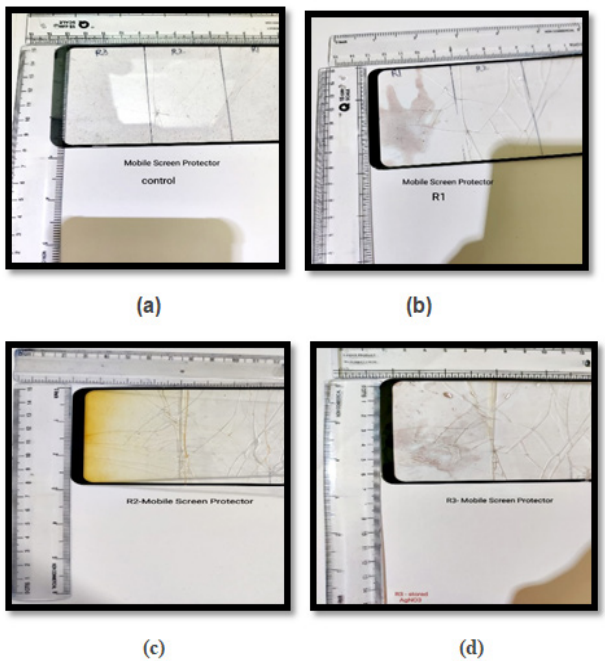


Figure 2: Representative latent fingermark development on a mobile screen protector (non-porous substrate): (a) control; (b) silver nitrate, immediate treatment, faint brown (CAST 3); (c) iodine fuming, immediate treatment, intense yellow-brown (CAST 4); (d) silver nitrate, 24-hour storage, light brown (CAST 2).

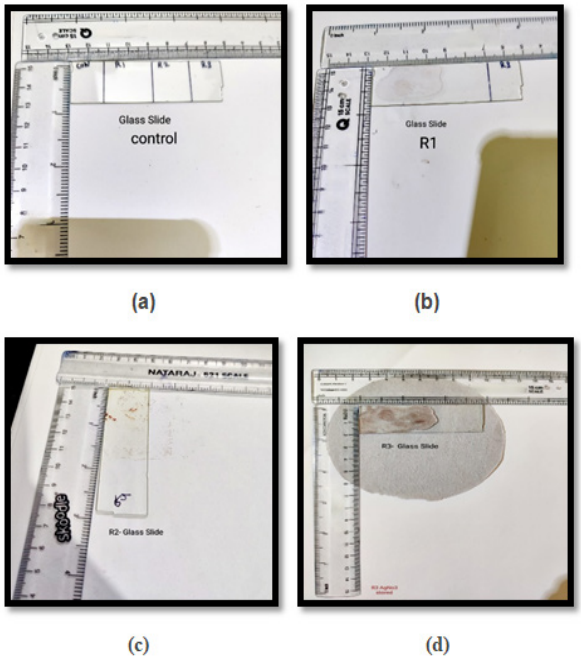


Figure 3: Representative latent fingermark development on a glass slide (non-porous substrate): (a) control; (b) silver nitrate, immediate treatment, light brown (CAST 2); (c) iodine fuming, immediate treatment, light yellow (CAST 2); (d) silver nitrate, 24-hour storage, brown (CAST 3).

Graphical Representation of Results

Figures 4, 5 and 6 provide graphical representations of CAST grades across all substrate and reagent combinations.

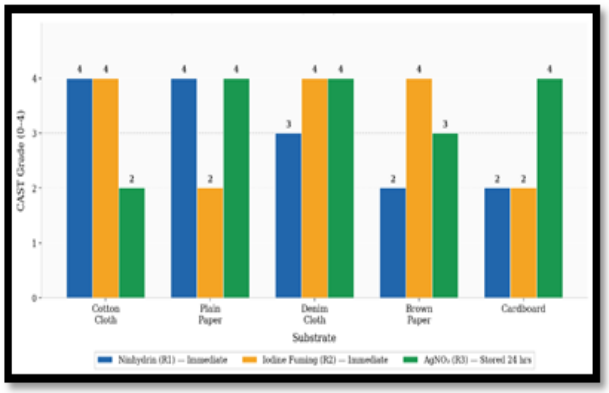


Figure 4: CAST Grades by Reagent Across Porous Substrates

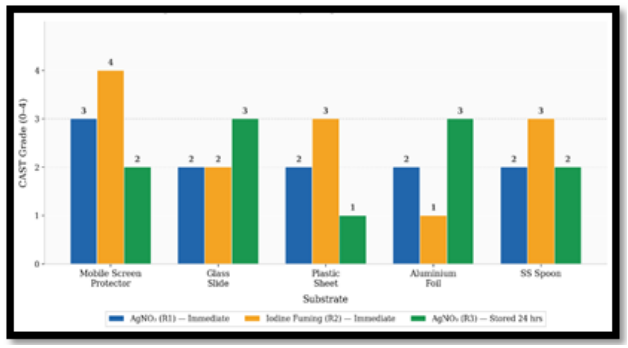


Figure 5: CAST Grades by Reagent Across Non-Porous Substrates

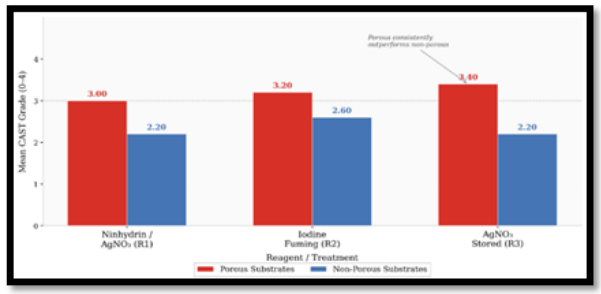


Figure 6: Mean CAST Grade per Reagent - Porous vs Non-Porous Substrates

Discussion

Sweat residues deposited on surfaces through skin contact consist of a complex mixture of eccrine and sebaceous secretions, including amino acids, chloride ions, lipids, and water. [1,3,19] The ability of any chemical reagent to visualise these residues depends not only on the target analyte but also on the extent to which the substrate retains that analyte following deposition. Ninhydrin, which reacts with free amino acids in eccrine sweat to produce the purple-coloured compound Ruhemann's purple, [6–8] performed strongly on plain paper and cotton cloth under immediate conditions, both yielding CAST grades of 4. This is consistent with the established efficacy of ninhydrin on cellulosic surfaces, where amino acids are concentrated near the surface and remain accessible to the reagent. This observation is consistent with the established use of ninhydrin on cellulosic surfaces. Differences among the porous substrates may also be related to their surface characteristics and the interaction between the deposited residue and the substrate. However, the distribution of amino acids within the substrates was not directly measured in the present study[6,16] The

weaker performance on brown paper and cardboard gives each scoring grade 2 that reflects the interference caused by surface texture and background colouration on these substrates, as well as the tendency of amino acids to diffuse rapidly into highly porous matrices, reducing their surface availability. [4,18]

Iodine fuming operates through physical adsorption rather than a true chemical reaction, forming transient complexes with lipids present in sebaceous sweat. [9,10] Its strongest result in this study was on the mobile screen protector, where it achieved a CAST grade of 4, consistent with reports of iodine responding well to lipid-rich deposits on smooth polymer surfaces. [9,11,21] The non-permanent nature of iodine visualisation is due to a known limitation arising from the volatility of iodine and the reversibility of lipid binding that was apparent in the lower and more variable grades recorded on plain paper and cardboard. [10] **Jasuja et al**. reported comparable variability and recommended fixing treatments to stabilise iodine-developed marks, a step not incorporated in the present study, which may partly account for the weaker results on certain substrates. [9,11]

Silver nitrate, applied to stored samples after a 24-hour delay, produced CAST scores that were, in several cases, comparable to or higher than those observed under immediate treatment on the same substrates. On plain paper, denim cloth, and cardboard, it achieved CAST grades of 4. The observed development may be related to the interaction of silver nitrate with chloride ions present in the deposited residue. However, chloride concentration was not directly measured in the present study, so the persistence or redistribution of chloride during the storage period cannot be confirmed from the present data.[12,13] This aligns with observations regarding the durability of chloride-based finger-mark residues on paper surfaces, and with the general understanding that chloride ions are less volatile than amino acids or lipids and therefore less susceptible to early degradation. [1,12] On non-porous surfaces such as glass slides and aluminium foil, stored samples also showed comparable or improved grades relative to immediate treatment, One possible explanation is that evaporation of the aqueous component may alter the distribution of less volatile constituents on the surface. However, this possibility was not directly investigated in the present study. [13,15]

The higher mean CAST grade observed for the porous substrates (3.20) compared with the non-porous substrates (2.33) describes the conditions evaluated in this study. This difference should be interpreted cautiously because the reagent and storage conditions were not identical across the two substrate categories. The observed difference is consistent with the established importance of substrate characteristics in fingerprints development; however, the present experimental design does not allow this finding to be generalized to all forensic surfaces. This difference should be interpreted cautiously, because the reagent and storage conditions were not identical across the two substrate categories. Overall mean 3.20compared to non-porous surfaces (overall mean 2.33) reflect the well-established forensic principle that absorbent materials retain sweat components more effectively by drawing them into the substrate matrix, protecting them from surface evaporation and mechanical loss. [4,16,17]Substrate porosity and surface energy have each been identified as primary determinants of finger-mark development success, [16,17] and the data from this study support that position across ten forensically relevant surfaces.

Among the porous substrates, plain paper and cotton cloth showed strong fingermark development under the treatment conditions evaluated, whereas cardboard showed comparatively lower scores under some conditions. The differences may be associated with surface texture, colour and material composition; however, these characteristics were not independently characterised in the present study and therefore represent possible explanations rather than confirmed causes. While cardboard presented the greatest challenge that a finding consistent with its the relatively lower scores observed on cardboard may be associated with its surface texture, colour and material composition. These characteristics were not independently characterised in the present study and therefore represent possible explanations rather than confirmed causes, which introduce background colouration and interfere with reagent specificity.

Among the non-porous substrates, the mobile screen protector showed a CAST score of 4 with

iodine fuming. This observation may be related to its smooth surface characteristics. However, the specific surface coating and its interaction with the deposited residue were not independently characterised in this study. The relatively higher score observed on the mobile screen protector under iodine fuming may be related to its smooth surface characteristics. However, the specific surface coating and its interaction with the deposited residue were not independently characterised in this study. and thereby enhances iodine fuming reactivity. [9,10] Taken together, the findings reinforce the position that.Taken together, the findings highlight the importance of considering substrate characteristics when evaluating chemical methods for fingermark development. However, the present observations were obtained under controlled laboratory conditions and should not be directly extrapolated to routine operational casework.[18,20]

Conclusion

The current study examined the development of latent fingerprint using selected chemical reagents on porous and non-porous substrates under controlled laboratory conditions. Results showed variability of CAST scores for the tested substrate–reagent combinations. Ninhydrin with immediate processing gave good fingerprint development on selected porous substrates and iodine fuming gave observable development on the non-porous substrates tested. Silver nitrate likewise led to observable development after 24 hours storage under the conditions tested.

The findings suggest that substrate characteristics and processing conditions should be considered when selecting a chemical method for latent fingerprint development. However, because all possible combinations of reagent, substrate and storage condition were not evaluated, the study does not establish an overall ranking or universal superiority of one reagent over another. Similarly, the observations obtained after 24 hours should not be interpreted as evidence of a general absence of storage-related effects.

These findings represent controlled laboratory observations from selected substrates and should therefore be interpreted cautiously in relation to operational forensic casework. Further studies incorporating a balanced experimental design, multiple donors, additional storage intervals, controlled environmental conditions and independent scoring would provide a stronger basis for comparing these chemical methods.

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References

1. Sato K, Kang WH, Saga K, Sato KT. Biology of sweat glands and their disorders. I. Normal sweat gland function. J Am Acad Dermatol 1989;20:537–63. https://doi.org/10.1016/S0190-9622(89)70063-3.
2. Bovell D. The human eccrine sweat gland: Structure, function and disorders. Journal of Local and Global Health Science 2015;2015. https://doi.org/10.5339/jlghs.2015.5.
3. Jain M, Bhattacharya B. Legal Studies & Criminal Justice Examining the Significance of Saliva and Sweat in Forensic Science. Journal of Legal Studies and Criminal Justice Page 2020;6:2020. https://doi.org/10.6084/m9.figshare.12524141.v1.
4. Kar AK, Patel R, Nandi T. Introduction to Sweat and Its Forensic Analysis. Fundamentals of Forensic Biology, Singapore: Springer Nature Singapore; 2024, p. 157–66. https://doi.org/10.1007/978-981-99-3161-3_11.
5. Wilke K, Martin A, Terstegen L, Biel SS. A short history of sweat gland biology. Int J Cosmet Sci 2007;29:169–79. https://doi.org/10.1111/j.1467-2494.2007.00387.x.
6. Jelly R, Patton ELT, Lennard C, Lewis SW, Lim () KF. The detection of latent fingermarks on porous surfaces using amino acid sensitive reagents: A review. Anal Chim Acta 2009;652:128–42. https://doi.org/10.1016/j.aca.2009.06.023.
7. Drochioiu G. Ion SANDU GIOIM. Ninhydrin-Based Forensic Investigations I. Fingerprints. International Journal of Criminal Investigation, n.d.
8. Markandeya N SCSS. Ninhydrin sweat test in the early detection of leprosy. International Journal of Leprosy and Other Mycobacterial Diseases 2002;70(2):125.
9. Jasuja OP, Kaur A, Kumar P. Fixing latent fingermarks developed by iodine fuming: A new method. Forensic Sci Int 2012;223:e47–52. https://doi.org/10.1016/j.forsciint.2012.09.013.

10. Jasuja OP, Singh G. Development of latent fingermarks on thermal paper: Preliminary investigation into use of iodine fuming. Forensic Sci Int 2009;192:e11–6. https://doi.org/10.1016/j.forsciint.2009.08.005.
11. Singh V, Mandal P, Sasho S, Najdoski M, Slobodan O, Jasuja OP. Stabilizing latent fingermarks developed with iodine fuming: A new method. Journal of the Indian Chemical Society 2025;102:101694. https://doi.org/10.1016/j.jics.2025.101694.
12. Sodhi GS, Kaur J. Physical developer method for detection of latent fingerprints: A review. Egypt J Forensic Sci 2016;6:44–7. https://doi.org/10.1016/j.ejfs.2015.05.001.
13. Cantu AA. Silver physical developers for the visualization of latent prints on paper. . Forensic Sci Rev 2001;13(1):29-64.
14. Sears VG, Bleay SM, Bandey HL, Bowman VJ. A methodology for finger mark research. Science & Justice 2012;52:145–60. https://doi.org/10.1016/j.scijus.2011.10.006.
15. Datta AK LHRRGR. Advances in fingerprint technology. 2001.
16. Champod C, Lennard CJ, Margot P, Stoilovic M. Fingerprints and Other Ridge Skin Impressions. CRC Press; 2004. https://doi.org/10.1201/9780203485040.
17. Houck MM SJA. Fundamentals of forensic science. . Academic Press; 2009.
18. Magro LSCS-IAALM. Chemical enhancement of fingerprints on various porous and non-porous surfaces. All India Institute of Medical Sciences Department of Forensic Medicine 2015.
19. Yadav PK, Sharma S. Forensic Analysis of Sweat. Advancements in Body Fluid Analysis in Forensics, Cham: Springer Nature Switzerland; 2024, p. 207–17. https://doi.org/10.1007/978-3-031-65804-4_7.
20. Saferstein R HPR. Criminalistics An introduction to forensic science. . pearson; 2001.
21. Kumari Sharma K, Kannikanti GH, Baggi TRR, Vaidya JR. Fixing Transient Iodine on Developed Latent Fingermarks. J Forensic Sci 2019;64:1859–66. https://doi.org/10.1111/1556-4029.14139.

Original Article

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AI-Driven Object Detection for Projectile Localization on Trauma X-rays: An Analytical Study of Diagnostic Accuracy and Forensic Utility

Balamanikandan S.[1], Vijayakumar Nair[2], Thumma Amar[3], Srimukesh A.[4]

[1]Postgraduate Resident; [2]Professor &; Head of Department; [3]Associate Professor ; [4]Senior Resident Department of Forensic Medicine & Toxicology, Vinayaka Mission's Medical College &; Hospital (VMMC&H), Karaikal, Puducherry, India.

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Abstract

Background: Trauma radiographs may reveal retained bullets or metallic fragments, and early localization can influence further imaging, surgical planning, safety assessment, and forensic documentation.

Methods: This feasibility study used a fully synthetic, computer-generated tabular dataset of 400 case-level records divided into 240 training-design records, 60 validation-design records, and a 100-record held-out test set. The 400 records were not pixel-level radiographs: no DICOM, PNG, or other X-ray images were generated for model training, and no YOLOv9 neural network was trained. Instead, ground-truth labels, confidence scores, simulated detector outputs, a simulated time-constrained radiologist first pass, object-level matches, and processing times were produced by a fixed-seed probabilistic simulation (seed 9173) to demonstrate an evaluation framework. The test set comprised 70 projectile-positive and 30 projectile-negative records.

Results: The simulated AI output yielded 65 true positives, 5 false negatives, 3 false positives, and 27 true negatives, corresponding to 92.0% accuracy, 92.9% sensitivity, 90.0% specificity, 95.6% precision, and a 94.2% F1 score; ROC-AUC was 0.972. The simulated radiologist first pass was correct in 83.0% of test records. In paired correctness analysis, AI alone was correct in 12 records and the radiologist alone in 3 (exact McNemar p=0.035). Of 145 synthetic annotated fragments, 136 were matched, with mean IoU 0.828, mAP@0.50=0.937, and mAP@0.50:0.95=0.593.

Conclusion: These values are simulation outputs and do not constitute evidence that YOLOv9 detects projectiles on patient radiographs. The study demonstrates a reproducible analysis plan that requires validation on authentic multicentre radiographs, independent annotation, external testing, calibration, human-factors evaluation, and forensic governance before any clinical use.

Keywords: artificial intelligence; object detection; YOLOv9; trauma radiography; retained projectile; gunshot injury; forensic radiology; diagnostic accuracy; bounding box localization

Introduction

Conventional radiography continues to serve as a fast primary imaging technique in several trauma protocols. Retained bullets and metallic fragments are typically prominent due to their high radiodensity; however, detection and comprehensive

Corresponding Author: Balamanikandan S., Postgraduate Resident, Department of Forensic Medicine & Toxicology, Vinayaka Mission's Medical College &; Hospital (VMMC&H), Karaikal, Puducherry, India.

E-mail: sbmkd138@gmail.com, ORCID: 0009-0004-6238-4115

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enumeration can be challenging when fragments are diminutive, numerous, superimposed on bone or medical apparatus, located near image peripheries, or obscured by exposure and positioning constraints. Localization is clinically significant as it informs computed tomography, surgical planning, vascular evaluation, magnetic resonance safety assessment, and subsequent monitoring. The quantity, distribution, and recorded position of projectiles are significant for forensic analysis since they may aid in reconstruction and evidence retrieval.

Object detection is distinct from picture categorization. A classifier determines the presence of a projectile, whereas a detector provides one or more bounding boxes, class labels, and confidence ratings. This output is operationally advantageous in trauma treatment as it may focus attention on a specific area while maintaining the whole radiograph for physician analysis. It is susceptible to minor item mistakes, duplicate boxes, overlooked pieces, and false positives induced by external objects, surgical clips, jewelry, monitoring devices, picture labels, or other thick materials.

Recent research has used deep learning to identify foreign objects in chest radiographs and other X-ray images [1-4]. Nonetheless, the typical performance of foreign objects cannot be presumed to apply to ballistic pieces. Projectile datasets may be limited in size, legally sensitive, varied, and enriched with complicated trauma. Transparent reporting and rigorous segregation of training and test data are therefore required. CLAIM, DECIDE-AI, TRIPOD+AI, and STARD-AI delineate pertinent reporting criteria for data provenance, reference standards, model formulation, error analysis, and clinical assessment [5-10,12,13]. A comprehensive systematic assessment of deep-learning diagnostic accuracy emphasizes the need for stringent reference criteria, transparent analysis, and external validation [14].

Forensic and Clinical Rationale

A projectile-detection system may serve three functions. Initially, it may function as a triage overlay, identifying probable pieces for prompt examination. Additionally, it may facilitate organized documentation by exporting coordinates, confidence levels, and picture IDs into a radiology or forensic spreadsheet. Third, it may enable longitudinal comparison when several pieces need to be monitored during imaging and surgical procedures. None of these positions allows for independent diagnosis or forensic determination. The radiologist retains responsibility for interpretation, whereas every retrieved physical object adheres to a distinct chain-of-custody protocol.

The forensic usefulness is contingent upon maintaining evidentiary neutrality. A detector may identify a radiopaque item associated with a bullet or fragment; however, it cannot ascertain the weapon, ammo, firing sequence, trajectory, or legal causality based only on a bounding box. Radiographs are two-dimensional pictures that lack depth perception; perceived overlap may cause confusion. Computed tomography, surgical results, autopsy, physical examination, and scene evidence may be necessary. AI output should be regarded as a repeatable measuring tool rather than an authoritative expert judgment.

Objectives and Hypotheses

The main aim was to provide an analytical procedure for assessing an AI detector that identifies trapped bullets or pieces on trauma radiographs. The secondary goals included comparing picture-level performance with a time-constrained first assessment by a radiologist, quantifying object-level localization, identifying image subgroups prone to errors, measuring processing duration, and establishing criteria for forensic application.

The first hypothesis posits that the simulated AI detector will attain sensitivity and accuracy above 90% on the withheld test set.

- Comparator hypothesis: The initial accuracy of AI and radiologists will vary in comparative analysis.
- Error hypothesis: diminutive fragments, compromised pictures, and moderate to severe artifacts would contribute significantly to the incidence of misses and false alarms.

The utility hypothesis posits that bounding-box output may facilitate attention and recording, although it would be inadequate for autonomous clinical or forensic decision-making.

Materials and Methods

Research Design and Synthetic Dataset Status

This study is a synthetic feasibility simulation of diagnostic-accuracy analysis, not a clinical imaging experiment. The dataset consists of 400 computer-generated tabular case records and contains no authentic radiographs, patients, institutions, or protected health information. The records were generated programmatically with a fixed random seed of 9173. Computer generation here refers only to pseudorandom generation of structured variables and simulated predictions; no GAN, diffusion model, CT forward projector, physics simulator, or other image-generation system was used to create training radiographs. No DICOM, PNG, JPEG, or comparable pixel-level X-ray dataset was created, and the radiograph-like illustration in Figure 7 is explanatory artwork rather than a training or test image. Each record contains variables describing anatomic region, projectile presence, fragment count, dominant fragment size, artifact severity, image quality, synthetic reference-standard information, and—where applicable—simulated AI and comparator outputs, confidence scores, and processing times. The simulation therefore evaluates the statistical workflow and reporting structure rather than the empirical performance of a trained medical AI device.

Dataset Size Rationale, Composition and Partitioning

The total of 400 records was specified a priori as a pragmatic feasibility size rather than derived from a formal power calculation. Two considerations guided this choice. First, it permits a simple 60%/15%/25% design split (240/60/100), retaining 100 completely held-out records for transparent confusion-matrix estimates, confidence intervals, ROC analysis, and paired McNemar testing. Second, the scale mirrors two prior proof-of-concept chest-radiograph foreign-object studies that each used 400 public CXRs [2,15], allowing the simulated framework to be discussed against a familiar sample scale without implying evidentiary equivalence. Across the 400 synthetic records, 268 were projectile-positive and 132 projectile-negative; the held-out test set was intentionally enriched to 70 positive and 30 negative records so that both sensitivity and specificity could be demonstrated with non-trivial denominators. In a real study, sample size should instead be justified prospectively from the primary endpoint, expected sensitivity/specificity, desired confidence-interval width, prevalence, clustering, and planned external validation. Patient-level partitioning would also be mandatory to prevent leakage across training, validation, and test sets.

The 70 projectile-positive test records represented 145 synthetic fragment annotations, with one to four fragments per positive record. Dominant fragment size was categorized as small (<5 mm), medium (5-15 mm), or large (>15 mm). Metallic artifact severity was categorized as none/minimal, mild, moderate, or severe, and image quality was represented as adequate or degraded. Projectile-negative records retained simulated hard-negative conditions to model possible false-positive scenarios. These categories are structured variables in the case-record simulation, not measurements obtained from actual X-ray pixels.

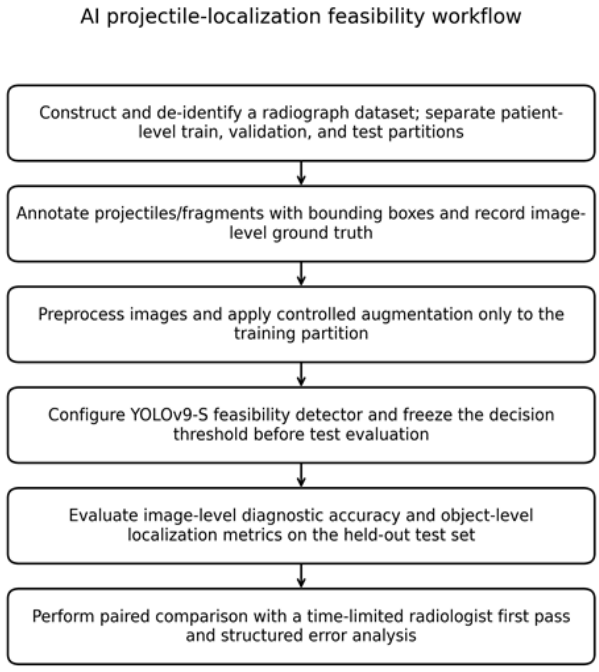


Figure 1. Planned AI development and held-out diagnostic-accuracy evaluation workflow.

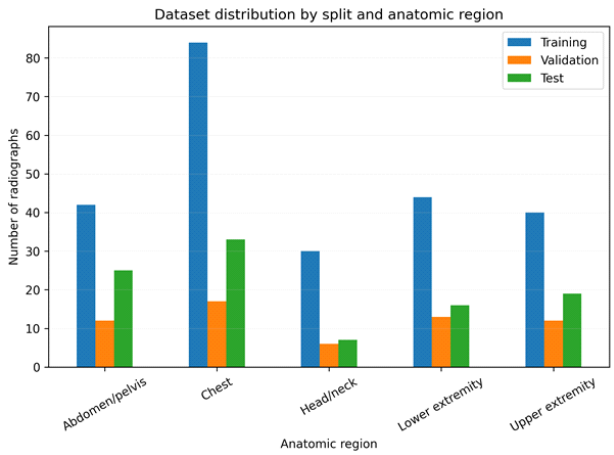


Figure 2. Synthetic dataset distribution by partition and anatomic region.

Ground Truth and Annotation Protocol

The suggested real-world reference standard would include independent bounding-box annotation by two fellowship-trained radiologists, with a third reader for adjudication and access to computed tomography, surgical results, or recovered-item data where available. An picture is deemed positive when at least one retained projectile or fragment is verified. Each visible part will be assigned a compact rectangular enclosure. Overlapping pieces would be marked individually only when their boundaries were discernible. External items and recognized medical equipment would be classified as hard negatives instead of being removed from the dataset.

For this simulation, the reference field was labelled "synthetic consensus annotation plus simulated confirmatory record." This is a generated reference-standard variable and must not be confused with a real consensus reading. A prospective study should document annotator expertise, annotation instructions, inter-reader agreement, adjudication frequency, access to CT/surgical/recovered-item evidence, and blinding to model outputs in accordance with current AI reporting guidance [5,6,10].

Planned AI Detector Specification

YOLOv9-S was selected only as the planned target architecture for a future empirical implementation because single-stage detectors can support low-latency, multi-scale object localization [11]. No YOLOv9 network was trained or executed in the present study, and none of the reported diagnostic or localization metrics should be attributed to YOLOv9. The model configuration in Table 1 is therefore a prespecified implementation template. A genuine study should train the architecture on pixel-level radiographs, compare it with at least one alternative detector, lock model selection on the validation set, and evaluate only the final frozen model on an unseen test set.

Table 1. Planned model configuration

Component	Pre-specified value
Architecture	YOLOv9-S feasibility configuration
Input	Single-view radiograph resized with aspect-ratio-preserving padding to 640 x 640 pixels
Output	Projectile/fragment bounding box, objectness confidence, class confidence
Initialization	Transfer learning from a non-medical object-detection checkpoint, followed by domain fine-tuning
Optimizer	AdamW with cosine learning-rate schedule
Batch size / epochs	16 / maximum 120 with early stopping
Augmentation	Small rotations, translations, scale changes, contrast variation, noise; no horizontal flip when laterality semantics are retained
Decision threshold	Fixed on the validation set before opening the test set
Post-processing	Non-maximum suppression with pre-specified intersection-over-union threshold
Primary test endpoint	Image-level sensitivity; object-level localization reported as secondary

Data Preprocessing and Augmentation

For a future authentic DICOM dataset, preprocessing should preserve clinically relevant acquisition metadata while applying validated de-identification. Photometric interpretation, pixel representation, orientation, laterality, and rescaling should be handled consistently. Training-

only augmentation may include small rotations, translations, scale changes, exposure variation, and noise; anatomically implausible transformations should be avoided. Validation and test images should remain unaugmented except in separately reported robustness analyses. These image-processing steps were not performed in the present tabular simulation.

Comparator Reading Conditions

The intended comparator is a radiologist's initial, time-constrained assessment before exposure to AI output. A future reader study should prespecify the number and specialty of readers, years of experience, display equipment, reading environment, available clinical history, time constraint, and whether the task is projectile detection alone or complete radiograph interpretation. Reference-standard annotators should be separated from comparator readers where feasible to reduce incorporation and review bias. In the present study, comparator decisions and reading times were simulated rather than observed.

Diagnostic and Localization Metrics

At case level, true positives, false negatives, false positives, and true negatives were used to calculate accuracy, sensitivity, specificity, precision, and F1 score with 95% confidence intervals. ROC-AUC summarized ranking performance across confidence thresholds, and average precision summarized the precision-recall relationship. Because the present study generated probability-like scores synthetically, these statistics demonstrate the evaluation procedure rather than calibration or discrimination of an actually trained detector.

At object level, a synthetic predicted box was considered matched when it was assigned to a reference fragment and met the prespecified IoU criterion without duplicate assignment. IoU is the area of overlap divided by the area of union. Object precision, recall, F1, mean IoU, mAP@0.50, and mAP@0.50:0.95 were reported. Image-level detection and object-level localization answer different questions: a case can be correctly classified positive even when one of several fragments is missed.

Statistical Analysis

The simulated AI output and simulated radiologist first pass were paired on the same 100 held-out records. Exact McNemar testing therefore used the discordant correctness cells: AI correct/radiologist incorrect versus AI incorrect/radiologist correct. The test evaluates whether the paired marginal error rates differ; it does not, by itself, establish clinical superiority. Subgroup analyses were descriptive because several strata were small and their error distribution was partly determined by the simulation rules.

Metric Definitions and Interpretation

Accuracy = (TP + TN) / (TP + TN + FP + FN).

Sensitivity = TP / (TP + FN), the proportion of projectile-positive case records correctly identified.

Specificity = TN / (TN + FP), the proportion of projectile-negative case records correctly rejected.

Precision = TP / (TP + FP), the proportion of positive AI alerts that are correct.

F1 score = 2 × (precision × sensitivity) / (precision + sensitivity).

Intersection over union (IoU) = area of overlap / area of union for a predicted and reference bounding box.

mAP@0.50 is mean average precision when a localization match requires IoU ≥0.50; mAP@0.50:0.95 averages AP across progressively stricter IoU thresholds from 0.50 to 0.95.

ROC-AUC can be interpreted as the probability that a randomly selected positive record receives a higher model score than a randomly selected negative record.

McNemar's test evaluates paired binary disagreement using the discordant correctness counts.

A 95% confidence interval reflects sampling uncertainty under the simulated data-generating process; it does not capture dataset shift, annotation uncertainty, clinical prevalence changes, or deployment bias.

Ethics, Privacy and Forensic Governance

No human participant data or identifiable medical images were used in this simulation. A real projectile-localization study would require

institutional ethics and data-governance approval, robust DICOM de-identification, controlled linkage to clinical or forensic outcomes, lawful secondary-use authority, and documented retention and access controls. When images may enter legal proceedings, the original image must remain immutable and AI outputs should be stored separately with model version, threshold, timestamp, and audit history. The broader legal and AI-governance framework is discussed in Section 5.

Use of Artificial Intelligence in Manuscript Preparation

A generative artificial-intelligence large language model was used only to assist with language editing, formatting, and readability of this manuscript. All study-design decisions, the synthetic data-generation procedure, the statistical analysis, the interpretation of results, and the final conclusions were conceived, checked, and approved by the authors, who take full responsibility for the integrity and accuracy of the work. No artificial-intelligence tool is listed or credited as an author.

Results

Dataset Characteristics

Table 2. Training, validation, and test partitions

Partition	Images	Positive	Negative	Positive %
Training	240	162	78	67.5
Validation	60	36	24	60.0
Test	100	70	30	70.0

The held-out partition consisted of 100 synthetic case records. No patient radiographs were used or generated.

Table 3. Held-out test-set characteristics (n=100)

Characteristic	Category	n	%
Anatomic region	Chest	33	33.0
	Abdomen/pelvis	25	25.0
	Upper extremity	19	19.0
	Lower extremity	16	16.0
	Head/neck	7	7.0
Artifact severity	None/minimal	45	45.0
	Mild	23	23.0
	Moderate	23	23.0
	Severe	9	9.0
Image quality	Adequate	84	84.0
	Degraded	16	16.0

Image-Level Diagnostic Accuracy

Within the 100-record synthetic test set, simulated AI outputs identified 65/70 projectile-positive records and correctly rejected 27/30 projectile-negative records, yielding 5 false negatives and 3 false positives. Overall accuracy was 92.0%, sensitivity 92.9%, specificity 90.0%, precision 95.6%, and F1 score 94.2%. ROC-AUC was 0.972 and average precision was 0.987. These are outputs of the fixed-seed simulation rather than estimates from a trained image detector.

Table 4. Confusion matrices for the held-out test set

Reader	True positive	False negative	False positive	True negative
AI detector	65	5	3	27
Radiologist first pass	59	11	6	24

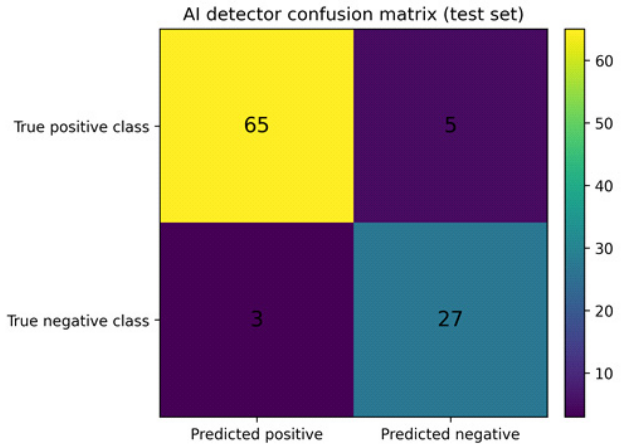


Figure 3. Confusion matrix for simulated AI outputs on the 100-record held-out test set.

Table 5. Image-level diagnostic performance

Reader	Metric	Estimate %	95% CI %
AI detector	Accuracy	92.0	85.0-95.9
AI detector	Sensitivity	92.9	84.3-96.9

Continue.....

AI detector	Specificity	90.0	74.4-96.5
AI detector	Precision	95.6	87.8-98.5
AI detector	F1 score	94.2	89.6-98.8
Radiologist first pass	Accuracy	83.0	74.5-89.1
Radiologist first pass	Sensitivity	84.3	74.0-91.0
Radiologist first pass	Specificity	80.0	62.7-90.5
Radiologist first pass	Precision	90.8	81.3-95.7
Radiologist first pass	F1 score	87.4	80.9-93.9

F1 confidence intervals use an approximate bounded standard-error method; other intervals use Wilson intervals.

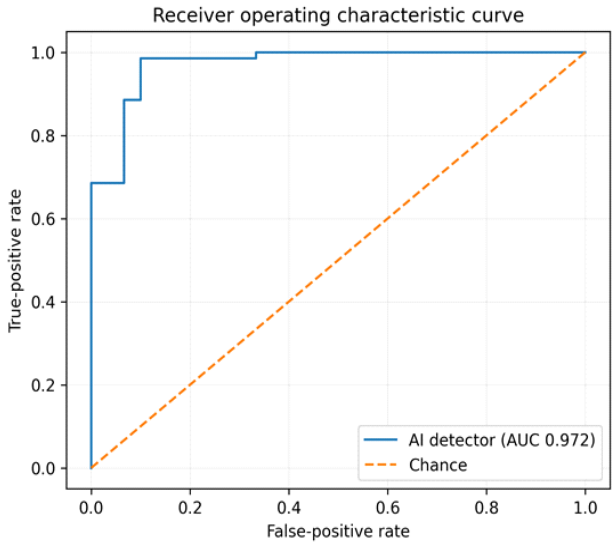


Figure 4. Receiver operating characteristic curve for the AI confidence score.

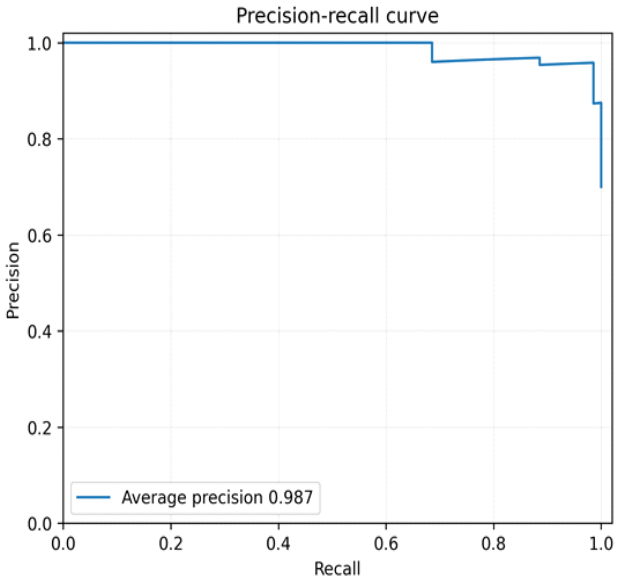


Figure 5. Precision-recall curve for the AI confidence score.

AI Versus Radiologist First Pass

The simulated radiologist first pass was correct in 83/100 records (83.0%). Both simulated methods were correct in 80 records and both were wrong in 5. AI alone was correct in 12 discordant records, while the radiologist alone was correct in 3. The exact McNemar p value was 0.035. This asymmetric disagreement is a property of the simulation and does not demonstrate clinical superiority.

Table 6. Paired correctness comparison

Paired outcome	n
Both AI and radiologist correct	80
AI correct; radiologist incorrect	12
AI incorrect; radiologist correct	3
Both incorrect	5

Exact McNemar test on discordant pairs: p=0.035.

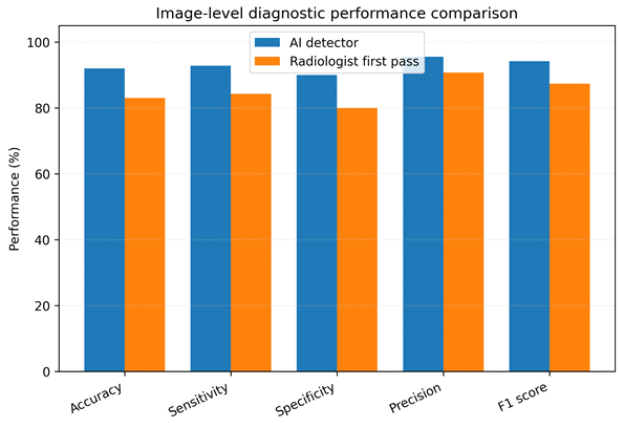


Figure 6. Simulated case-level performance of AI output and a time-limited radiologist first pass.

Object-Level Localization

The 70 projectile-positive test records contained 145 synthetic annotated fragments. Simulated detections matched 136 fragments and missed 9; 9 false-positive boxes were also generated. Object-level precision, recall, and F1 were each 93.8%.

Mean IoU among matched boxes was 0.828, with mAP@0.50=0.937 and mAP@0.50:0.95=0.593. These localization statistics were generated from synthetic object-level records and not from a trained detector operating on real images.

Table 7. Object-detection and localization metrics

Metric	Result
Ground-truth fragments	145
Matched fragments	136
Missed fragments	9
False-positive boxes	9
Object precision	93.8%
Object recall	93.8%
Object F1	93.8%
Mean IoU	0.828
mAP@0.50	0.937
mAP@0.50:0.95	0.593

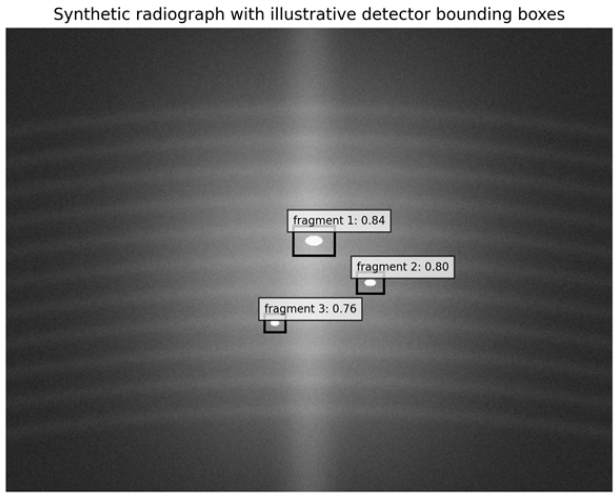


Figure 7. Synthetic radiograph-like illustration with example bounding boxes. This is not a patient image and is not diagnostic.

Processing Time

Median simulated AI processing time was 38.2 ms per record (IQR 34.8-41.8 ms), whereas the simulated radiologist first-pass time was 43.9 s (IQR 36.2-48.9 s). These assigned values exclude image transfer, queueing, display, integration, and human response time and must not be presented as observed clinical turnaround times.

Error Analysis by Region and Artifact

Table 8. Exploratory AI error analysis

Grouping	Level	n	Accuracy %	Sensitivity %	Specificity %	Errors
Region	Abdomen/pelvis	25	84.0	81.0	100.0	4
Region	Chest	33	97.0	100.0	92.3	1
Region	Head/neck	7	100.0	100.0	100.0	0
Region	Lower extremity	16	93.8	100.0	87.5	1
Region	Upper extremity	19	89.5	93.3	75.0	2
Artifact severity	Mild	23	100.0	100.0	100.0	0
Artifact severity	Moderate	23	82.6	84.2	75.0	4
Artifact severity	None/minimal	45	100.0	100.0	100.0	0
Artifact severity	Severe	9	55.6	71.4	0.0	4

Subgroup estimates are descriptive; several denominators are small and confidence intervals would be wide.

Errors clustered in higher-difficulty synthetic cases by construction. Small dominant fragments, degraded quality, and moderate/severe artifact increased the simulated difficulty score used to assign misses and false alarms. In a real study, these factors should be prespecified for subgroup analysis, and failure images should undergo blinded review for annotation error, overlap, hard negatives, threshold effects, duplicate detections, and fragment merging.

Discussion

Main Findings

The simulation met its prespecified feasibility targets, including case-level sensitivity and accuracy above 90%, high ROC-AUC, and internally consistent object-level localization metrics. These findings show that the proposed statistical workflow can reproduce the expected relationships among confusion-matrix measures, paired-reader analysis, and localization measures. They do not validate a medical AI system because the inputs and outputs were synthetically generated.

The simulated AI output was more often correct than the simulated first-pass comparator in discordant cases. Object-level performance was slightly lower than the case-level indication of "at least one projectile," illustrating an important operational distinction: a system may correctly flag a positive radiograph while still failing to enumerate every fragment. For trauma and forensic use, completeness of fragment localization may matter as much as image-level detection.

The deliberately imperfect error profile also highlights the clinical asymmetry of missed fragments. A false negative adjacent to a vessel, joint, spinal canal, airway, or orbit could be consequential even when overall sensitivity is high. Threshold selection should therefore be tied to the intended use. A triage assistant may prioritize sensitivity and accept more false positives, whereas structured documentation requires higher positive predictive value, stable calibration, and explicit human confirmation.

Quantitative Comparison with Previous Literature

Quantitatively, the simulated results fall within the broad range reported for related foreign-object detection tasks, but direct superiority claims would be invalid because the present study did not train or test a model on radiographs. Santosh et al. evaluated Faster R-CNN on 400 public CXRs and reported 97% precision, 90% recall, and 93% F1 for circle-like foreign objects [2]. In a later 400-CXR YOLOv4 study, Santosh et al. reported 91% accuracy, 85% precision, 93% recall, 89% F1, and mAP@0.50 of 83.75% [15]. Kufel et al. screened 112,120 CXRs and trained YOLOv8 to identify four foreign-object categories; the reported mAP@0.50:0.95 was 0.815 and the area under the precision-recall curve at IoU 0.50 was 0.987 [1]. The present simulation produced 92.0% accuracy, 95.6% precision, 92.9% sensitivity/recall, 94.2% F1, mAP@0.50 of 0.937, and mAP@0.50:0.95 of 0.593. The apparent numerical differences primarily demonstrate how metrics change across task definitions, object classes, IoU thresholds, datasets, and simulation assumptions.

Table 9. Quantitative comparison with related foreign-object detection studies

Study	Dataset / method	Accuracy	Precision / Recall / F1	Localization
Santosh et al., 2020 [2]	400 public CXRs; Faster R-CNN	NR	97% / 90% / 93%	NR
Santosh et al., 2022 [15]	400 public CXRs; YOLOv4	91%	85% / 93% / 89%	mAP@0.50 = 83.75%
Kufel et al., 2023 [1]	112,120 CXRs screened; YOLOv8	NR	NR / NR / NR	mAP@0.50:0.95 = 0.815; PR-AUC@ IoU0.50 = 0.987
Present simulation	400 synthetic tabular records; fixed-seed simulation	92.0%	95.6% / 92.9% / 94.2%	mAP@0.50 = 0.937; mAP@0.50:0.95 = 0.593

NR, not reported in the cited source. Metrics are not directly comparable across studies because object classes, datasets, IoU thresholds, and validation designs differ; present-study values are simulated rather than empirical model performance.

The comparison also clarifies why mAP values should not be mixed without specifying the IoU range. The present mAP@0.50 (0.937) is numerically higher than the 0.8375 mAP@0.50 reported for the 2022 YOLOv4 study [15], while the stricter present mAP@0.50:0.95 (0.593) is lower than Kufel et al.'s 0.815 mAP@0.50:0.95 [1]. Because the present values are synthetic, these differences are not evidence that one architecture or workflow is better. They instead define quantitative targets and reporting conventions for a future authentic trauma-radiograph study.

Data generation is relevant when authentic annotated radiographs are scarce, but the generation method itself must be validated. Andriiashen et al. demonstrated a CT-based approach for generating X-ray projections for foreign-object detection and showed that synthetic projection data could support classification performance in the studied non-medical objects [4]. Unlike that physics-based approach, the present study generated tabular case records rather than synthetic X-ray pixels. Future work could evaluate whether clinically realistic projection simulation or controlled compositing can augment real projectile radiographs without introducing shortcuts or unrealistic fragment appearances.

Clinical Integration

Initially, the most secure position is that of a concurrent or secondary reader assistant. The model may display boxes and confidence ratings while the radiologist maintains access to the original picture and either rejects, modifies, or adds discoveries. Anatomy should remain unobscured by notifications. The system should provide the version and threshold used. It should indicate if the picture is outside the validated domain. Ultimately, it should fail quietly instead of generating errors when the necessary pre-processing is not performed. Reader studies are to be used to assess integration using metrics of sensitivity, time to interpretation, false-positive load, automation bias, and behavior about corrections.

Clinically evaluate the negative pictures that may resemble common artifacts such as surgical clips, orthopedic hardware, monitoring leads, snaps, jewelry, zippers, coins, external trash, image markers, and scanner artifacts. The criteria must include penetrating trauma while omitting retained pieces to prevent injury context from serving as a shortcut for positive prediction. The critical domains for external assessment include portable anteroposterior pictures, appropriate pediatric patients, obesity, motion artifacts, underexposure, and discrepancies in equipment across many institutions.

Forensic Utility

For forensic value, it must be repeatable, auditable, and diagnostically accurate. Each exported box must have the picture identification, coordinates in the original pixel space, confidence level, model version, threshold, date, and any human modifications, if applicable. The original DICOM object must stay unchanged. A medico-legal report using AI output must delineate that the automated recommendation differs from the radiologist's conclusive assessment and reveal significant limitations.

The localization on a projection radiograph cannot be converted into a three-dimensional trajectory. A box cannot serve as evidence that a subsequently found article is identical unless there exists an independent chain of custody and comparative analysis. Examples of potentially beneficial applications include prioritized picture review, compilation of a fragment inventory, correlation of radiographic and surgical sites, and identification of discrepancies between preoperative imaging and retrieved materials. A performance specification must be confirmed for each unique application.

Error Analysis and Mitigation

Table 10. Anticipated Failure Modes and Mitigation

Failure mode	Potential consequence	Mitigation
Small or low-contrast fragment missed	Incomplete inventory; missed clinically important object	Multi-scale training; high-resolution crops; CT correlation; human review
Hardware or external object called projectile	False forensic documentation or unnecessary imaging	Hard-negative enrichment; object taxonomy; confidence threshold; reader confirmation

Continue...

Multiple adjacent fragments merged	Under-counting and poor localization	Instance-aware annotation; higher resolution; alternative detector/ segmenter
One fragment receives duplicate boxes	Over-counting	Calibrated non-maximum suppression; duplicate audit
Domain shift across equipment or sites	Unrecognized performance degradation	External validation; drift monitoring; site-specific acceptance testing
Incorrect laterality or coordinate export	Wrong operative/forensic location	DICOM orientation validation; pixel-coordinate unit tests; visible laterality checks
Automation bias	Reader accepts incorrect box	Independent first look; editable overlay; training; periodic blinded review

Ethical, Legal, and Regulatory Considerations

Global AI and Health-Governance Frameworks

The World Health Organization guidance on ethics and governance of artificial intelligence for health emphasizes protection of human autonomy, promotion of well-being and safety, transparency and explainability, responsibility and accountability, inclusiveness and equity, and responsive, sustainable AI [16]. For projectile localization, these principles translate into radiologist control, documented intended use, auditable outputs, subgroup evaluation, transparent performance limits, and prospective safety monitoring. The system should function as decision support rather than as an autonomous diagnostic or forensic authority.

The European Union Artificial Intelligence Act, Regulation (EU) 2024/1689, establishes a risk-based legal framework and specific obligations for high-risk AI systems [17]. Depending on intended purpose and regulatory classification, medical-device AI may fall within the high-risk regime under Article 6 together with applicable product-safety legislation. Requirements relevant to medical imaging include risk management, data and data-governance controls, technical documentation, logging and record keeping, transparency to deployers, human oversight, and appropriate accuracy, robustness, and cybersecurity. A clinical projectile-localization product in the EU would therefore require formal regulatory classification and conformity assessment based on its intended purpose rather than on algorithm type alone.

Indian Data Protection and Medical-AI Governance

In India, the Digital Personal Data Protection Act, 2023 establishes the statutory framework for processing digital personal data, including lawful processing, notice and consent or other permitted grounds, data-fiduciary obligations, security safeguards, breach handling, and data-principal rights [18]. Trauma radiographs and linked clinical or forensic metadata can constitute digital personal data when an individual is identifiable. A real study should therefore minimize collection, separate identifiers from research images, restrict role-based access, document the lawful basis for secondary use, apply retention controls, and maintain breach-response procedures. De-identification should address DICOM headers, burned-in annotations, accession numbers, and linked metadata, not merely visible patient names.

The Indian Council of Medical Research's 2023 Ethical Guidelines for Application of Artificial Intelligence in Biomedical Research and Healthcare provide a health-specific framework addressing ethics review, informed consent, safety, privacy, transparency, accountability, fairness, and human oversight [19]. For projectile localization, institutional ethics review should explicitly consider the dual clinical-forensic sensitivity of gunshot imaging, legitimacy of data access, annotation procedures, potential bias across populations and equipment, limits on secondary disclosure, and separation of clinical decision support from law-enforcement functions.

Operational, Forensic, and Accountability Controls

- Bias assessment should examine age, sex, body habitus, anatomic region, acquisition device, projection, image quality, hospital/site, and mechanism while preventing protected or legally sensitive variables from becoming shortcut features.
- Gunshot-related imaging may carry heightened privacy and medico-legal sensitivity; access, retention, secondary use, disclosure, and cross-system linkage require explicit governance and an auditable lawful basis.
- Model versions, thresholds, software dependencies, and updates should be controlled and traceable. Performance claims from one version must not be silently transferred to another.
- An AI detector should not independently trigger law-enforcement notification, evidence collection, or legal conclusions. Those actions depend on applicable law, institutional policy, consent where relevant, and professional judgment.
- Radiologists and trauma clinicians require training on false negatives, false positives, calibration, automation bias, and the limits of two-dimensional projectile localization.
- Prospective deployment should monitor safety events, overrides, subgroup drift, calibration, false-positive burden, workflow time, downstream imaging, and clinically or forensically consequential misses.

Limitations

This work is a simulation and not an experiment that developed or validated an image-detection model. No neural network was trained, and no authentic or computer-generated pixel-level radiograph dataset was used. The 400 observations are synthetic tabular case records created by a fixed-seed probabilistic process. Confidence scores, processing times, bounding-box matches, diagnostic classifications, and comparator outputs are therefore simulated variables. They must not be cited as evidence that YOLOv9 detects projectiles on patient radiographs. The comparator is also simulated rather than a real radiologist reader study. In addition, the enriched 70% projectile prevalence in the test set does not represent routine clinical prevalence, so positive predictive value would change in deployment.

The radiograph-like bounding-box figure is illustrative and not a medical image. Object-level statistics were generated from synthetic matched-detection records rather than end-to-end pixel inference. Subgroup denominators are small, and error clustering partly reflects the rules used by the simulation. A definitive study should include prospectively justified sample size, patient-level partitioning, authentic multicentre radiographs, independent multi-reader annotation, external validation, calibration analysis, prespecified subgroup testing, alternative detector comparison, locked test data, human-factors reader studies, versioned software, and forensic auditability. Where synthetic images are later introduced, realism and domain-gap validation should be separately demonstrated against authentic radiographs.

Conclusion

This study presents a structured, reproducible framework for evaluating AI-assisted projectile localization in trauma radiography using 400 synthetic case-level records. The simulated outputs yielded 92.0% accuracy, 92.9% sensitivity, 90.0% specificity, and 94.2% F1, with object-level mAP@0.50 of 0.937 and mAP@0.50:0.95 of 0.593. These figures are simulation results, not YOLOv9 performance on radiographs. Quantitative comparison with published foreign-object detection studies shows that the proposed reporting framework covers the major image-level and localization metrics used in prior work, while also emphasizing paired-reader analysis and forensic auditability. The next step is empirical development on authentic multicentre trauma radiographs with independent ground truth, external validation, calibration, human-factors assessment, and compliance with applicable AI, health-research, data-protection, and forensic-governance requirements. AI should remain a decision-support layer under radiologist control and should never replace the original image, chain of custody, or expert clinical and forensic interpretation.

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References

1. Kufel J, Bargieł-Łączek K, Koźlik M, Czogalik Ł, Dudek P, Magiera M, et al. Chest X-ray foreign objects detection using artificial intelligence. J Clin Med. 2023;12(18):5841. doi:10.3390/jcm12185841.
2. Santosh KC, Dhar MK, Rajbhandari R, Neupane A. Deep neural network for foreign object detection in chest X-rays. In: 2020 IEEE 33rd International Symposium on Computer-Based Medical Systems (CBMS). Piscataway (NJ): IEEE; 2020. p. 538-541. doi:10.1109/CBMS49503.2020.00107.
3. Eibschutz L, Lu MY, Abbassi MT, Gholamrezanezhad A. Artificial intelligence in the detection of non-biological materials. Emerg Radiol. 2024;31(3):391-403. doi:10.1007/s10140-024-02222-4.
4. Andriiashen V, van Liere R, van Leeuwen T, Batenburg KJ. CT-based data generation for foreign object detection on a single X-ray projection. Sci Rep. 2023;13:1881. doi:10.1038/s41598-023-29079-w.
5. Mongan J, Moy L, Kahn CE Jr. Checklist for Artificial Intelligence in Medical Imaging (CLAIM): a guide for authors and reviewers. RadiolArtif Intell. 2020;2(2):e200029. doi:10.1148/ryai.2020200029.
6. Tejani AS, Klontzas ME, Gatti AA, Mongan JT, Moy L, Park SH, et al. Checklist for Artificial Intelligence in Medical Imaging (CLAIM): 2024 update. RadiolArtif Intell. 2024;6(4):e240300. doi:10.1148/ryai.240300.
7. Vasey B, Nagendran M, Campbell B, Clifton DA, Collins GS, Denaxas S, et al. Reporting guideline for the early-stage clinical evaluation of decision support systems driven by artificial intelligence: DECIDE-AI. Nat Med. 2022;28(5):924-933. doi:10.1038/s41591-022-01772-9.
8. Collins GS, Moons KGM, Dhiman P, Riley RD, Beam AL, Van Calster B, et al. TRIPOD+AI statement: updated guidance for reporting clinical prediction models that use regression or machine learning methods. BMJ. 2024;385:e078378. doi:10.1136/bmj-2023-078378.
9. Sounderajah V, Ashrafian H, Golub RM, Shetty S, De Fauw J, Hooft L, et al. Developing a reporting guideline for artificial intelligence-centred diagnostic test accuracy studies: the STARD-AI protocol. BMJ Open. 2021;11(6):e047709. doi:10.1136/bmjopen-2020-047709.
10. Sounderajah V, Guni A, Liu X, Collins GS, Karthikesalingam A, Markar SR, et al. The STARD-AI reporting guideline for diagnostic accuracy studies using artificial intelligence. Nat Med. 2025;31:3283-3289. doi:10.1038/s41591-025-03953-8.
11. Wang CY, Yeh IH, Liao HYM. YOLOv9: learning what you want to learn using programmable gradient information. In: Computer Vision - ECCV 2024. Lecture Notes in Computer Science. Cham: Springer; 2024. doi:10.1007/978-3-031-72751-1_1.
12. Liu X, Rivera SC, Moher D, Calvert MJ, Denniston AK; SPIRIT-AI and CONSORT-AI Working Group. Reporting guidelines for clinical trial reports for interventions involving artificial intelligence: the CONSORT-AI extension. Nat Med. 2020;26(9):1364-1374. doi:10.1038/s41591-020-1034-x.
13. Rivera SC, Liu X, Chan AW, Denniston AK, Calvert MJ; SPIRIT-AI and CONSORT-AI Working Group. Guidelines for clinical trial protocols for interventions involving artificial intelligence: the SPIRIT-AI extension. Nat Med. 2020;26(9):1351-1363. doi:10.1038/s41591-020-1037-7.
14. Aggarwal R, Sounderajah V, Martin G, Ting DSW, Karthikesalingam A, King D, et al. Diagnostic accuracy of deep learning in medical imaging: a systematic review and meta-analysis. NPJ Digit Med. 2021;4:65. doi:10.1038/s41746-021-00438-z.
15. Santosh KC, Roy S, Allu S. Generic foreign object detection in chest X-rays. In: Santosh KC, Hegadi R, Pal U, editors. Recent Trends in Image Processing and Pattern Recognition. Communications in Computer and Information Science. Vol. 1576. Cham: Springer; 2022. p. 93-104. doi:10.1007/978-3-031-07005-1_10.
16. World Health Organization. Ethics and governance of artificial intelligence for health: WHO guidance. Geneva: World Health Organization; 2021.
17. European Parliament, Council of the European Union. Regulation (EU) 2024/1689 of 13 June 2024 laying down harmonised rules on artificial intelligence (Artificial Intelligence Act). Off J Eur Union. 2024;L 2024/1689.
18. Government of India. The Digital Personal Data Protection Act, 2023 (Act No. 22 of 2023). New Delhi: Ministry of Law and Justice; 2023.
19. Indian Council of Medical Research. Ethical guidelines for application of artificial intelligence in biomedical research and healthcare. New Delhi: ICMR; 2023.

Original Article

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Efficacy of Energy Dispersive X-Ray Fluorescence Spectroscopy in the Characterisation of Ballpoint Pen Inks

Bhawana Dahiya[1], Dipika Mistry[2], Shakti Prakash[3], J. R. Gaur[4]

[1]Research Scholar, School of Behavioural Sciences and Forensic Investigations, Rashtriya Raksha University, Gandhinagar, Gujarat, India, https://orcid.org/0009_0000_7563_0160, [2]Scientific Assistant, School of Behavioural Sciences and Forensic Investigations, Rashtriya Raksha University, Gandhinagar, Gujarat, India, [3]Research Officer, School of Behavioural Sciences and Forensic Investigations, Rashtriya Raksha University, Gandhinagar, Gujarat, India, https://orcid.org/0000_0003_2761_9121, [4]Lifetime Professor, School of Behavioural Sciences and Forensic Investigations, Rashtriya Raksha University, Gandhinagar, Gujarat, India.

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Abstract

This paper presents the results of a scientific study conducted on ballpoint pen ink of blue, red and black colour samples to find out the elemental composition of ballpoint pen inks from twelve available brands in the market viz Pentonic, Cello (Butterflow), Goldex, Flair, Hauser XO, and Unomax. The samples from each brand were analysed using Energy Dispersive X-ray Fluorescence (EDXRF) spectroscopy. The levels of elements S, Si, Cl, K, P, V, Ho, Cu, Mn, Fe, Zn, Ag, Ni etc. were quantified. This non-destructive analytical method was used to determine and quantify the elemental constituents in the inks, which would indicate their distinct chemical make-up. The results indicate notable differences in the elemental profiles between brands and ink colours, which may be used as markers for brand identification and differentiation. These results are very important in forensic document analysis as they allow questioned documents to be compared with reference samples. The research proves the suitability of EDXRF as a versatile working technique for ink characterisation and its possible use in forensic investigation.

Keywords: Forensic Document Examination, Ballpoint Pen inks, Energy Dispersive X-ray Fluorescence, Elemental analysis, Forensic Investigation

Introduction

Questioned Document Examination in forensic science plays a vital role in verifying the authenticity of documents. This examination identifies whether the document is genuine or a forged one. The identification of forged signatures and handwriting on suspected and fraudulent documents is necessary to serve the ends of justice. In this examination, the

Corresponding Author: Bhawana Dahiya, Research Scholar, School of Behavioural Sciences and Forensic Investigations, Rashtriya Raksha University, Gandhinagar, Gujarat, India

E-mail: bhawana.dahiya_phd@rru.ac.in

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analysis and comparison of ink is also an integral part of the process.

Ink is one of the most common evidence encountered in document examination. Different inks have unique chemical compositions. It helps in determining whether the same ink has been used in making the document when the document was written or if it has been tampered. Analysis of ink involves studying various ingredients that make up the ink composition, including pigments, dyes, chemicals, modifiers and fillers used in ink formulation. [1] Every ink bears a unique combination of these compounds, which form a distinctive "chemical fingerprint." By identifying the specific elements and chemicals present in an ink composition, forensic experts can often determine the source of the manufacturer or brand of the ink used in the document. Different inks may be linked to a specific time duration or manipulation, which can indicate the age, geographic location, or manufacturer. Various studies have been conducted using Energy Dispersive X-Ray Fluorescent spectroscopy to determine the elemental composition of different materials. Pessanha et al. (2009) conducted a study using EDXRF to determine the elemental composition of paper from an early 20th-century book in order to identify various paper manufacturers.[2] Whereas, Manso et al. (2007) analysed various papers dating from 1555 to 2005 to identify the elemental composition across different years. Their study found that papers from 1779 and 1787 had high concentrations of toxic metals. [3] Manso et al. (2007) conducted another research study of elemental characterisation using EDXRF. In this study, the author utilised ancient and modern papyrus and parchment along with newspaper from different years.[4]

Further, Shih & Chen (2026) highlighted the use of EDXRF technology for numerous forensic applications. Their work emphasised its use in the elemental analysis of trace evidence, such as glass, fibres, soil, paint, and hair, as well as in document analysis, which includes the elemental profiling of coins, ink, and paper. [5] Verma et al. (2019) explored the utility of EDXRF with field emission scanning electron microscopy to enhance accuracy and reliability. The author analysed printed documents containing black toner from laser printers and photocopier machines to determine the contributing elements in the composition of toner. [6]

An EDXRF study analyses elemental compositions in writing, copying, and computer printing papers, revealing significant variations by type, manufacturer, and printing method. Navigator and Copier papers had the lowest element concentrations, while Fabriano paper showed higher levels of lead (Pb), strontium (Sr), and zirconium (Zr). Laser printing added cobalt (Co), manganese (Mn), iron (Fe), chromium (Cr), and titanium (Ti) to Navigator paper. Organic content and components such as chromium (Cr) and copper (Cu) rose with grammage in Fabriano papers, whereas others such as Pb and Sr fell. These results are very useful for forensic and analytical investigations. [7]

The study focused on the elemental composition of ink using the sophisticated instrument technique EDXRF (Energy Dispersive X-ray Fluorescence) spectroscopy. EDXRF yields detailed information regarding the elemental composition that makes up the distinctive makeup of ink. Use of this technique enhances the understanding of the elemental makeup of inks used in everyday writing devices, in particular, ballpoint pens. Ballpoint pen inks, typically blue, red, and black, are in common usage for writing and signing a document. Although extensively utilised, not much research has been conducted on the particular ink formulation used in such pens and how they can be distinguished according to their chemical and elemental content.[8] [9]

Ultimately, this study hopes to further advance the art of more accurate and trustworthy methods of ink analysis, which is very important in the general framework of forensic science and the criminal justice system. The determination of the elemental make-up of inks not only assists in establishing the genuineness of documents but also adds to the overall body of forensic document examination knowledge.

The basic aim of the study is to analyse the elemental composition of blue, red, and black ballpoint pen inks using Energy Dispersive X-ray Fluorescence (EDXRF) technique to attain the following objectives:

- Identify the elemental components present in the ink samples of different brands and colours.

- Compare the elemental composition of different brands and colours to assess potential similarities and differences.
- Explore the potential application of EDXRF as a tool for ink discrimination in forensic document analysis.

Materials and Methods

Sample Selection

Material for the present study consisted of twelve commonly available ballpoint pen brands selected for this study: Cello (Butter flow), Pentonic, Flair Yolo, Hauser XO, Unomax boldtron RT, Goldex, Pentonic (BRT) 4001-B, Linc (Petronic)08-24 India, Flair (Czee click), Flair Woody, Cello Gripper and Saino Softex. A total of 36 ball point pen utilised for the study. For each brand, three colours of pen ink (red, blue, and black) were chosen. Commonly available and highest selling ballpoint pens in the market were chosen for the study, aiming to represent a diverse range of market-available options.

Sample Preparation and Methods

To prepare the ink samples, a small quantity of ink was blown out of the ballpoint pen refills on the mylar sheet in the sample holder after removing the nozzle or pen tip of the refill to make the mylar sheet opaque in the area where the ink was applied. The sample was analysed under Energy Dispersive X-ray Fluorescence Spectrometer. The sample was enough to be able to record the composition. The thickness of the Mylar sheet is 6mm. Composition formula ($_{C10H8O4}$), having a density of 1.38g/cm^3. The Mylar film, P/N 202-86501-56, has an outer diameter of 70 mm.

Sample Analysis

Energy Dispersive X-ray Fluorescence (EDXRF) spectroscopy was employed to analyse and determine the elemental composition of the different ink samples. EDXRF is a non-destructive technique that can identify and quantify elements present in a sample by measuring the characteristic X-rays emitted when the sample is irradiated with high-energy X-rays. The analysis was performed under air atmosphere, Collimator- 3mm, and time duration- 15 minutes.

Result

The results presented in the table 1 to 3 showed common and unique elemental composition of blue, black and red ballpoint pen inks of twelve different make and model. Figures 1 to 3 illustrates the percentage of elements that is common in all brands of pen.

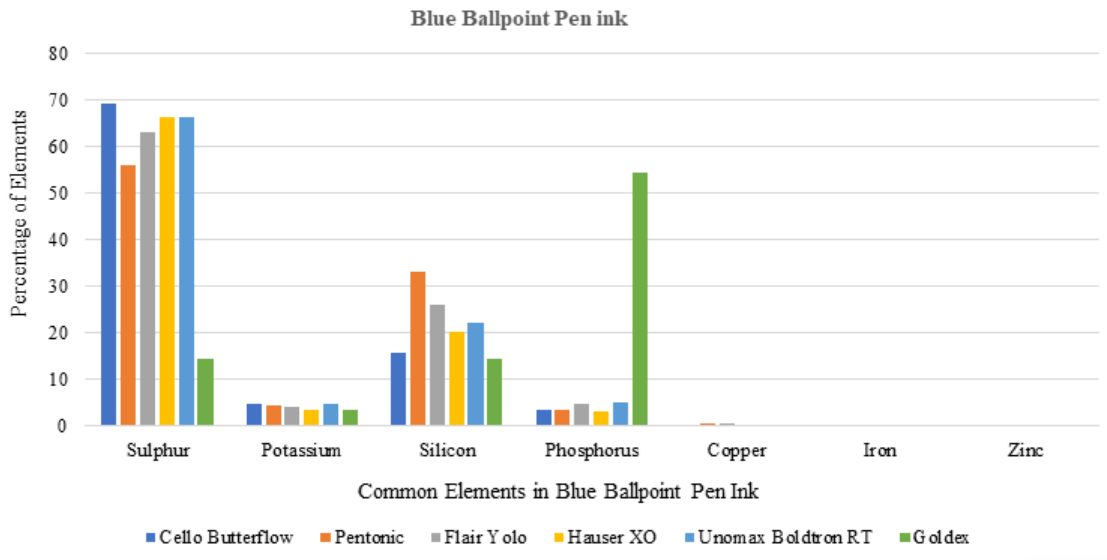


Figure 1. (A) Percentage of various elements present in different brands of blue ballpoint pen ink

Table 1. Percentage of Elements in Blue Ballpoint Pen Inks of Different Manufacturers

Sr. No.	Elemental Composition	Percentage of Elements in Blue ballpoint pen ink											
		Cello Butterflow %	Pentonic %	Flair Yolo%	Hauser XO %	Unomax Boldtron RT %	Goldex %	Pentonic (BRT) 4001-B	Linc (Petronic) 08-24 India	Flair (Czee click)	Flair Woody	Cello Gripper	Saino Softex
1	Sulphur (S)	69.280	56.104	62.976	66.130	66.283	14.488	9.727	60.045	58.538	62.794		7.113
2	Silicon (Si)	15.673	32.976	26.143	20.158	22.214	14.448	14.153	23.772	27.123	29.730	5.015	4.377
3	Chlorine (Cl)	5.127	-	-	5.118	-	9.350	9.460	7.154	3.967	-	19.580	11.314
4	Potassium (K)	4.689	4.569	4.121	3.650	4.783	3.466	2.722	3.346	3.304	-	2.047	2.935
5	Phosphorus (P)	3.397	3.482	4.755	3.062	5.217	54.209	61.414	4.066	3.369	6.744	66.280	66.305
6	Vanadium (V)	0.586	-	0.693	0.914	0.277	1.576	1.587	0.486	0.551	6.744	3.286	4.688
7	Holmium (Ho)	0.581	0.874	-	-	-	0.577	0.317	-	0.576	-	0.778	0.984
8	Copper (Cu)	0.272	0.538	0.703	0.449	0.379	0.275	0.179	0.230	0.238	-	1.123	0.509
9	Manganese (Mn)	0.165	0.185	-	-	-	0.153	-	-	-	-	-	-
10	Iron (Fe)	0.121	0.142	0.249	0.184	0.104	0.074	0.068	0.120	0.102	0.045	0.148	0.122
11	Zinc (Zn)	0.058	0.081	0.085	0.071	0.080	0.061	0.068	0.120	0.102	0.045	0.148	0.122
12	Silver (Ag)	0.027	-	-	-	0.110	-	-	-	-	-	-	-
13	Nickel (Ni)	0.024	-	-	0.146	-	-	0.042	-	0.012	-	0.348	-
12	Hafnium (Hf)	-	-	-	-	-	-	-	0.191	-	-	-	-
13	Lead (Pb)	-	-	-	-	-	0.095	-	-	-	-	-	-
14	Bromine (Br)	-	0.025	-	-	-	-	-	-	-	-	-	0.028
15	Calcium (Ca)	-	-	-	-	-	-	-	-	1.554	-	-	-
16	Praseodymium (Pr)	-	-	-	-	-	0.036	-	-	-	-	-	-
17	Selenium (Se)	-	-	-	-	-	-	-	-	-	-	-	0.153
18	Gadolinium (Gd)	-	-	-	-	-	0.628	-	-	0.514	-	-	-
19	Titanium (Ti)	-	-	-	-	-	-		-	-	0.335	-	-
20	Ytterbium (Yb)	-	0.424	-	-	-	-	-	-	-	-	-	-
21	Lutetium (Lu)	-	-	-	-	0.393	-	-	-	-	-	-	0.640
22	Chromium (Cr)	-	-	-	-	-	0.073	0.048	-	-	0.049	-	-

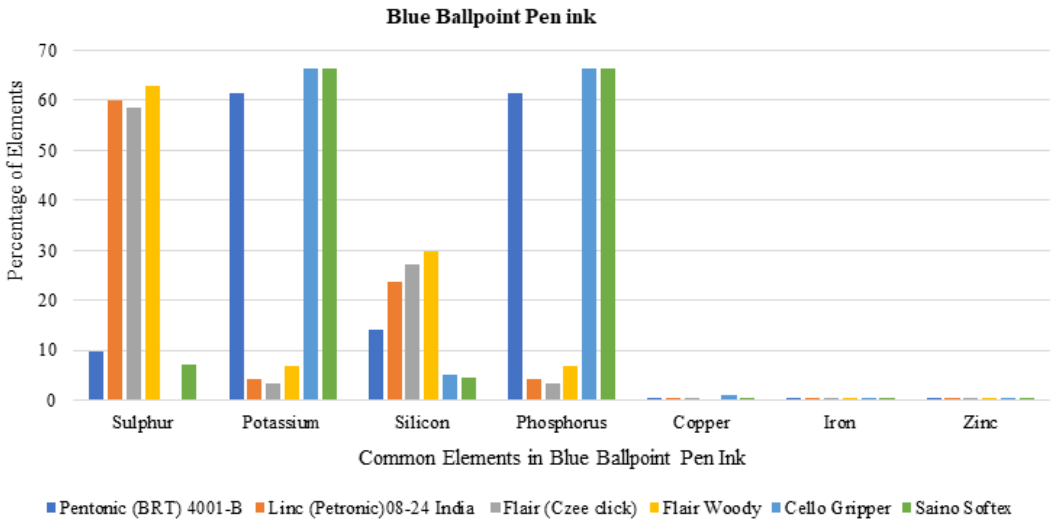


Figure 1. (B) Percentage of various elements present in different brands of blue ballpoint pen ink

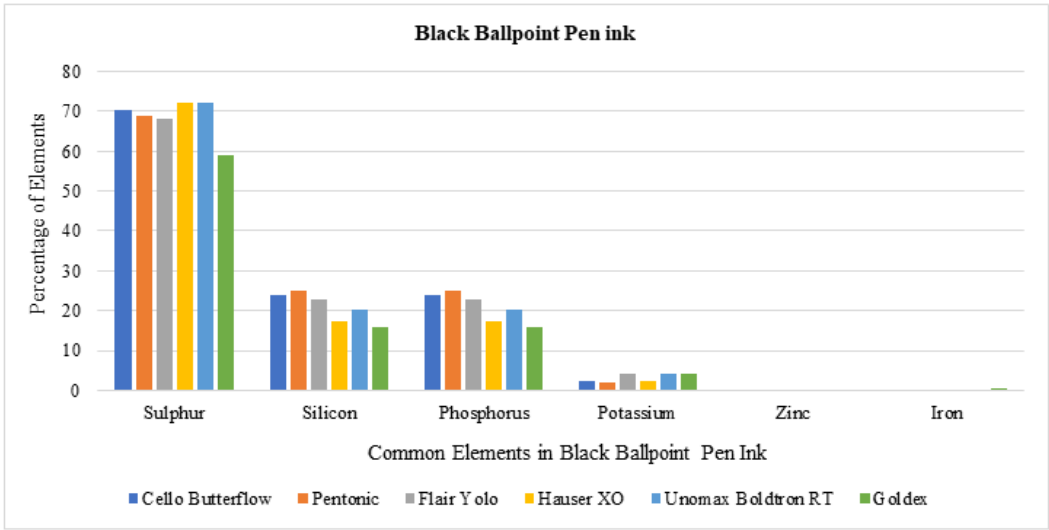


Figure 2. (A) Percentage of various elements present in different brands of black ballpoint pen ink

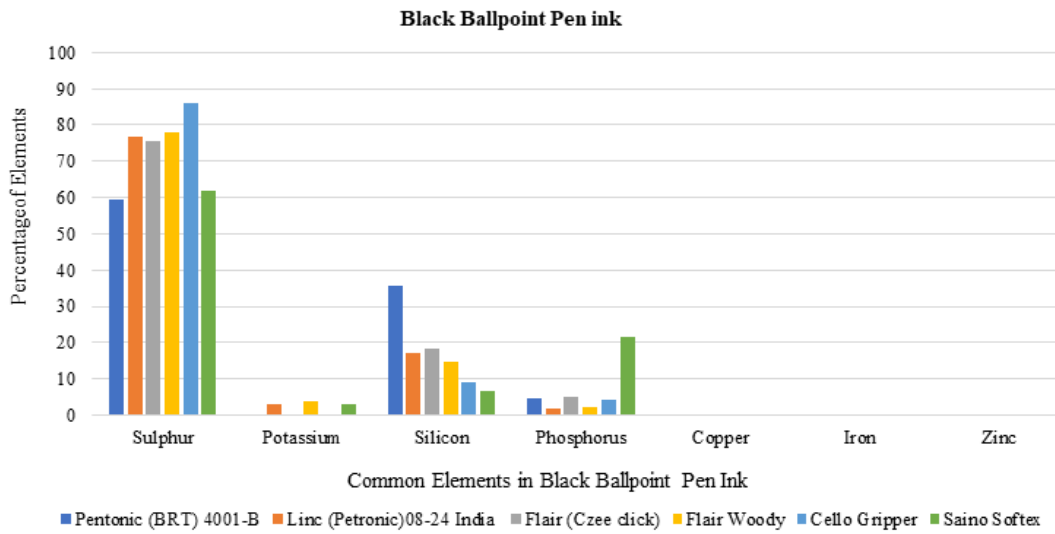


Figure 2. (B) Percentage of various elements present in different brands of black ballpoint pen ink

Table 2. Percentage of Elements in Black Ballpoint Pen Inks of Different Manufacturers

Sr. No.	Elemental Composition	Percentage of Elements in BlackBall Point Pen Ink											
		Cello Butterflow %	Pentonic %	Flair Yolo%	Hauser XO %	Unomax Boldtron RT %	Goldex %	Pentonic (BRT) 4001-B	Linc (Petronic) 08-24 India	Flair (Czee click)	Flair Woody	Cello Gripper	Saino Softex
1	Sulphur (S)	70.196	68.737	68.245	72.008	71.954	58.922	59.570	76.614	75.450	77.807	86.188	62.046
2	Silicon (Si)	23.889	24.858	22.723	17.320	20.098	15.841	35.679	17.211	18.386	14.623	8.966	6.589
3	Phosphorus (P)	23.889	24.858	22.723	17.320	20.098	15.841	4.505	1.937	5.238	2.096	4.353	21.636
4	Potassium (K)	2.440	2.151	4.338	2.364	4.160	4.067	-	3.093	-	3.670	-	3.228
5	Copper (Cu)	0.374	0.110	0.170	0.286	0.176	-	0.055	0.335	0.102	0.210	0.159	0.219
6	Gadolinium (Gd)	0.258	-	-	-	-	0.361	-	-	-	0.321	-	-
7	Cobalt (Co)	0.107	0.055	-	0.205	0.087	-	-	0.239	0.074	0.231	-	-
8	Nickel (Ni)	0.080	-	-	0.140	-	-	-	0.151	-	0.010	-	0.051
9	Zinc (Zn)	0.041	0.025	0.077	0.054	0.069	0.093	-	0.061	-	0.052	-	0.038
10	Iron (Fe)	0.030	0.142%	0.046	0.057	0.056	0.373	0.031	0.091	0.028	0.058	0.055	0.055
11	Holmium (Ho)	-	0.364	0.085	0.971	0.419	0.286	-	-	-	0.589	-	0.358
12	Titanium (Ti)	-	-	0.310	-	0.264	-	0.126	-	-	-	-	-
13	Calcium (Ca)	-	-	-	-	0258	0.767	-	-	-	-	-	-
14	Silver (Ag)	-	-	-	-	-	-	0.010	0.030	-	-	-	0.028
15	Hafnium (Hf)	-	-	-	0.735	-	-	-	-	-	-	-	-
16	Lutetium (Lu)	-	-	-	0.094	-	-	-	-	-	-	-	-
17	Osmium (Os)	-	-	-	0.110	-	-	-	-	-	-	-	-
18	Erbium (Er)	-	-	-	-	-	-	-	-	-	-	-	0.212
19	Lanthanum (La)	-	--	-	-	-	-	-	-	0.461	-	-	-
20	Manganese (Mn)	-	0.074	-	-	-	-	-	-	-	-	-	-

Table 3. Percentage of Elements in Red Ballpoint Pen Inks of Different Manufacturers

Sr. No.	Elemental Composition	Percentage of Elements in RedBall Point Pen Ink											
		Cello Butterflow %	Pentonic %	Flair Yolo%	Hauser XO %	Unomax Boldtron RT %	Goldex %	Pentonic (BRT) 4001-B	Linc (Petronic) 08-24 India	Flair (Czee click)	Flair Woody	Cello Gripper	Saino Softex
1	Sulphur (S)	41.173	41.595	43.495	44.993	44.872	33.473	46.159	46.821	39.631	49.500	51.666	20.810
2	Silicon (Si)	33.421	29.368	39.434	31.977	33.295	34.925	29.275	34.127	39.273	33.763	22.711	24.951
3	Phosphorus (P)	19.110	22.290	8.299	15.474	15.300	30.258	17.486	10.814	15.796	8.652	16.983	40.756
4	Potassium (K)	3.694	4.742	4.335	5.146	3.871	-	5.280	5.364	3.367	6.198	6.405	5.248
5	Zinc (Zn)	0.066	0.063	0.099	0.060	0.082	0.110	0.081	0.080	0.071	0.078	0.084	0.185
6	Iron (Fe)	0.110	0.093	0.260	0.138	0.688	0.137	0.443	0.245	0.305	0.133	0.348	0.891
7	Holmium (Ho)	0.543	-	1.378	0.452	-	-	-	1.203	-	0.638	-	2.513
8	Copper (Cu)	0.354	0.278	0.523	0.286	0.530	0.402	0.466	0.506	0.413	0.293	0.260	0.616
9	Gadolinium (Gd)	0.701	0.507	1.255	0.541	-	-	-	-	0.693	0.590	-	-
10	Erbium (Er)	0.473	0.402	-	-	-	-	-	-	-	-	0.435	2.220
11	Lutetium (Lu)	0.355	-	-	-	0.616	-	-	0.151	-	-	-	-
12	Titanium (Ti)	-	0.327	-	-	-	0.561	-	-	-	-	0.497	-
13	Terbium (Tb)	-	-	-	0.933	-	-	-	-	-	-	-	-
14	Manganese (Mn)	-	-	-	-	0.235	-	0.474	-	-	-	-	-
15	Osmium (Os)	-	-	-	-	0.179	-	-	-	-	-	-	-
16	Hafnium (Hf)	-	-	-	-	0.330	-	-	-	-	-	0.245	-
17	Silver (Ag)	-	0.041	-	-	-	-	-	0.148	-	-	-	-
18	Calcium (Ca)	-	-	0.606	-	-	-	-	-	-	-	-	1.583

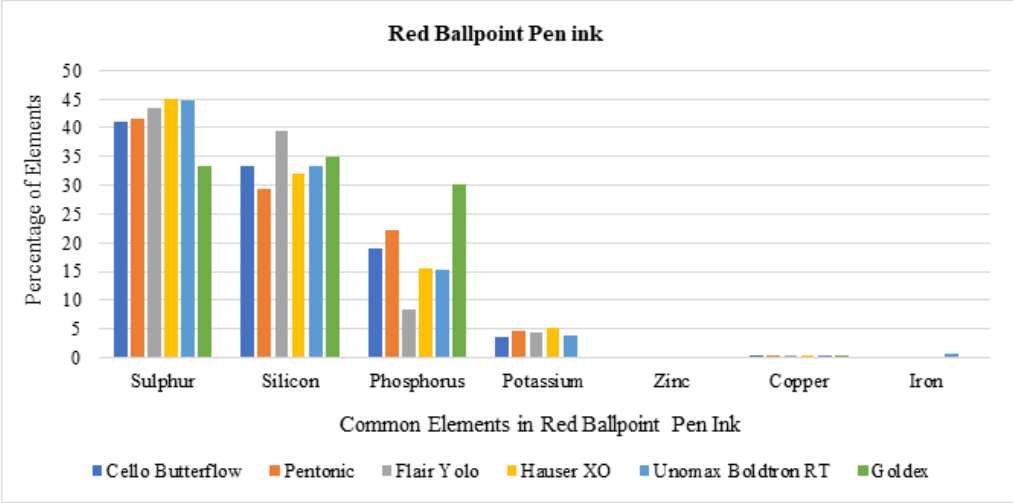


Figure 3. (A) Percentage of various elements present in different brands of red ballpoint pen ink

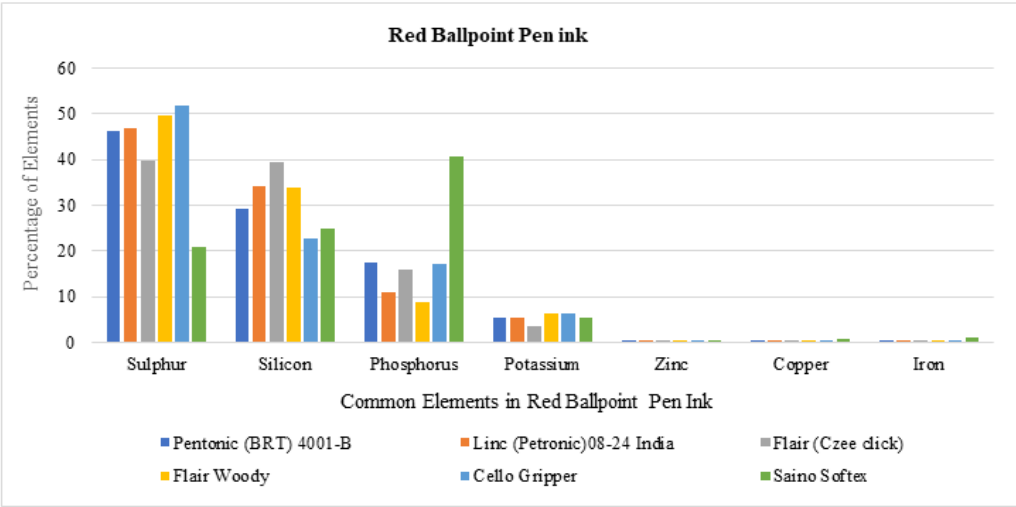


Figure 3. (B) Percentage of various elements present in different brands of red ballpoint pen ink

Table 4. Percentage of Elements in the Mylar sheet

Element	Concentration in %
Silicon (Si)	0.194
Sulphur (S)	0.106
Copper (Cu)	0.016
Caesium (Cs)	0.015
Molybdenum (Mo)	0.010
Gold (Au)	0.006
Chromium (Cr)	0.005
Iron (Fe)	0.004
Lanthanum (La)	0.003
Iridium (Ir)	0.003
Manganese (Mn)	0.000
Plastic	99.638

The background readings of the mylar sheet for the presence of inorganic materials were taken, and the results showed that the percentage of inorganic materials present was found in traces in the mylar sheet and were negligible, as shown in Table 4 mentioned above. The plastic material was found to be 99.6385%.

Discussion

As indicated in Table 1-3, findings are based on varying percentages of elements found in blue, black and red ballpoint pen inks from different manufacturers. The percentage of elements shown in the table and graphic figures 1-3 indicates that all samples of inks contained some common and different elements in varying percentages. These percentages specify the ink type and making it

unique from other inks. EDXRF analysis can be used to distinguish between inks of different brands and models to determine their elemental composition. The results demonstrated that some elements, such as sulphur, potassium, silicon, phosphorus, zinc, and iron, were consistently present across all inks in varying percentages, regardless of their hue or brand. These elements are crucial to the general formulation of pen inks.

In blue ballpoint pen ink, as presented in Table 1, unique elements were identified as follows: Lutetium (Lu) was present only in Unomax ink, while Silver (Ag) was detected in the compositions of both Cello Butterflow and Unomax. Lead (Pb) and Praseodymium (Pr) were exclusive to Goldex ink, whereas Molybdenum (Mo), Chromium(Cr)are present in both Goldex and Pentonic (BRT) 4001-B. Additionally, Osmium (Os), Ytterbium (Yb), Bromine (Br), and Erbium (Er) were found only in Pentonic ink. Hafnium is only found in Linc (Petronic)08-24 India and Titanium (Ti) is unique to Flair Woody, making a unique identifier for these brands. Saino Softex brand contains Selenium (Se), Lutetium (Lu), Bromine (Br).

For black ballpoint pen ink mentioned in Table 2, apart from the commonly shared elements, Titanium (Ti) was detected in Flair, Unomax and Pentonic (BRT) 4001-Binks, while Calcium (Ca) was found in Goldex and Unomax inks in varying percentages. Hafnium (Hf), Lutetium (Lu), and Osmium (Os) were uniquely identified in Hauser XO ink. Erbium (Er) was only present in the inks from Saino Softex brand of ink, whereas Lanthanum (La) can be identified in Flair Czee Click Black ink. It was observed that the Cello Gripper does not bear any unique element; instead, it has the same elements but in different percentage. Gadolinium is found in Cello Butterflow, Goldex, and Flair Woody.

The red ballpoint pen ink elements presented in Table 3, Lutetium (Lu) was detected in Cello Butterflow and Unomax Boldtron Rt inks, while Titanium (Ti) was found in Pentonic, Cello Griper and Goldex inks. Terbium (Tb) was exclusive to Hauser XO ink. Additionally, Hafnium (Hf), Manganese (Mn), and Osmium (Os) were found only in Unomax ink. Erbium (Er) is common to Cello Butterflow and Saino Softex manufacturers. whereas Lanthanum (La) can be identified in Flair Czee Click red ink. Holmium (Ho) is particular to Flair Yolo, Linc Petronic and Flair Woody.

These findings demonstrate that certain elements are common across all pen inks, each brand and ink colour, whereas these contain some unique elements which make them distinguishable. This variability is significant for forensic and analytical applications, particularly in identifying the origin of inks. Overall, the study demonstrates that EDXRF is a powerful tool for ink analysis, offering valuable insights into the elemental composition and providing a scientific basis for distinguishing between various inks in cases of forensic document examination.

Conclusion

- The foregoing results and discussion revealed that these inks contained some common elements that is Sulphur (S), Silicon (Si), Chlorine (Cl), Potassium (K), Phosphorus (P), Vanadium (V), Holmium (Ho), Copper (Cu), Manganese (Mn), Iron (Fe), Zinc (Zn) in different percentages graphically presented in the figures 1-3.
- The inks also contained some unique elements in their composition, as shown in Tables 1-3.
- The non-destructive elemental analysis by the use of EDXRF can be carried out to find out the similarity or differences in inks in questioned document examination.

The research laid its importance by distinguishing between inks by their elemental make-up, which is invaluable in document forgery, alteration of ink, and verification of authorship. The findings can be applied to various aspects such as law enforcement organisations and forensic document examiners, to track the origin of a document. Future studies need to widen the ambit of investigation by looking at a greater variety of ink brands and colours in order to increase accuracy in differentiating inks.

Declarations

Funding: This research received no specific grant from any funding agency.

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

Ethical Approval: Not applicable, as the study includes analysis of inks extracted from pens that are commercially available on the market.

Reference

1. Ferrero J, Roldan C, Juanes D, Carballo J, Pereira J, Ardid M, et al. Study of inks on paper engravings using portable EDXRF spectrometry. Nucl Instrum Methods Phys Res B. 2003;213:729-34. doi:10.1016/S0168-583X(03)01694-X.
2. Pessanha S, Manso M, Guilherme A, Costa M, Carvalho ML. Investigation of historical documents for forensic purposes by X-ray fluorescence spectrometry. Surf Interface Anal. 2010;42:419-22. doi:10.1002/sia.3085.
3. Manso M, Costa M, Carvalho ML. Comparison of elemental content on modern and ancient papers by EDXRF. Appl Phys A. 2008;90:43-48. doi:10.1007/s00339-007-4235-y.
4. Manso M, Costa M, Carvalho ML. From papyrus to paper: elemental characterization by X-ray fluorescence spectrometry. Nucl Instrum Methods Phys Res A. 2007;580:732-34. doi:10.1016/j.nima.2007.05.136.
5. Shih Y, Chen Y. The development of X-ray fluorescence for trace evidence detection and document analysis in forensic science. Forensic Sci J. 2022;21(1):1-15. doi:10.6593/FSJ.202212_21(1).0002.
6. Verma N, Sharma V, Kumar R, Sharma R, Joshi MC, Umapathy GR, et al. On the spectroscopic examination of printed documents using field emission scanning electron microscopy with energy-dispersive X-ray spectroscopy (FE-SEM-EDS) and chemometric methods: application in forensic science. Anal Bioanal Chem. 2019;411:3477-95. doi:10.1007/s00216-019-01824-z.
7. Rožić M, Mačefat MR, Oreščanin V. Elemental analysis of ashes of office papers by EDXRF spectrometry. Nucl Instrum Methods Phys Res B. 2005;229:117-22. doi:10.1016/j.nimb.2004.11.011.
8. Whiting T. Discrimination of ballpoint pen inks by high-performance capillary electrophoresis and high-performance liquid chromatography. J Am Soc Questioned Doc Examiners. 1998;1:2-16.
9. Dhara S, Misra N, Maind SA, Kumar SA, Chattopadhyay N, Aggarwal S. Forensic application of total reflection X-ray fluorescence spectrometry for elemental characterization of ink samples. Spectrochim Acta Part B At Spectrosc. 2010;65:167-70. doi:10.1016/j.sab.2010.01.004.
10. Egan JM, Brewer JD, Hagan KA, Strelko CL. Capillary electrophoresis of ballpoint pen inks. J Am Soc Questioned Doc Examiners. 2006;9:37-45. doi:10.69525/jasqde.130.

Original Article

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Clustering of HIV, Hepatitis C and Tuberculosis in Custodial Deaths: Role of Drug Abuse and Socio-demographic Factors

Preetinder Singh Chahal[1], Merry Pal Kaur Chawla[2], Akashdeep Aggarwal[3], Amandeep Kaur[4], Didar Singh Walia[5]

[1]Associate Professor, Department of Forensic Medicine, Govt. Medical College, Patiala. [2]Assistant Professor, Department of Forensic Medicine, Guru Gobind Singh Medical College, Faridkot. [3]Professor and Head, Department of Forensic Medicine, Govt. Medical College, Patiala. [4]Senior resident, Department of Forensic Medicine, Govt. Medical College, Patiala. [5]Associate Professor, Department of Forensic Medicine, Govt. Medical College, Patiala.

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Abstract

Background: Custodial populations are recognised as high-risk groups for blood-borne viral infections and tuberculosis, largely due to pre-incarceration behavioural and socio-demographic vulnerabilities. Custodial deaths often attract medico-legal scrutiny, yet systematic data examining infectious disease burden and its contribution to mortality remain limited.

Objectives: To analyse the epidemiological profile of custodial deaths with respect to HIV, hepatitis C virus (HCV), tuberculosis (TB), and drug abuse; to assess infection clustering; and to examine their association with socio-demographic factors, custody status, terminal hospital stay, and cause of death.

Materials and Methods: A retrospective analytical study was conducted on 143 custodial deaths at a tertiary care centre in North India. Anonymised data were extracted from medico-legal autopsy records, hospital files, and laboratory reports. The study was carried out from 1 April 2020 to 31 March 2025.

Results: HIV, HCV and TB were detected in 33.6%, 28.0% and 27.3% of cases respectively. Drug abuse showed a strong association with HIV, HCV and HIV–HCV co-infection ($p < 0.001$). Multiple infections were present in 21.7% of cases, with HIV–HCV–TB triple infection in 14.0%. Natural causes accounted for 93.7% of deaths, predominantly due to opportunistic infections, pulmonary tuberculosis and chronic liver disease.

Conclusion: Custodial deaths were largely attributable to advanced pre-existing infectious disease burden compounded by drug abuse, underscoring the need for early screening, harm reduction and integrated care in custodial settings.

Keywords: Custodial Death; Prisoners; HIV Infections; Hepatitis C; Tuberculosis; Substance-Related Disorders; Drug Users; Coinfection; Mortality; Forensic Medicine

Corresponding Author: Merry Pal Kaur Chawla, Assistant Professor, Department of Forensic Medicine, Guru Goblnd Singh Medical College, Faridkot.

E-mail:merrypalkaur@gmail.com

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Introduction

Custodial settings represent a unique intersection of public health vulnerability, social marginalisation, and medico-legal scrutiny. Globally, incarcerated populations bear a disproportionately high burden of communicable diseases, particularly human immunodeficiency virus (HIV), hepatitis C virus (HCV), and tuberculosis (TB), compared with the general population[1,2]. This excess burden is largely attributed to pre-incarceration risk behaviours such as injecting drug use, unsafe sexual practices, poor access to healthcare, and socio-economic deprivation, which are subsequently concentrated within custodial environments.

Drug abuse, especially injecting drug use, has been consistently identified as the most important determinant for transmission of blood-borne viral infections in prisons[3,4]. HCV transmission is particularly efficient via percutaneous exposure, often resulting in higher prevalence rates than HIV among incarcerated individuals, while HIV–HCV co-infection is common due to shared transmission routes[1]. TB further complicates this landscape, as immunosuppression associated with HIV, coupled with overcrowding and delayed diagnosis, increases the risk of active disease and adverse outcomes[5].

Despite growing epidemiological evidence on infection prevalence in prisons, limited data exist that specifically examine custodial deaths as a distinct subgroup. Deaths in custody frequently attract judicial, human rights, and public attention, necessitating robust scientific evaluation to differentiate natural disease-related mortality from allegations of custodial violence or neglect. Indian forensic literature has repeatedly demonstrated that the majority of custodial deaths are due to natural causes, with TB, HIV-related opportunistic infections, and chronic liver disease being prominent contributors.[3,6–8] However, systematic analyses exploring infection clustering, behavioural correlates, and terminal hospitalisation patterns in custodial deaths remain sparse.

Understanding the interplay between socio-demographic factors, drug abuse, infectious disease burden, and cause of death is therefore crucial from both public health and medico-legal perspectives. Identification of single and multiple infection patterns, particularly HIV–HCV and HIV–HCV–TB co-infections, provides insight into disease severity, healthcare utilisation, and mortality risk in this vulnerable population.

The present study was undertaken to analyse the epidemiological profile of custodial deaths with respect to HIV, HCV, TB, and drug abuse, to examine patterns of infection clustering, and to assess their association with socio-demographic variables, terminal hospital stay, and causes of death. By doing so, the study aims to contribute evidence-based clarity to custodial death investigations and inform targeted preventive and therapeutic interventions within correctional systems.

Materials and Methods

This was a retrospective observational, record-based study of 143 custodial deaths reported at a tertiary care teaching hospital in North India from 1 April 2020 to 31 March 2025. Data were retrieved from medico-legal post-mortem records, hospital case files, prison medical records, and laboratory reports. All data were fully anonymised prior to analysis, and no personal identifiers were accessed or retained. Serological status for HIV and HCV, tuberculosis diagnosis was based on documented clinical evidence. Drug abuse history and presence of injection marks were obtained from clinical records and detailed post-mortem examination. Socio-demographic variables, custody status, terminal hospital stay, infectious disease burden, and cause of death were systematically recorded. Statistical analysis was performed using appropriate tests of association, with a p-value <0.05 considered statistically significant. All custodial deaths during the study period that underwent medico-legal post-mortem examination at the study centre and had the relevant records available were included. Cases in which custodial status could not be confirmed or the essential records were incomplete were excluded. Associations were assessed using the chi-square test or Fisher's exact test, as appropriate. For the main binary comparisons, unadjusted odds ratios (ORs) with 95% confidence intervals (CIs) were calculated from the 2×2 tables. Adjusted estimates could not be calculated from the aggregate data available for this study.

Results

A total of 143 custodial deaths were analysed, comprising 140 males (97.9%) and 3 females (2.1%). The majority of inmates were undertrials (81.1%), while convicts constituted 18.9%. Most deaths were due to natural causes (93.7%), with only 6.3% attributed to unnatural causes (Table 1).

Infectious Disease Profile and Age Distribution

Overall, HIV infection was detected in 48 cases (33.6%), HCV in 40 cases (28.0%), and TB in 39 cases (27.3%). HIV and HCV positivity showed a strong age association, with the highest prevalence observed in individuals aged ≤30 years, followed by a progressive decline with increasing age (χ^2 = 51.57 and 43.33 respectively; $p < 0.001$). TB showed a weaker but statistically significant association with age ($\chi^2 = 9.76$; $p = 0.045$), with cases distributed across all age groups (Table 1).

Custody Status, Behavioural Factors and Co-infections

HIV and HCV positivity were significantly higher among undertrial prisoners compared to convicts ($p = 0.006$ and $p = 0.028$ respectively), whereas TB showed no association with custody type (Table 1). Drug abuse was significantly associated with HIV ($p < 0.001$; OR 16.00, 95% CI 6.80–37.62) and HCV ($p < 0.001$; OR 9.71, 95% CI 4.20–22.46), while no significant association was seen with TB. Injection-related prick marks demonstrated highly significant associations with HIV, HCV, and drug abuse (all $p < 0.001$), reinforcing the role of injecting practices in viral transmission (Table 1). Prick marks suggestive of injection were significantly associated with HIV (OR 19.57, 95% CI 6.75–56.69; $p < 0.001$) and HCV (OR 9.30, 95% CI 3.78–22.87; $p < 0.001$). HIV (OR 3.51, 95% CI 1.14–10.84; $p = 0.006$) and HCV (OR 3.75, 95% CI 1.06–13.24; $p = 0.028$) were also more frequently observed among undertrials than convicts.

HIV–HCV co-infection was identified in 31 cases (21.7%), and a statistically significant association was observed between TB and HIV–HCV co-infection ($p < 0.001$). HIV–HCV co-infection was significantly associated with drug abuse ($p < 0.001$; OR 59.72, 95% CI 17.70–201.50). It was also associated with TB (OR 8.90, 95% CI 3.67–21.58; $p < 0.001$) (Table 1).

Table 1. Socio-demographic profile of custodial deaths and association with HIV, HCV, TB and Drug abuse (n = 143) (Males = 140, 97.9% and Females = 3, 2.1%)

Parameters		n	% + (n=48)	HIV		HCV		TB		Drug abuse	
				- (n=95)	+ (n=40)	- (n=103)	+ (n=39)	- (n=104)	+ (n=51)	- (n=92	
Age group (years)	≤30	44	30.8	32	12	27	17	19	25	28	16
	31–40	34	23.8	11	23	10	24	9	25	12	22
	41–50	22	15.4	4	18	3	19	5	17	7	15
	51–60	19	13.3	1	18	0	19	3	16	1	18
	>60	24	16.8	0	24	0	24	3	21	3	21
	χ^2			51.57		43.33		9.76		28.41	
	p			< 0.001		< 0.001		0.045		< 0.001	
Custody type	Convict	27	18.9	4	23	3	24	6	21	43	73
	Undertrial	116	81.1	44	72	37	79	33	83	8	19
	χ^2			7.64		4.82		0.02		0.62	
	p			0.006		0.028		0.882		0.432	
Manner of death	Natural	134	93.7	43	91	36	98	36	98	51	83
	Unnatural	9	6.3	5	4	4	5	3	6	0	9
	χ^2			2.54		1.18		0.11		3.79	
	p			0.113		0.282		0.743		0.051	

Continue.....

Prick marks	Present	30	21.0	25	5	20	10	10	20	27	3
	Absent	113	79.0	23	90	20	93	29	84	24	89
	χ^2			39.39		25.84		0.37		45.90	
	p			< 0.001		< 0.001		0.542		< 0.001	
HIV co-infection	Present	48	33.6	-	-	31	17	20	28	36	12
	Absent	95	66.4	-	-	9	86	19	76	15	80
	χ^2			-		45.37		6.49		46.18	
	p			-		< 0.001		0.011		< 0.001	
HIV–HCV co-infection	Present	31	21.7	-	-	-	-	20	11	25	6
	Absent	112	78.3	-	-	-	-	19	93	26	86
	χ^2			-		-		27.70		38.70	
	p			-		-		< 0.001		< 0.001	
Drug abuse	Present	51	35.7	36	15	29	22	14	37	-	-
	Absent	92	64.3	12	80	11	81	25	67	-	-
	χ^2			46.18		30.65		0.00		-	
	p			< 0.001		< 0.001		0.999		-	
Marital status	Married	97	67.8	23	74	17	80	22	75	26	71
	Unmarried	43	30.1	23	20	22	21	17	26	24	19
	Divorced	3	2.1	2	1	1	2	0	3	1	2
	χ^2			13.35		16.77		5.42		10.93	
	p			0.0013		< 0.001		0.067		0.004	
Religion	Sikh	101	70.6	35	66	28	73	28	73	35	66
	Hindu	36	25.2	14	22	10	26	9	27	14	22
	Muslim	6	4.2	2	4	2	4	2	4	2	4
	χ^2			0.41		0.59		0.36		0.22	
	p			0.821		0.740		0.843		0.891	
Physical built	Average	26	18.2	5	21	4	22	6	20	5	21
	Poor	46	32.2	24	22	18	28	17	29	19	27
	Well	71	49.7	19	52	18	53	16	55	27	44
	χ^2			11.01		5.13		3.21		3.87	
	p			0.004		0.077		0.200		0.140	
Terminal hospital stay	0-1 days	61	42.7	14	47	12	49	13	48	17	44
	2-7 days	50	35.0	21	29	18	32	14	36	22	28
	1-3 weeks	26	18.2	12	14	10	16	11	15	11	15
	4-5 weeks	6	4.2	1	5	0	6	1	5	1	5
	χ^2			7.29		7.44		4.41		4.57	
	p			0.063		0.059		0.22		0.21	
Area	Rural (n=86)	86	60.1	24	62	21	65	23	63	27	59
	Urban (n=57)	57	39.9	24	33	19	38	16	41	24	33
	χ^2			2.49		0.95		0.00		1.28	
	p			0.11		0.33		1.00		0.26	

Table 2. Single vs Multiple Infectious Disease Burden among Inmates (n = 143)

Infectious disease burden	n	%
No infection (HIV− / HCV− / TB−)	67	46.9
Single infection (any 1)	45	31.5
HIV only	17	11.9
HCV only	9	6.3
TB only	19	13.3
Multiple infections (≥2)	31	21.7
HIV + HCV	11	7.7
HIV + TB	0	0.0
HCV + TB	0	0.0
HIV + HCV + TB	20	14.0
Total	143	100.0

Table 3. Key unadjusted associations with HIV, HCV and HIV–HCV co-infection

Association	Odds ratio (OR)	95% CI	P-value
Drug abuse → HIV	16.00	6.80–37.62	<0.001
Drug abuse → HCV	9.71	4.20–22.46	<0.001
Drug abuse → HIV–HCV co-infection	59.72	17.70–201.50	<0.001
Prick marks → HIV	19.57	6.75–56.69	<0.001
Prick marks → HCV	9.30	3.78–22.87	<0.001
Undertrial vs convict → HIV	3.51	1.14–10.84	0.006
Undertrial vs convict → HCV	3.75	1.06–13.24	0.028
TB → HIV–HCV co-infection	8.90	3.67–21.58	<0.001
Unmarried vs married/divorced → HIV	4.65	2.17–9.98	0.0013
Unmarried vs married/divorced → HCV	5.24	2.38–11.51	<0.001
Poorly built vs average/well built → HIV	3.32	1.58–6.95	0.004

Note: ORs and 95% CIs are unadjusted estimates calculated from the 2×2 counts in Table 1. Adjusted ORs were not calculated because individual-level data were not available.

Single vs Multiple Infectious Disease Burden

Nearly half of the inmates (46.9%) had no evidence of HIV, HCV, or TB. Single infection was present in 31.5%, most commonly TB alone (13.3%), followed by HIV alone (11.9%). Multiple infections were observed in 21.7%, with HIV–HCV–TB triple infection accounting for 14.0% of all cases (Table 2).

Socio-demographic and Clinical Correlates

Marital status showed a significant association with HIV and HCV, with higher prevalence among unmarried inmates ($p = 0.001$ and $p < 0.001$ respectively), whereas TB showed no significant association. Religion and area of residence (rural/urban) demonstrated no association with HIV, HCV, TB, or drug abuse (Table 1). When unmarried inmates were compared with married/divorced inmates, the unadjusted OR was 4.65 (95% CI 2.17–9.98) for HIV ($p = 0.0013$) and 5.24 (95% CI 2.38–11.51) for HCV ($p < 0.001$).

Physical built was significantly associated with HIV infection ($p = 0.004$), with higher positivity among poorly built inmates. No significant association was seen with HCV, TB, or drug abuse. On comparing poorly built inmates with those having average or good physical built, the unadjusted OR for HIV was 3.32 (95% CI 1.58–6.95). Terminal hospital stay was not significantly associated with any of the infections or with drug abuse (Table 1).

Cause of Death

Among infected inmates, deaths were predominantly due to natural disease processes. HIV-positive cases most commonly died due to opportunistic infections and sepsis, HCV-positive cases due to chronic liver disease, and TB-positive cases due to pulmonary complications. Drug abusers, particularly those with HIV or HCV, predominantly succumbed to infectious or systemic complications.

Discussion

The present study offers a comprehensive epidemiological and medico-legal evaluation of custodial deaths, focusing on the burden and clustering of HIV, hepatitis C virus (HCV), tuberculosis (TB), and drug abuse. The findings demonstrate a strikingly high prevalence of these infections, strong behavioural associations, and an overwhelming predominance of natural causes of death. Together, these observations provide critical insights into custodial mortality and reinforce the importance of viewing prison health as an extension of community health.

Magnitude of Infectious Disease Burden

The prevalence of HIV (33.6%), HCV (28.0%), and TB (27.3%) among custodial deaths in this study is substantially higher than national averages and exceeds prevalence figures reported in many prison-based screening studies. Indian studies from Punjab and central India have reported HIV prevalence ranging from 6–10% and HCV prevalence from 20–32% among inmates, with dual HIV–HCV infection between 8–10%.[3,6–8] The higher proportions observed in the present study are plausibly explained by the exclusive focus on custodial deaths, which represent a biologically more vulnerable subgroup with advanced disease.

Internationally, prison studies from Pakistan, Iran, and Nigeria have consistently documented elevated prevalence of HIV and HCV compared with the general population, although with regional variation in absolute rates.[2,9–11] Global meta-analyses estimate pooled prevalence of HIV at 3–4% and HCV at 15–22% in incarcerated populations, figures that are markedly lower than those observed in the present cohort, again underscoring the severity of disease burden among custodial deaths.[1,2]

Age Pattern and Early Acquisition of Infection

A pronounced inverse association between age and both HIV and HCV was observed, with the highest positivity among inmates aged ≤30 years. Similar age gradients have been reported from prisons in India, Pakistan, and Iran, where younger inmates with a history of injecting drug use account for the majority of HIV and HCV cases.[2,3,9] This pattern strongly suggests early acquisition of infection in the community, well before incarceration. In contrast, TB showed a wider age distribution with only a modest association, reflecting its endemicity and cumulative lifetime exposure rather than age-specific risk behaviour.

Drug Abuse and Injection Practices

Drug abuse showed a strong association with HIV and HCV infection in this study, with highly significant associations supported by objective evidence in the form of injection-related prick marks. These findings are consistent with overwhelming global evidence identifying injecting drug use as the primary driver of blood-borne viral transmission in custodial settings.[1,5] The disproportionately higher prevalence of HCV compared to HIV among drug abusers mirrors international observations, attributable to the higher efficiency of HCV transmission via percutaneous exposure.[1,3]

The lack of association between drug abuse and TB observed in the present study has also been reported elsewhere and reinforces the concept that TB transmission is influenced more by immune status, overcrowding, and background prevalence than by injecting practices per se.[5,9]

Infection Clustering and Triple Burden

One of the most important contributions of this study is the demonstration of infection clustering, with more than one-fifth of custodial deaths harbouring multiple infections and a substantial proportion showing HIV–HCV–TB triple infection. While HIV–HCV co-infection has been widely reported in prisons, triple infection is less frequently documented due to limited TB ascertainment in

many studies.[1,2] Studies from Pakistan and India that have actively screened for TB have similarly highlighted co-morbidity patterns, though at lower frequencies.[6–9]

The significant association between TB and HIV–HCV co-infection observed in this study underscores the synergistic effect of immunosuppression, chronic viral infection, and poor nutritional status. This clustering has profound clinical and prognostic implications and likely explains the high mortality observed in this subgroup.

Custody Status and Socio-demographic Correlates

The significantly higher prevalence of HIV and HCV among undertrial prisoners compared to convicts may indicate that infection was present before incarceration; however, the available records do not allow us to determine when the infection was acquired. This finding aligns with international evidence indicating that prisons act as reservoirs that concentrate pre-existing disease rather than serving as the primary site of transmission.[2,5] TB showed no association with custody type, further supporting its endemic nature.

Marital status demonstrated a significant association with HIV and HCV, with higher prevalence among unmarried inmates. Similar associations have been reported from African and South Asian prison studies, where unmarried status reflects social instability and high-risk behaviour.[9–11] In contrast, religion and rural–urban residence showed no association with infection or drug abuse, an important observation that excludes sociocultural bias and reinforces behavioural causation.

Physical Built, Hospital Stay and Care Access

Poor physical built was significantly associated with HIV infection, likely reflecting advanced disease and chronic catabolism rather than an etiological role of body habitus. There was no significant association between terminal hospital stay and HIV, HCV, TB, or drug abuse. This finding is particularly relevant medico-legally, as it suggests that infected inmates were not systematically deprived of hospital care or referred late compared to non-infected inmates.

Cause of Death and Medico-legal Relevance

Natural causes accounted for nearly all custodial deaths in this study, with opportunistic infections, TB-related pulmonary complications, and chronic liver disease being the dominant causes. This pattern is consistent with Indian forensic studies and global prison mortality reviews, which identify HIV/AIDS and TB as the leading infectious causes of death in custody.[5–8] Importantly, deaths among drug abusers were overwhelmingly due to natural disease rather than overdose or violence, a finding of considerable significance in custodial death inquiries.

Overall, most deaths in this study were associated with advanced disease and the presence of behavioural risk factors. Since prison-entry screening information and a non-custodial comparison group were not available, the study cannot establish when the infections were acquired or assess the contribution of custodial conditions to infection.

Limitations

The retrospective design limits control over data completeness and accuracy, particularly for behavioural variables and treatment history. The study includes only custodial deaths, preventing direct comparison with living inmates or estimation of mortality risk. Advanced immunological and virological parameters were unavailable for analysis. Being a single-centre study, generalisability may be limited. Nevertheless, systematic record review, objective post-mortem findings, and comprehensive infection profiling strengthen the validity of the conclusions. Prison-entry screening data were not available, so it was not possible to determine whether the infections had been acquired before or during incarceration. Only three female deaths were included, which did not allow meaningful sex-based comparison. There was also no non-custodial comparison group. In addition, the available data were in aggregate form, which limited further multivariable analysis.

Suggestions

Universal screening for HIV, HCV, and TB at prison entry should be mandatory, particularly for young and undertrial inmates. Integration of harm-reduction and de-addiction services is essential to address injecting drug use. Early identification and integrated management of co-infected inmates may reduce mortality. From a medico-legal perspective, meticulous documentation of pre-existing disease and

treatment is vital to ensure transparency in custodial death investigations. Prospective multicentric studies with treatment outcome data are recommended.

Conclusion

This study demonstrates a high burden of HIV, HCV and tuberculosis among custodial deaths, with strong clustering of multiple infections and a clear association with drug abuse and injecting practices. The higher frequency of infection among younger and undertrial inmates may reflect a substantial pre-existing disease burden. However, in the absence of prison-entry screening data, the timing of infection cannot be established. Natural disease processes, particularly opportunistic infections, tuberculosis and chronic liver disease, accounted for the vast majority of deaths, underscoring advanced pre-existing morbidity. These findings highlight the critical need for early screening, integrated management of co-infections and effective harm-reduction strategies in custodial settings, while providing robust medico-legal evidence to support natural causation in custodial death investigations.

Declartions

Ethical Clearance: Ethical clearance video letter no. Trg9(310)2024/32860-61 dated 25/10/24

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References

1. Dolan K, Wirtz AL, Moazen B, Ndeffo-Mbah M, Galvani A, Kinner SA, et al. Global burden of HIV, viral hepatitis, and tuberculosis in prisoners and detainees. Lancet. 2016 Sept 10;388(10049):1089–102.
2. Mehmandoost S, Khezri M, Mousavian G, Tavakoli F, Mehrabi F, Sharifi H, et al. Prevalence of HIV, hepatitis B virus, and hepatitis C virus among incarcerated people in Iran: a systematic review and meta-analysis. Public Health. 2022 Feb;203:75–82.
3. Singh GP, Lata S, Swu AK, Virk NS, Singh J, Thakkar S. A retrospective study to find the prevalence of HIV, HCV and dual HIV-HCV infection in the prison inmates. J Fam Med Prim Care. 2022 Oct;11(10):6250.
4. Gahrton C, Westman G, Lindahl K, Öhrn F, Dalgard O, Lidman C, et al. Prevalence of Viremic hepatitis C, hepatitis B, and HIV infection, and vaccination status among prisoners in Stockholm County. BMC Infect Dis. 2019 Nov 9;19(1):955.
5. Bosworth RJ, Borschmann R, Altice FL, Kinner SA, Dolan K, Farrell M. HIV/AIDS, hepatitis and tuberculosis-related mortality among incarcerated people: a global scoping review. Int J Prison Health. 2022 Jan 25;18(1):66–82.
6. Mittal DR, Jasbir S, Rai G, Kumar K, Sharma RK. A Two-Year Prospective Study of Custodial Deaths from Punjab Region of India. Medico Leg Update. 2019 Feb 28;19(1):757–62.
7. Jangid A, Verma A, Sivasankary R, Meshram R, Parate SV, Tyagi A, et al. Epidemiological Characteristics of Custodial Deaths: An Autopsy Study at a Tertiary Care Institute in Rishikesh. Cureus. 2025 Sept 6;17(9):e91737.
8. Bansal YS, Rao M, Singh D. Custodial Deaths - An Overview of the Prevailing Healthcare. J Indian Acad Forensic Med. 2010 Oct 1;32(4):315–7.
9. Wali A, Khan D, Safdar N, Shawani Z, Fatima R, Yaqoob A, et al. Prevalence of tuberculosis, HIV/AIDS, and hepatitis; in a prison of Balochistan: a cross-sectional survey. BMC Public Health. 2019 Dec 4;19(1):1631.
10. Uchechukwu OF, Abdulrahman Y, Aliyu AU, Mustaphar U, Zama I, Charles AT, et al. Seroprevalence of HIV, HBV and HCV among Prisoners in Sokoto, Nigeria. Asian J Med Health. 2018 Dec 31;1–8.
11. Adepoju VA, Abdulrahim A, Udah DC, Jamil S, Olaniyi BO, Gulumbe BH, et al. Prevalence and risk factors of hepatitis B and C among prison inmates in Nigeria: a systematic review and meta-analysis. BMC Public Health. 2025 Nov 24;26:10.

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Associations of Mortuary Ambient Temperature, Relative Humidity and Gross Nutritional Status with Rigor Mortis and Postmortem Lividity in the Mahakaushal Region of Central India

Abhishek Soni

MD (Forensic Medicine), Class II Gazetted Medical Officer and Forensic Doctor, Department of Public Health and Medical Education, Government of Madhya Pradesh; posted at Civil Hospital Berasia, District Bhopal, Madhya Pradesh, India.

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Abstract

Objective: To assess the associations of mortuary ambient temperature, relative humidity and gross nutritional status with the stage of rigor mortis, pattern of postmortem lividity and lividity fixation in medicolegal autopsies.

Methods: This prospective autopsy-based observational study included 100 consecutive eligible cases with a documented time of death over 18 months in the Mahakaushal region of Central India. Rigor mortis was staged by systematic manual examination; lividity was classified as mottled, coalesced or fully developed, with fixation assessed by a one-minute blanching test. Mortuary temperature and humidity were measured digitally, and nutritional status was graded from gross body habitus. Sparse contingency tables were evaluated by Monte Carlo exact conditional tests; association strength was summarized with Cramer's V.

Results: Rigor mortis was fully developed in 60.0% (95% CI, 50.2%-69.1%), lividity was fully developed in 54.0% (95% CI, 44.3%-63.4%), and lividity was fixed in 69.0% (95% CI, 59.4%-77.2%) of cases. Fully developed rigor occurred in 80.4% of cases examined at 30-35°C and all cases at 35-40°C; fixed lividity occurred in 84.3% and 100%, respectively. Temperature and humidity were associated with all three postmortem outcomes (all $p<0.001$). Relative humidity showed the largest effect sizes for rigor stage (V=0.973; 95% CI, 0.814-1.000), lividity pattern (V=0.710; 95% CI, 0.544-0.826) and fixation (V=0.843; 95% CI, 0.626-1.000). Nutritional status was associated with rigor and lividity pattern (both $p<0.001$), but not conclusively with fixation ($p=0.052$).

Conclusions: Observed rigor and lividity differed substantially across mortuary environmental and nutritional categories. These factors should be documented when interpreting early postmortem signs, but the cross-sectional findings do not constitute a stand-alone time-since-death equation; prospective validation against measured postmortem intervals is required.

Keywords: Cramer's V; forensic pathology; livor mortis; postmortem interval; relative humidity; rigor mortis

Corresponding Author: Abhishek Soni, MD (Forensic Medicine), Class II Gazetted Medical Officer and Forensic Doctor, Department of Public Health and Medical Education, Government of Madhya Pradesh; posted at Civil Hospital Berasia, District Bhopal, Madhya Pradesh, India

E-mail: drabhisheksonimbbs@gmail.com

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Introduction

Estimating the postmortem interval is central to reconstruction of death and interpretation of medicolegal evidence. Rigor mortis and dependent lividity remain accessible early postmortem signs, but neither functions as an invariant chronometer; their appearance depends on elapsed time, physiology and the environment.[1-3]

Temperature, relative humidity, body habitus, premortem exertion and cause of death can modify the biochemical and vascular processes underlying muscular stiffening and hypostasis.[4] Evidence from Indian autopsy series also shows geographic and seasonal variability in rigor and lividity, limiting uncritical application of fixed timelines derived in other climates.[5-7]

Published Indian autopsy studies document regional and seasonal variation in rigor mortis and postmortem lividity, but evidence concurrently evaluating mortuary temperature, relative humidity and gross nutritional status against rigor stage, lividity pattern and lividity fixation within one regional cohort remains limited. The present study addressed this gap in the hot, seasonally humid Mahakaushal region by simultaneously assessing these three routinely documented modifiers across three early postmortem outcomes and quantifying association strength. We hypothesized that outcome distributions would differ across environmental and nutritional categories.

Materials and Methods

Study design, setting and participants

A prospective observational autopsy-based study was undertaken over 18 months at a tertiary teaching-hospital mortuary in the Mahakaushal region of Central India. The planned sample comprised 100 consecutive eligible medicolegal autopsy cases. Hospital deaths with a documented time of death were eligible. Bodies exposed to mortuary refrigeration before assessment, or showing cold stiffening, heat stiffening, cadaveric spasm or decomposition, were excluded; fetal autopsies and drowning cases were also excluded.

Measurements and operational definitions

Each body was examined in adequate light before refrigeration. Mortuary ambient temperature and relative humidity at examination were recorded with a digital hygrometer. Rigor mortis was assessed systematically by passive manipulation of the eyelids, mandible, neck, trunk, intercostal and abdominal musculature, limb joints, fingers and toes, and was classified as appearing, fully developed or disappearing according to the resistance encountered.

Postmortem lividity was classified as mottled (discrete patches), coalesced (merged patches not yet uniformly covering the dependent surface) or fully developed (uniform dependent discoloration). Fixation was tested by sustained right-thumb pressure for one minute; blanching denoted unfixed lividity and absence of blanching denoted fixation. Gross nutritional status was visually graded from body habitus as poorly, moderately or well nourished. Cause of death was abstracted from the autopsy record.

Outcomes and statistical analysis

Primary outcomes were rigor-mortis stage, lividity pattern and lividity fixation. Exposures were categorized mortuary temperature, relative humidity and gross nutritional status. Categorical variables were summarized as n (%), with percentages recalculated within exposure categories for interpretability. All 100 cases had complete data for the analyzed variables. Because several contingency tables contained sparse or zero cells, two-sided p values were obtained from exact conditional Monte Carlo tests based on Pearson's chi-square statistic with fixed margins and 1,000,000 sampled tables. Cramer's V summarized unadjusted association strength. Wilson 95% confidence intervals (CIs) were calculated for proportions; approximate 95% CIs for Cramer's V were obtained by inversion of the noncentral chi-square distribution and constrained to 0-1. Statistical significance was set at $p<0.05$. Analyses were performed with IBM SPSS Statistics version 20 and independently verified in SciPy version 1.17.0.

Ethical considerations

The study was approved by the institutional ethics committee; identifying committee details are supplied in the separate title-page file for double-blind review. Written informed consent for examination was obtained from the next of kin. Bodies were handled with dignity, personal identifiers were removed, and only aggregate results are reported.

Ethics approval: Approved by the Institutional Ethics Committee, Netaji Subhash Chandra Bose Medical College, Jabalpur, Madhya Pradesh (IEC/2024/4559; committee meeting 21 March 2024; approval letter dated 17 May 2024).

Results

The analysis included 100 cases. Road traffic accidents accounted for 63 cases, followed by poisoning (n=11), hanging (n=8) and falls from height (n=5); other causes together accounted for 13 cases. Rigor mortis was fully developed in 60%, lividity was fully developed in 54%, lividity was fixed in 69%, and 52% of bodies were classified as well nourished.

Rigor-mortis stage differed across temperature, humidity and nutritional categories (all p<0.001; Table 1). Fully developed rigor was observed in 41/51 (80.4%) cases at 30-35°C and 19/19 (100%) at 35-40°C, whereas none of the 30 cases examined below 30°C had fully developed rigor. Full rigor was present in 40/52 (76.9%) well-nourished but only 1/14 (7.1%) poorly nourished bodies.

Table 1. Rigor-mortis stage across environmental and nutritional categories (N=100)

Exposure	Category	Fully developed n (%)	Appearing n (%)	Disappearing n (%)	p value
Temperature (°C)	20-25	0 (0.0)	0 (0.0)	4 (100.0)	<0.001
	25-30	0 (0.0)	15 (57.7)	11 (42.3)	
	30-35	41 (80.4)	10 (19.6)	0 (0.0)	
	35-40	19 (100.0)	0 (0.0)	0 (0.0)	
Relative humidity (%)	<60	0 (0.0)	0 (0.0)	9 (100.0)	<0.001
	60-69	0 (0.0)	0 (0.0)	6 (100.0)	
	70-79	0 (0.0)	23 (100.0)	0 (0.0)	
	80-89	40 (95.2)	2 (4.8)	0 (0.0)	
	>90	20 (100.0)	0 (0.0)	0 (0.0)	
Gross nutritional status	Poor	1 (7.1)	7 (50.0)	6 (42.9)	<0.001
	Moderate	19 (55.9)	10 (29.4)	5 (14.7)	
	Well	40 (76.9)	8 (15.4)	4 (7.7)	

Lividity pattern also differed by temperature, humidity and nutrition (all p<0.001; Table 2). Fully developed lividity was present in 37/51 (72.5%) cases at 30-35°C and 17/19 (89.5%) at 35-40°C, compared with none below 30°C. It was recorded in 37/52 (71.2%) well-nourished and 1/14 (7.1%) poorly nourished bodies.

Table 2. Postmortem lividity pattern across environmental and nutritional categories (N=100)

Exposure	Category	Fully developed n (%)	Mottled n (%)	Coalesced n (%)	p value
Temperature (°C)	20-25	0 (0.0)	4 (100.0)	0 (0.0)	<0.001
	25-30	0 (0.0)	15 (57.7)	11 (42.3)	
	30-35	37 (72.5)	8 (15.7)	6 (11.8)	
	35-40	17 (89.5)	0 (0.0)	2 (10.5)	
Relative humidity (%)	<60	0 (0.0)	9 (100.0)	0 (0.0)	<0.001
	60-69	0 (0.0)	6 (100.0)	0 (0.0)	
	70-79	0 (0.0)	10 (43.5)	13 (56.5)	
	80-89	36 (85.7)	2 (4.8)	4 (9.5)	
	>90	18 (90.0)	0 (0.0)	2 (10.0)	
Gross nutritional status	Poor	1 (7.1)	9 (64.3)	4 (28.6)	<0.001
	Moderate	16 (47.1)	11 (32.4)	7 (20.6)	
	Well	37 (71.2)	7 (13.5)	8 (15.4)	

Fixation varied across temperature and humidity categories (both p<0.001; Table 3). Fixed lividity was observed in 43/51 (84.3%) cases at 30-35°C and 19/19 (100%) at 35-40°C, compared with 7/26 (26.9%) at 25-30°C and none at 20-25°C. Fixation increased from 6/14 (42.9%) in poorly nourished to 40/52 (76.9%) in well-nourished bodies, but the exact association with nutrition did not meet the prespecified threshold (p=0.052).

Table 3. Fixation of postmortem lividity across environmental and nutritional categories (N=100)

Exposure	Category	Fixed n (%)	Not fixed n (%)	p value
Temperature (°C)	20-25	0 (0.0)	4 (100.0)	<0.001
	25-30	7 (26.9)	19 (73.1)	
	30-35	43 (84.3)	8 (15.7)	
	35-40	19 (100.0)	0 (0.0)	
Relative humidity (%)	<60	0 (0.0)	9 (100.0)	<0.001
	60-69	0 (0.0)	6 (100.0)	
	70-79	8 (34.8)	15 (65.2)	
	80-89	41 (97.6)	1 (2.4)	
	>90	20 (100.0)	0 (0.0)	
Gross nutritional status	Poor	6 (42.9)	8 (57.1)	0.052
	Moderate	23 (67.6)	11 (32.4)	
	Well	40 (76.9)	12 (23.1)	

Relative humidity had the largest unadjusted association with rigor-mortis stage (V=0.973; 95% CI, 0.814-1.000), lividity pattern (V=0.710; 0.544-0.826) and fixation (V=0.843; 0.626-1.000). Corresponding V estimates (95% CIs) for temperature were 0.659 (0.498-0.780), 0.542 (0.376-0.659) and 0.667 (0.453-0.850); for nutritional status they were 0.346 (0.177-0.465), 0.324 (0.152-0.443) and 0.246 (0.000-0.426), respectively (Figure 1).

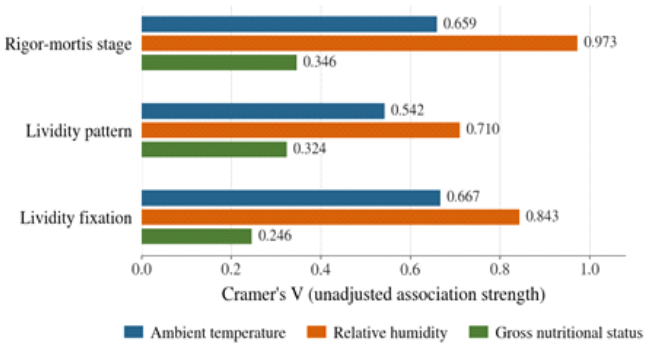


Figure 1. Unadjusted association strength of environmental and nutritional factors with postmortem outcomes. Cramer's V ranges from 0 to 1; larger values indicate stronger association and do not imply causation.

Discussion

Principal findings and research question

The stage of rigor mortis, morphology of postmortem lividity and fixation status were distributed very differently across mortuary temperature and humidity strata. Gross nutritional status showed smaller associations with rigor and lividity morphology, while its association with fixation was borderline and was not significant by the exact analysis. The study question was therefore answered at the level of unadjusted association: environmental and intrinsic factors were related to observed postmortem signs, but the data cannot establish that they accelerated or delayed those signs.

Existing work and interpretation

The findings reinforce the established principle that rigor should be interpreted within its environmental and physiological context rather than against a universal timetable.[1,4,14] Recent autopsy studies from Tripura and Varanasi described variation in rigor and lividity across known intervals and seasons, with winter conditions producing greater temporal variability.[5,6] Indian studies of rigor have similarly identified temperature, humidity,

body build, cause of death and premortem activity as relevant modifiers.[9-12] The present high Cramer's V values for mortuary conditions are consistent with climate-sensitive interpretation, although the absence of interval-stratified or multivariable analysis prevents direct comparison of timing.

Prior studies describe lividity as a progressive transition from patchy to coalescent dependent discoloration and then fixation, with substantial variation by circulation, blood loss and environment.[7,8] Objective spectrophotometric work has also shown that lividity contains time-dependent information but requires standardized measurement.[13] The present gross morphological observations extend this literature in a warm regional setting, but should not be treated as quantitative temporal calibration.

Novel contribution and forensic relevance

The novel contribution is the simultaneous comparison of three readily documented modifiers with three routine early postmortem outcomes in one Mahakaushal autopsy series, together with effect-size reporting. Humidity showed near-complete separation of some stages, a striking result that is useful for hypothesis generation but also warrants source-data and coding validation. In practice, mortuary temperature, humidity and body habitus should be recorded alongside rigor and lividity. These observations should complement, not replace, case history, body temperature, ocular findings, decomposition and other validated postmortem-interval methods.[2,3] Standardized regional documentation may improve consistency for forensic physicians, investigators and courts without implying a stand-alone clock.

Limitations and future research

This single-center series comprised 100 known-time hospital deaths, and each body was assessed once. Because postmortem interval was not included in an adjusted analysis, the study could not determine whether ambient temperature or relative humidity was independently associated with rigor-mortis stage, lividity pattern or lividity fixation after accounting for elapsed time since death. Temperature and humidity were measured once in the mortuary rather than continuously from the death scene. Rigor, lividity and nutrition were visually or manually graded by one observer without blinding or reliability testing. Age- and sex-stratified data were unavailable, and the analyses did not adjust for cause of death, premortem activity, body position, clothing, season or transport. Sparse, highly separated tables limited precision despite exact testing. Future multicenter studies should use prespecified postmortem intervals, continuous environmental monitoring, objective anthropometry, blinded duplicate assessment and multivariable prediction with internal and external validation.

Conclusion

Mortuary ambient temperature, relative humidity and gross nutritional status were associated with observed rigor-mortis stage and lividity morphology; temperature and humidity were also strongly associated with fixation. These factors should be documented when interpreting early postmortem signs in Central India. The results do not generate a correction factor or validated time-since-death equation, and prospective interval-stratified validation is required before quantitative use.

Declarations

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References

1. Henssge C, Madea B. Estimation of the time since death in the early post-mortem period. Forensic Sci Int. 2004;144(2-3):167-175. doi:10.1016/j.forsciint.2004.04.051.
2. Shedge R, Zubair M. Postmortem changes [Updated 2026 Jun 19]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2026 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK539741/.
3. Stigter H, Krap T, Duijst WLJM. Estimation of the post-mortem interval: added value of mechanical excitability of human skeletal muscle. J Forensic Leg Med. 2024;103:102664. doi:10.1016/j.jflm.2024.102664.

4. Emam A, Mujalid H, Altamimi N, Faraj W, Almutairi M, Alresheedi Z, et al. Classification of post-mortem changes and factors affecting it. J Healthc Sci. 2022;2(9):213-218. doi:10.52533/JOHS.2022.2901.

5. Das N, Chakraborty JS, Chakraborty PN. Estimation of time since death from rigor mortis, postmortem staining, and decomposition: an autopsy-based study in a tertiary care hospital, Tripura. SSR Inst Int J Life Sci. 2024;10(6):6454-6459. doi:10.21276/SSR-IIJLS.2024.10.6.14.

6. Singh J, Kumar A, Mishra R, Pandey SK. Impact of winter season and other contributory variables on estimation of time since death using livor and rigor mortis in Varanasi region. Indian J Forensic Med Pathol. 2023;16(2):123-131. doi:10.21088/ijfmp.0974.3383.16223.5.

7. Arikeri SM, Abhinandana R, Patil BS. Post-mortem lividity and its determinants: a cross-sectional study. J Indian Acad Forensic Med. 2019;41(2):86-88. doi:10.5958/0974-0848.2019.00024.1.

8. Chintalwar RS, Patond S, Ninave S. Estimation of time since death from postmortem lividity. Indian J Forensic Med Toxicol. 2019;13(4):164-166. doi:10.5958/0973-9130.2019.00281.0.

9. Kaur N, Chaudhary RK, Dalal JS, Rai H. Effect of factors influencing rigor mortis: a medico-legal study to estimate postmortem interval. Med Leg Update. 2015;15(2):25-28. doi:10.5958/0974-1283.2015.00041.9.

10. Oberoi SS, Singh P, Aggarwal AD, Walia DS, Bhullar DS, Aggarwal KK. Factors affecting estimation of time since death by rigor mortis. J Punjab Acad Forensic Med Toxicol. 2015;15(2):81-85.

11. Khartade HK, Tasgaonkar G, Sukhadeve RB, Parchake MB, Meshram VP, Hosmani AH. Study of rigor mortis and factors affecting its development for determination of postmortem interval. Indian J Forensic Med Toxicol. 2017;11(2):70-74. doi:10.5958/0973-9130.2017.00068.8.

12. Dalal JS, Tejpal HR, Chanana A, Kaur N. Medicolegal study of rigor mortis to estimate postmortem interval. J Indian Acad Forensic Med. 2006;28(2):49-51. doi:10.1177/0971097320060203.

13. Usumoto Y, Hikiji W, Sameshima N, Kudo K, Tsuji A, Ikeda N. Estimation of postmortem interval based on the spectrophotometric analysis of postmortem lividity. Leg Med (Tokyo). 2010;12(1):19-22. doi:10.1016/j.legalmed.2009.09.008.

14. Bate-Smith EC, Bendall JR. Factors determining the time course of rigor mortis. J Physiol. 1949;110(1-2):47-65. doi:10.1113/jphysiol.1949.sp004420.

Original Article

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Observational Analysis of Alcohol in Postmortem Vitreous Humor: A Forensic Perspective

Niranjan Sahoo[1], Yukti Awasarkar[2], Atul S. Keche[3], Deeksha Thakur[4]

[1]Associate professor, Department of Forensic Medicine and Toxicology, AIIMS, Bhopal, [2]Student, 3rd year BSc. Forensic Science, IEHE, Bhopal, [3]Additional professor, Department of Forensic Medicine and Toxicology, AIIMS, Bhopal, [4]Assistant professor, Department of Forensic Science, IEHE, Bhopal

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Abstract

Post-mortem toxicological analysis of alcohol is a critical aspect of forensic investigations, particularly in cases of suspected poisoning or unexplained deaths. Among biological specimens, the vitreous humor offers distinct advantages owing to its anatomical isolation, chemical stability, and resistance to putrefaction. This study aimed to detect the presence of alcohol in vitreous samples using classical chemical screening methods to evaluate their sensitivity and effectiveness. Our findings indicate that the dichromate and iodoform tests are effective in detecting ethanol but yield positive results only up to a certain concentration range. Similarly, the Schiff reagent test demonstrates specificity for methanol but is also limited by concentration sensitivity. The study highlights that both the time elapsed since alcohol consumption and the concentration of alcohol in the vitreous humor significantly influenced the outcome. This variation is especially crucial for interpreting results during forensic autopsies, where decomposition and post-mortem redistribution can alter chemical profiles.

Background: This study aimed to establish an alternative method for preliminary alcohol detection in resource-limited or time-sensitive settings.

Objective: This study aimed to detect the presence of alcohol in spiked post-mortem vitreous samples and compare the diagnostic performance of dichromate and iodoform tests for ethanol detection. It further seeks to evaluate the Schiff's reagent test for identifying methanol, while also assessing the role of concentration thresholds and the time elapsed since alcohol consumption in the outcomes of these tests. Ultimately, this study endeavored to validate these analytical chemical methods as reliable spot tests for application in forensic toxicological investigations.

Methods: This observational study was conducted over 45 days. The tests were initially applied to pure ethanol and methanol samples, and the effect of dilution on the results was evaluated. Later, post-mortem vitreous samples from accident cases were collected at the mortuary, AIIMS, Bhopal, during autopsies.

Results: The findings of this study provide insightful observations regarding the effectiveness and limitations of classical chemical tests (dichromate, iodoform, and Schiff's reagent) for detecting alcohol in postmortem vitreous humor.

Corresponding Author: Yukti Awasarkar, Student, 3rd year BSc. Forensic Science, IEHE, Bhopal.

E-mail: yukti2845a@gmail.com

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Conclusions: These classical screening tests provide cost-effective and field-applicable methods for detecting alcohol in vitreous samples. This comparative study contributes to the understanding of the practical limitations and utility of traditional qualitative tests in modern forensic toxicology.

Keywords: Alcohol, Dichromate test, Iodoform test, Schiff'stest, Vitreous humor

Introduction

Alcohol is frequently associated with sudden or unexplained deaths, particularly in cases involving accidents, poisoning, or suicide. In forensic practice, determining whether alcohol was consumed and in what quantity is crucial for understanding its possible role in the cause of death. Among the various biological samples available for analysis, vitreous humor, the transparent gel-like fluid inside the eye, has proven to be one of the most reliable [1] .

Vitreous humor is anatomically well-protected and chemically stable, making it less susceptible to postmortem changes such as contamination or microbial activity [2]. Its high-water content (approximately 98%) means that alcohol is often found in higher concentrations in vitreous humor compared to blood, which contains around 83% water [3]. Moreover, vitreous alcohol levels tend to reflect blood alcohol concentrations approximately one to two hours before death, offering forensic investigators a more stable and temporally informative marker [4][5].

While advanced techniques like gas chromatography (GC) and headspace GC remain the gold standard for alcohol detection due to their precision and sensitivity, they are costly. They may not be accessible in all forensic laboratories, especially those in rural or low-resource settings [6]. This creates a need to evaluate and validate simpler, affordable chemical methods that could serve as effective alternatives for alcohol screening.

The present study focuses on the application of three classical chemical tests to detect alcohol in postmortem vitreous samples:

- The dichromate test, which relies on the oxidation of ethanol and a visible color change.
- The iodoform test, which identifies methyl ketones and ethanol by producing a characteristic yellow precipitate.
- Schiff's test, used to detect methanol by identifying formaldehyde formed during its metabolism.

This study also investigates the effect of different alcohol concentrations and time intervals on test performance, aiming to validate these classical methods for use in modern forensic toxicology, particularly in settings where advanced instrumentation is not available.

Methods

Type of study:

A pilot study was conducted over 45 days.

Sample preparation:

The tests were initially applied to pure ethanol and methanol samples, and the effect of dilution on the results was observed. Later, post-mortem vitreous samples from 10 cases were collected at the mortuary, AIIMS, Bhopal, during autopsies after obtaining written informed consent from next of kin. These vitreous samples were impregnated with a mixture of ethanol and methanol, and then the tests were conducted on them. To prepare the spiked samples, we first made a combined stock solution by mixing equal parts (10 mL each) of the 50% dilute ethanol and 50% dilute methanol. This gave us a starting mixture where the ethanol concentration was **4.28 M,** and the methanol concentration was **6.18 M**. From there, we spiked the postmortem vitreous humor samples at two different levels. For the first sample, we added 1 mL of our alcohol mixture to 4 mL of the vitreous fluid, bringing the final concentration in the sample to **0.86 M ethanol** and **1.24 M methanol**. For the second sample, we mixed 1 mL of the alcohol solution into 3 mL of the vitreous fluid, which resulted in a higher final concentration of **1.07 M ethanol** and **1.55 M methanol**.

Inclusion criteria:

Vitreous samples were collected from cases with a history of road traffic accident, fall from a height, suspected alcohol poisoning, sudden death, drowning, suicide, homicide, or fire-related death for the study.

Exclusion criteria:

Cases in which the body of the deceased was decomposed, burned, or charred; had severe ocular trauma; or had any history of eye infection.

Principle of the tests:

Dichromate test -

The dichromate test is a qualitative chemical test used to detect ethanol (or other primary and secondary alcohols). It is based on the oxidation of alcohols by acidified potassium dichromate ($K_2Cr_2O_7$). During the reaction, the orange dichromate ions ($Cr_2O_7^{2-}$) are reduced to green chromium ions (Cr^{3+}), and the alcohol is oxidized to an aldehyde or carboxylic acid, depending on the conditions [7][8].

$$C_2H_5OH + 2K_2Cr_2O_7 + 8H_2SO_4 \rightarrow 3CH_3COOH + 2Cr_2(SO_4)_3 + 2K_2SO_4 + 11H_2O$$

Iodoform test -

The iodoform test is a qualitative reaction used to detect methyl ketones (R–CO–CH_3) and alcohols that can be oxidized to methyl ketones, most commonly ethanol (CH_3CH_2OH) and secondary alcohols of the form $CH_3CH(OH)R$. In alkaline iodine solution, the alcohol is first oxidized to the corresponding carbonyl compound, which then undergoes successive iodination at the methyl ketone position. When three iodine atoms have substituted the methyl group, the triiodomethyl ketone fragment cleaves to give iodoform (CHI_3), a pale yellow, crystalline precipitate with a characteristic antiseptic odor and hexagon-shaped crystals when observed under a microscope [8][9][10].

$$CH_3CH_2OH \rightarrow CH_3CHO \text{ [Oxidation]}$$

$$CH_3CHO + 3I_2 + 4NaOH \rightarrow CHI_3\text{(yellow precipitate)} + HCOONa + 3NaI + 2H_2O$$

Schiff's reagent test -

Schiff's reagent test mainly detects aldehydes, not alcohols directly. However, primary alcohols can produce a positive result if they are first oxidized to aldehydes. In alcohol analysis, especially with methanol, Schiff's test is used after chemical oxidation to identify formaldehyde (HCHO), an oxidation product of methanol. Schiff's reagent is a solution of fuchsin dye (rosaniline hydrochloride) that has been decolorized by treatment with sulfurous acid (H_2SO_3). When an aldehyde is present, it reacts with the decolorized dye and reestablishes the purple-pink color. This is a colorimetric test. Therefore, when methanol is oxidized to formaldehyde, the Schiff test can detect the aldehyde [8][10].

$$CH_3OH \rightarrow HCHO \text{ [Oxidation]}$$

HCHO (formaldehyde) + Schiff's reagent → Magenta complex

Reagent preparation for ethanol detection (Test 1):

An oxidizing reagent was prepared as a 0.49% (w/v) acidic potassium dichromate by dissolving 0.009 g of anhydrous $K_2Cr_2O_7$ in 2 mL of distilled water in a beaker with gentle stirring until fully dissolved. This reagent is prepared fresh to ensure accurate test results. Additionally, a 50 mL solution of 50% concentrated H_2SO_4 was made to provide an acidic medium for reducing dichromate ions ($Cr_2O_7^{2-}$) to Cr^{3+} ions [9].

Reagent preparation for ethanol rescreening (Test 2):

A 10% (w/v) solution of sodium hydroxide was prepared by dissolving 10.0 g of NaOH in 100.0 mL of distilled water in a 500-mL beaker. The beaker was placed in a bowl of water to absorb the heat generated by the dissolution process. A 6.54% (w/v) iodine solution was prepared by dissolving 1.27 g of anhydrous iodine (I_2) and 2 g of potassium iodide (KI) into 50.0 mL of distilled water. This iodine solution was kept in a brown container to avoid light and stored in the refrigerator (4 °C) [9][11].

Reagent preparation for detecting methanol (Test 3):

Schiff's reagent, with a concentration likely between 1 to 6% (w/v), is used in addition to potassium dichromate and sulfuric acid [9].

Classical analytical procedure:

For Test 1 (dichromate test), a glass test tube was used as a sample container to facilitate the chemical reaction. The analytical process involves two steps. In step 1, 1.00 mL of the sample was added to the glass test tube containing 1.00 mL of potassium dichromate solution. In step 2, the H_2SO_4 solution was finally added dropwise, causing the product to develop a bluish-green color. Detection is performed visually for qualitative analysis [9].

Test 2 (iodoform test) would then be performed if ethanol was found positive but still doubtful. A glass test tube was filled with 1.00 mL of 10 % (w/v) sodium hydroxide solution. A 1.0 mL sample was then added to the same vial. The mixture in the glass test tube was gently swirled for a minute. To achieve the iodoform reaction, 2.00 mL of 6.54 % (w/v) iodine solution was finally added to the test tube and subsequently gently swirled for 1 minute. The yellow precipitates are then formed and observed as turbidity [9][11].

For Test 3 (Schiff's reagent test), a glass test tube was used as a sample container to facilitate the chemical reaction. The analytical procedure involves three steps. In step 1, 1.00 mL of the sample was added to the glass test tube containing 1.00 mL of potassium dichromate solution. In step 2, H_2SO_4 solution was added dropwise. The oxidation reaction was allowed to proceed for 5 minutes with gentle swirling. In step 3, 2.00 mL of Schiff's reagent was added, resulting in a product with a pinkish-purple color. Detection is performed visually for qualitative analysis [9][11].

Results

The analytical chemical tests conducted on postmortem vitreous samples revealed varying sensitivities for detecting different types of alcohol.

The dichromate test, a common method for alcohol detection, showed positive results for ethanol up to a 1% dilution. This test relies on the oxidation of ethanol by dichromate ions, resulting in a color change from orange to green. The higher sensitivity of this test indicates its potential usefulness in detecting lower concentrations of ethanol in postmortem vitreous samples. (Table 1)

Table 1:

Concentration of ethanol	Colour of the solution obtained	Result
100%	Dark green	Positive
80%	Dark green	Positive
60%	Dark green	Positive
40%	Dark green	Positive
20%	Blue-green	Positive
10%	Blue-green	Positive
5%	Light blue-green	Positive
1%	Light blue-green	Positive

We have observed that the green color of the solution gradually fades as ethanol is diluted. At a 100% concentration, it appears dark green, whereas at a 1% concentration, it appears light blue-green.



Figure 1: Results of Dichromate tests performed on different concentrations of alcohol (From higher to lower concentration)

In contrast, the iodoform test showed lesser sensitivity, yielding positive results for ethanol only up to a 10% dilution. This test, also known as the haloform reaction, involves the formation of iodoform (a yellow precipitate) when ethanol reacts with iodine in the presence of a base. Although effective, its limited sensitivity indicates it may not be suitable for detecting very low ethanol levels in vitreous samples. (Table 2)

Table 2:

Concentration of ethanol	**Colour of the solution obtained**	**Result**
100%	Yellow-colored solution with turbidity	Positive
80%	Yellow-colored solution with turbidity	Positive
60%	Yellow-colored solution with turbidity	Positive
40%	Yellow-colored solution with turbidity	Positive
20%	Yellow-colored solution with turbidity	Positive
10%	Yellow-colored solution with turbidity	Positive
5%	Yellow-colored solution without any turbidity	Negative
1%	Yellow-colored solution without any turbidity	Negative

We have observed that the yellow color of the solution remains unchanged with ethanol dilution, but the amount of precipitate formed decreases gradually as dilution increases. At 100% concentration, the result shows a yellow color with turbidity, and if the solution is set aside for some time, a precipitate appears at the bottom of the test tube. This occurs down to 10% ethanol concentration. However, at 5% and 1% ethanol concentrations, the solution displays only a yellow color without any turbidity.

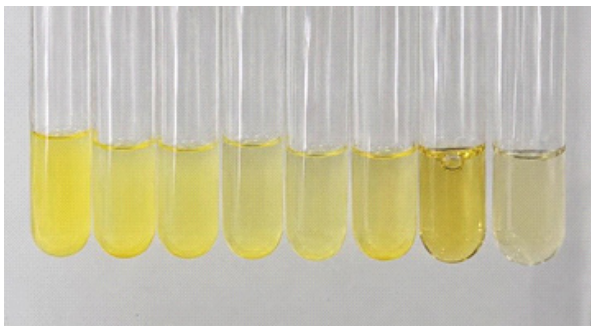


Figure 2: Results of Iodoform tests performed on different concentrations of alcohol(From higher to lower concentration)

When the result of 100% concentration was left undisturbed, a yellow precipitate (iodoform) settled at the bottom of the test tube. *He*xagon-shaped crystals of iodoform were observed under a light microscope. These crystals can be observed in the precipitate of any concentration, which gives a positive result.



Figure 3: Crystals of Iodoform viewed under a compound microscope

For methanol detection, the Schiff's reagent test produced positive results up to a 1% dilution. This test is based on the reaction of methanol with Schiff's reagent, resulting in a characteristic color change. The sensitivity of this test suggests its potential application in identifying methanol intoxication cases, which can be crucial in differentiating between ethanol and methanol poisoning. (Table 3)

Table 3:

Concentration of ethanol	Colour of the solution obtained	Result
100%	Dark magenta	Positive
80%	Magenta	Positive
60%	Magenta	Positive
40%	Magenta	Positive
20%	Magenta	Positive
10%	Magenta	Positive
5%	Purple	Positive
1%	Light purple	Positive

We have observed that the magenta of the solution gradually fades with the dilution of ethanol. At 100% concentration, the result shows a dark magenta color. Whereas, at 1% concentration of ethanol, the result shows a light purplish color.

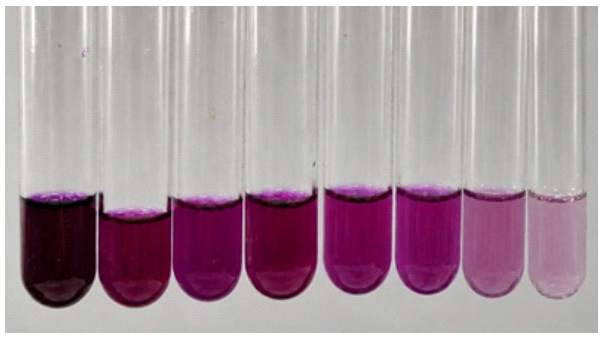


Figure 4: Results of Schiff's tests performed on different concentrations of alcohol(From higher to lower concentration)

Initial attempts with a low-concentration spiked vitreous sample (4 mL of the vitreous sample was spiked with 1 mL of alcohol) did not yield positive results, even after 24 hours of observation. This finding highlights the importance of considering detection limits in postmortem alcohol analysis. To address this issue, a second spiked vitreous sample with a higher alcohol concentration (3 mL of the vitreous sample was spiked with 1 mL of alcohol) was utilized to assess the temporal progression of results.

This higher-concentration sample showed positive results for the dichromate and iodoform tests from 6 to 72 hours after impregnation. The longer detection window indicates that these tests might be reliable for identifying alcohol in postmortem samples over an extended period. This information could be useful in forensic investigations where the time of death is unknown or when there is a delay in sample collection and analysis. (Table 4)

Table 4:

Time elapsed	Dichromate test results	Iodoform test results	Schiff's reagent test results
Immediate	Negative	Negative	Negative
20 mins	Negative	Negative	Positive
1 hr	Negative	Negative	Positive
3 hrs	Negative	Negative	Positive
6 hrs	Positive	Positive	Positive
12 hrs	Positive	Positive	Positive
18 hrs	Positive	Positive	Positive
24 hrs	Positive	Positive	Positive
48 hrs	Positive	Positive	Positive
72 hrs	Positive	Negative	Positive
80 hrs	Negative	Negative	Negative

The Schiff's reagent test demonstrated the earliest detection, showing positive results from 20 minutes to 72 hours after impregnation. This rapid detection capability, combined with its extended window of positivity, indicates that Schiff's reagent test may be particularly useful for quick screening of methanol presence in postmortem vitreous samples.



Figure 5: Result of all three tests on vitreous after 20 minutes

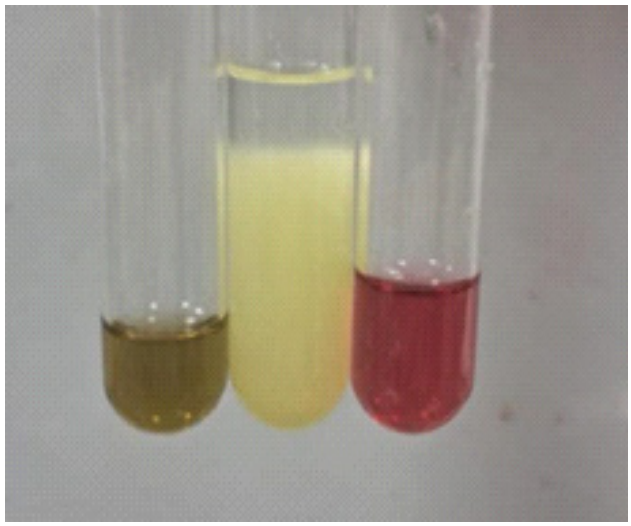


Figure 6: Results of all three tests on vitreous at 6 hours

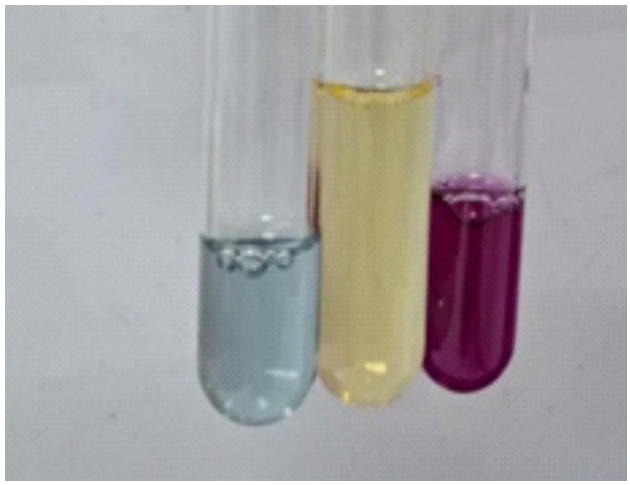


Figure 7: Results of all three tests on vitreous at 72 hours

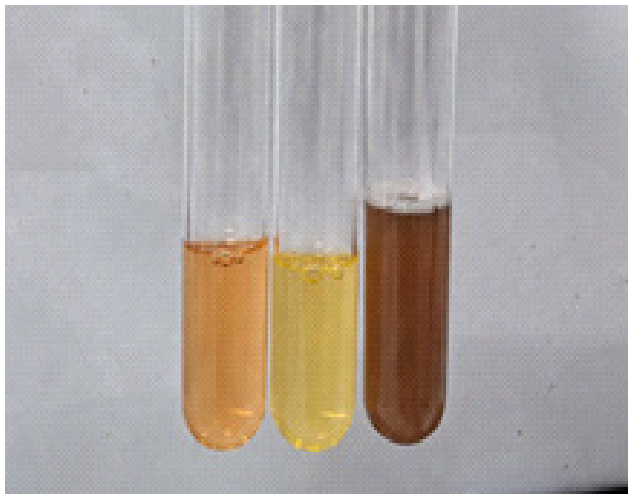


Figure 8: Results of all three tests on vitreous at 80 hours

Discussion

The findings from this study provide insightful observations regarding the effectiveness and limitations of classical chemical tests like dichromate, iodoform, and Schiff's reagent for detecting ethanol and methanol in postmortem vitreous humor. While each test demonstrated certain strengths in terms of sensitivity and detection window, several scientific and methodological limitations must be considered when interpreting the results.

The dichromate test exhibited a relatively higher sensitivity for ethanol, giving positive results only up to a 1% dilution. The reaction is stoichiometric and dependent on the redox potential of the sample environment [12]. On the other hand, the iodoform test showed lower sensitivity, detecting ethanol up to a 10% dilution. This can be attributed to the test's reliance on the haloform reaction, which is highly specific for methyl ketones and primary alcohols, such as ethanol. The yellow precipitate of iodoform (CHI_3) formed during the reaction is visually distinctive even at relatively low concentrations, enhancing the test's detection limit [13].

Schiff's reagent test proved effective for methanol detection, producing positive results at dilutions up to 1%. The early color change suggests rapid interaction between methanol's aldehyde metabolite (formaldehyde) and the Schiff reagent. This is chemically plausible since methanol is metabolized by alcohol dehydrogenase into formaldehyde, which readily forms a magenta-colored complex with Schiff's reagent [14].

The temporal aspect of detection also revealed critical insights. The failure to detect low-concentration spiked samples initially may reflect the minimum threshold requirement of these colorimetric reactions. Additionally, postmortem diffusion dynamics, enzymatic degradation, and sample matrix complexity (proteins, electrolytes) may affect the availability of alcohols for response. Only at higher concentrations were the tests able to consistently detect ethanol and methanol over time. The extended window (6 to 72 hours) of positive results in the second spiked sample supports the relative stability of vitreous humor, which resists putrefaction longer than blood or other tissues [2].

Schiff's test, which showed a positive result within 20 minutes and remained stable up to 72 hours, emphasizes its utility as a rapid screening tool for methanol poisoning. Given methanol's severe toxicity and the need for timely differentiation from ethanol, this test could be particularly useful in forensic screening scenarios [11].

Limitations of the Study and Tests:

Despite their simplicity and cost-effectiveness, these classical tests possess several limitations:

1. Lack of Quantitative Precision: All tests used are qualitative at best. They do not provide actual concentrations, which are often essential in determining fatal or toxic levels.
2. Interference and Specificity: The iodoform test, although sensitive, is not specific to ethanol and can give positive results with other alcohols like isopropanol or acetone. Similarly, Schiff's reagent reacts with any aldehyde, not just formaldehyde from methanol metabolism.
3. Postmortem Variables: Decomposition, enzymatic activity, and bacterial action postmortem can alter ethanol or methanol levels, either by producing or degrading alcohols, leading to false positives or negatives [15].
4. Sample Size and Generalizability: This study involved limited experimental setups using spiked samples under controlled conditions. Real-world forensic cases involve complex biological variation, including interactions with other drugs, diseases, and varying time since death.

These chemical screening tests are relatively simple, cost-effective, and time-efficient, making them well-suited for preliminary alcohol detection in resource-limited or time-sensitive settings. They can be effectively applied to postmortem vitreous humor, blood, or urine samples collected from deceased individuals in cases involving alcohol consumption or spurious liquor ingestion, such as road traffic accidents (RTAs), alcohol-induced suicides, or homicides. These tests are particularly beneficial in forensic toxicology side laboratories within autopsy-performing institutions, especially in situations where delays in sample transportation to

forensic science laboratories (FSLs) may result in the loss of detectable alcohol levels due to postmortem degradation.

Conclusion

These tests provide valuable preliminary screening tools, especially in low-resource settings; they should be supplemented with instrumental methods such as UV-Visible Spectrophotometry, Gas Chromatography (GC) or Headspace GC-MS for accurate identification and quantification of alcohol. Furthermore, integrating these chemical tests into a multi-step diagnostic workflow may enhance the reliability of toxicological investigations. Future studies involving a larger number of real postmortem samples, alongside comparisons with instrumental gold standards, are essential for the validation and wider adoption of these methods in forensic practice.

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Source of Funding: Nil

References

1. Gilliland, M. G. F., & Bost, R. O. (1993). Alcohol in vitreous humor and blood: A comparative study. Journal of Forensic Sciences, 38(2), 442-447.
2. Coe, J. I. (1989). Vitreous potassium as a measure of the postmortem interval: A historical review and critical evaluation. Forensic Science International, 42(3), 201-213.
3. Pelissier-Alicot, A. L., Gaillard, Y., & Pépin, G. (2003). Comparison of blood and vitreous humor ethanol concentrations in 341 forensic cases. Journal of Forensic Sciences, 48(3), 1–4.
4. Sturmer, W. Q. (1963). The vitreous humor: Postmortem potassium changes and forensic medicine. Journal of the American Medical Association, 186(6), 544–547.
5. Issa, S., & Khattab, F. (2024). Ethanol concentrations in various biological specimens: Living and postmortem forensic toxicology analysis and comprehensive literature review. Journal of Forensic and Legal Medicine. Advance online publication.
6. Winek, C. L., Wahba, W. W., & Winek, C. L., Jr. (1996). Blood, breath, and urine alcohol concentrations in a live subject: A comparative study. Forensic Science International, 77(2), 115-123.
7. Zehner, R. (2005). Chromate-dichromate tests. In J. A. Siegel, P. J. Saukko, & G. C. Knupfer (Eds.), Encyclopedia of forensic sciences (Vol. 1, pp. 387–390). Academic Press.
8. Clark, E. G. C. (1986). Isolation and identification of drugs (2nd ed.). The Pharmaceutical Press.
9. Jaiswal, AK, Milo, T, Handbook of Forensic Analytical Toxicology, Page no. 88-89, 978-935152-224-9.
10. Furniss, B. S., Hannaford, A. J., Smith, P. W. G., & Tatchell, A. R. (1989). Vogel's textbook of practical organic chemistry (5th ed.). Longman.
11. Patel, P., Joshi, N., & Ghosh, S. (2023). Exploitation of Schiff's test and iodoform test for an effective quality assessment of alcohol-based hand sanitizers. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 312, 123076.
12. Spillane, L. (2023). Alcohol toxicity. Medscape.
13. discrepancy between blood and vitreous humor concentrations. Forensic Science International, 74(2-3), 135-148.
14. Kugelberg, F. C., & Jones, A. W. (2010). Interpreting results of ethanol analysis in postmortem specimens: A review of the literature. Forensic Science International, 207(1-3),
15. Watson, J. T., Sparkman, O. D., & Glish, G. L. (2005). Introduction to mass spectrometry: Instrumentation, applications, and strategies for data interpretation (4th ed.). Wiley.

Original Article

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Advanced Techniques in Mosquito Blood Meal DNA Analysis: Technological Innovations in Forensic Methodologies

Eni-Yimini Solomon Agoro[1], Teddy Charles Adias[2], Charles German Ikimi[1]

[1]Department of Biochemistry, Facultyof Science, Federal University Otuoke, Bayelsa State, Nigeria,
[2]Department of Microbiology, Facultyof Science, Federal University Otuoke, Bayelsa State, Nigeria

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Abstract

Objectives: Detecting human DNA in a mosquito's bloodmeal is a crucial aspect of forensic entomology that canaidin identifying crime scenes and serve as evidence in court. Mosquitoes serve as a potential source of human DNA due to their feeding habits.

Methods: This study utilized electrophoretic and molecular techniques to validate the effectiveness of mosquito blood meal in crime investigation and jurisprudence. The sample size was calculated using the G*Power formula at a 0.05 confidence level, yielding samples offifty (50) human subjects and fifty (50) mosquitoes. Similarly, the methodologies approved by the World Health Organization (WHO) were used for the laboratory analysis. Data analysis was performed on SPSS(version18-20).Gel electrophoresis confirmed the presence of DNA in a mosquito blood meal. Furthermore, the Sanger Nucleotide Sequencing alignment was performed on the NCBI platform, upon which the phylogenetic tree was built.

Results: The result affirmed the presence of *homo sapiens* DNA in the BLAST search conducted. Therefore, blood-fed mosquitoes found at a crime scene are forensically valuable, as they can indicate the presence of human blood, but are hampered by the lack of human identity indication due to method limitations.

Novelty: This study identified human detection in Anopheles mosquito blood meals using natural, real-time scenarios.

Keywords: Forensic science, mosquito blood meal, *Anopheles* mosquito, Sanger nucleotide sequencing, PCR.

Introduction

Mosquitoes are delicate, long-legged, two-winged flies (Order: Diptera, Family: Culicidae) easily recognized by their long proboscis and scaly wings and legs [1,2]. They are common flying insects that transmit many medically important pathogens and parasites [3,4]. Mosquitoes obtain a blood meal from invertebrates and/or vertebrates, an essential nutrient for vitellogenesis (egg production) and other metabolic activities [1,2]. The medical importance of mosquitoes has extended beyond their usual role

Corresponding Author: Eni-Yimini Solomon Agoro, Department of Biochemistry, Facultyof Science, Federal University Otuoke, Bayelsa State, Nigeria.

E-mail: agoroes@fuotuoke.edu.ng

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in causing disease to include applications in crime investigations and jurisprudence [5-9].

Violent crimes are universally committed intentionally and/or unintentionally, with evidence of blood stains in many instances. Nigeria, with a crime index of 66.2, faces a complex set of challenges, including insurgency, cybercrime, kidnappings, and communal clashes, without prosecutable and scientifically admissible evidence [10-11]. Most of these crimes involve blood and could be of jurisprudential use when applying techniques such as DNA profiling and other blood identification methods[12-13]. However, scenes involving blood spills could be tampered with, or samples could be contaminated or destroyed to evade precise investigation. This could be resolved by applying entomological techniques.

Entomology, as a crime-investigation tool, has long been used, dating back to the Chinese Revolution [14-15]. Traditional approaches, such as serological and molecular methods, have been used to identify mosquitoes' blood meals. Assays such as precipitin tests and Enzyme-Linked Immunosorbent Assay (ELISA) were common before polymerase chain reaction (PCR)-based blood meal analysis [16-17]. Forensic entomology has helped resolve a handful of criminal cases. A classic example is the use of the blood of a single dead mosquito found at the home of a businessman; the forensic police were able to identify the victim's DNA [18].

Mosquito blood meal is increasingly used in crime investigations. The blood a mosquito has fed on can be sequenced for DNA and used for forensic and disease surveillance purposes [19-21]. In modern blood meal analysis, host DNA is extracted from a mosquito, amplified by polymerase chain reaction (PCR), and sequenced. This is among the most direct and specific approaches for host identification [8-19]. This study used Sanger nucleotide sequencing to target cytochrome b [23-24], a well-characterized protein of complex III in the mitochondrial oxidative phosphorylation system. Cytochrome b has proven useful for identifying host blood meals because of its high copy number in mitochondrial genomes. Cyt-b found in mosquito blood meals after degradation can be used to match human DNA [24-25].

The use of mosquito bloodmeal in crime investigation is a well-established scientific approach spanning decades [8-9]. However, the mosquito species used and the diversity of environmental conditions pose challenges to reproducibility in results and applications [19-21]. Furthermore, a handful of studies rely on laboratory-bred mosquito species and restricted laboratory conditions [9,16]. This study addresses these gaps by using female Anopheles mosquitoes and, in a real-life scenario, mosquito bloodmeals to include or exclude crime suspects.

Materials and Methods

Study Location

This study was conducted in Otuoke, Ogbia Local Government Area, Bayelsa State [26-27]. The laboratory component of the study took place at Eni-Yimini Laboratories LTD in Yenagoa, Yenezue-Gene Epie, and at the famous Inqaba Biotech Laboratory in Ibadan, Oyo State. The research duration spanned from November 2022 to November 2023.

Population/Sample Size

The sample size was calculated using the G-power formula at a 0.05 significance level and 80% power, yielding a result of fifty (50) subjects [28]. Of the humans and mosquitoes used, only 30 engorged mosquitoes were selected for the study due to their level of engorgement and viability. The digestion time points tested were restricted to a 12-hour nocturnal period (7 am-7 pm).

The research was classified into two groups: A - those exposed to mosquito bites and engorged; and B - those protected from mosquitoes and unengorged.

Ethical Approval

The Department of Biochemistry and the Directorate of Research and Quality Assurance (DR&QA) in Bayelsa State approved the ethical clearance and experimental protocol. The study subjects provided individual consent without coercion or inducement. Ethical approval was obtained on October 1, 2022.

Selection Criteria

The medical team at the university medical center confirmed that all subjects were healthy. This study included both fully engorged and unengorged mosquitoes.

Collection of Sample

Collection of a humanblood sample

Blood samples were collected via venipuncture from subjects who were bitten by mosquitoes and from those who were not, and were placed in EDTA tubes for further laboratory analysis and DNA studies. Subjects recruited for the study received prophylaxis and were monitored for insect-borne diseases for 6 months.

Collection of mosquitoes

Subjects willingly offered their arms for mosquitoes to land on and remained still for 5 to 10 minutes. Once the mosquitoes had fully engorged themselves with blood, each was captured in a microtube. This process occurred both in the late evening (between 10 pm and 11 pm) and early the following morning (between 5 am and 6 am). Similarly, resting mosquitoes without a blood meal were collected in the field during these periods. Collections were carried out at Otuoke, Ogbia Local Government Area,

Bayelsa State. The study was conducted at 25 °C, and samples were transported in a cool chain to the laboratory for analysis.

However, the collected samples were sealed in well-sealed, bar-coded materials and transmitted to the laboratory using an internationally accepted chain-of-custody procedure, as documented by Kumar and Kashyap [29]. The post-feeding maximum interval used in the study was set at 24 hrs, as established by Ahmed et al. [9] in an STR study using a mosquito blood meal.

Sample Preparation

We used the method of Kent and Norris [30] for sample preparation. Mosquito heads/thoraces were separated from abdomens, and only the DNA isolated from engorged abdomens was used in the study. Unengorged mosquito samples were prepared as described above and tested simultaneously for nonspecific amplification using the mammalian primer set.

Laboratory Procedure

Figure 1 presents the flowchart of the experimental protocol. The figure enumerates the experimental protocol followed sequentially during the bench work.

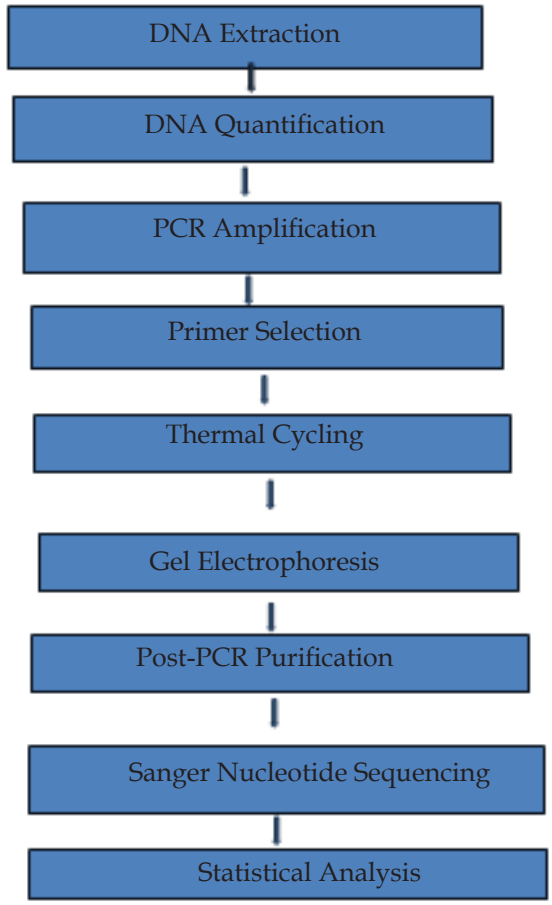


Figure 1: Flowchart of the Experimental Protocol

Materials and Reagents

The following materials and reagents were used for the study; solid tissue buffer, proteinase K, DNA pre-wash buffer, genomic binding buffer, DNA elution buffer, Nimagen, brilliant dye™ terminator cycle sequencing, 1% agarose gel with ethidium bromide stain, template DNA, 10µM forward primer, 10µM reverse primer, one Taq Quick Load 2X master mix with standard buffer, nuclease-free water, 50µl of 20U/µl exonuclease I (Catalogue No. NEB M0293L), and 200µl of 1U/µl shrimp alkaline phosphatase.

DNA Extraction

Genomic DNA was extracted from the samples using the Quick-DNA Miniprep Plus Kit (Zymo Research, catalog no. D4068).

The protocol followed is highlighted below.

For the insects

Place a sample of ≤ 25 mg in a microcentrifuge tube. Then, add 95 µL of nuclease-free water, 95 µL of solid tissue buffer, and 10 µL of proteinase K. Vortex the tube contents from step 1 above for 10 to 15 seconds, then incubate at 55°C for 1 to 3 hours. Centrifuge the tube at 12,000 x g for 1 minute, then transfer the aqueous supernatant to another microcentrifuge tube. Next, 2 volumes of genomic binding buffer (i.e., 200 µL of buffer to 100 µL of supernatant) were added to the supernatant from step 2 above. Vortex the tube for 10-15 seconds to mix the contents. Transfer the mixture from step 3 into a Zymo-Spin™ IIC-XLR Column in a collection tube and centrifuge at 12,000 x g for 1 minute. Discard the collection tube and flow-through. Transfer the Zymo-Spin™ IIC-XLR column to a new collection tube. Then, add 400 µL of DNA pre-wash buffer to the spin column. Centrifuge the assembly at 12,000 x g for 1 minute. The flow-through was discarded. Next, 700 µL of g-DNA wash buffer was added to the spin column. Centrifuge the assembly at 12,000 x g for 1 minute. The flow-through was discarded. Then, add 200 µL of g-DNA wash buffer to the spin column. Centrifuge the assembly at 12,000 x g for 1 minute, and discard the flow-through collection tube. Transfer the spin column to a clean 1.5 mL microcentrifuge tube, and add 50 µL of DNA Elution Buffer directly to the column matrix.Centrifuge the assemblyat12,000 xg for 30 seconds to elute the DNA.

Forthe blood

Place 200 µL of the received sample in a microcentrifuge tube. Afterward, 200 µL of BioFluid and cell buffer and 20 µL of Proteinase K were added, respectively. The tube contents from step 1 above were thoroughly mixed by vortexing for 10 to 15 seconds, then incubated at 55°C for 10 minutes. The tube was then centrifuged at 12,000 x g for 1 minute, and the aqueous supernatant was transferred to another microcentrifuge tube. This was followed by the addition of 1 volume of genomic binding buffer (i.e., 100 µL of buffer to 100 µL of supernatant) to the supernatant from step 2 above. Vortex the tube for 10-15 seconds to mix the contents. Transfer the mixture from step 3 into a Zymo-Spin™ IIC-XLR Column in a collection tube and centrifuge at 12,000 x g for 1 minute. Afterward, discard the collection tube with the flow-through. Transfer the Zymo-Spin™ IIC-XLR column to a new collection tube. Then, add 400 µL of DNA pre-wash buffer to the spin column.

Centrifuge the assembly at 12,000 x g for 1 minute. The flow-through was discarded. Next, add 700 µL of g-DNA wash buffer to the spin column. Centrifuge the assemblyat12,000xg for1minute. The flow-through was again discarded. Then, add 200 µL of g-DNA wash buffer to the spin column. Centrifuge the assembly at12,000 x gfor1 minute. Discard the flow-through into the collection tube. Transfer the spin column to a clean 1.5 mL microcentrifuge tube, and add 50 µL of DNA elution buffer directly to the column matrix. The assembly was thereafter centrifuged at 12,000 x g for 30 seconds to elute the DNA

DNA Quantification

The quality and quantity of the extracted DNA were measured using a NanoDrop One Microvolume UV-Vis spectrophotometer (Thermo Scientific™). The system was blanked with 1 µL of DNA Elution Buffer. Afterward, 1 µL of the DNA was placed on the pedestal and measured. The sample concentration (ng/µL), A260/280 ratio, and A260/230 ratio were then recorded.

PCR Amplification

The target region was amplified using OneTaq® Quick-Load® 2X Master Mix (New England Biolabs, USA, Catalog No. M0486) under the conditions listed in Table1 below.

Table 1: PCR amplification conditions.

COMPONENT	Volumesfora 25 µL reaction
TemplateDNA	2-3µL
10µMForward Primer	0.5µL(10nM)
10µMReverse Primer	0.5µL(10nM)
One Taq Quick Load 2X Master Mix with Standard Buffer	12.5µL
Nuclease-freewater	20- (sumofabove) µL

Primers

The following primers were used in the reaction.

- VF– GAGGMCAAATATCATTCTGAG
- VR – TAGGGCVAGGACTCCTCCTAGT

Thermal Cycling

The samples were then subjected to the following thermal cycling conditions on the Eppendorf Mastercycler Nexus Gradient 230 (Germany).Table 2 summarizes the steps and stages.

Table 2: Thermal cycling steps and stages

Step	Stage of PCR	Temperature (°C)	Time (min: secs)
1	Initial Denaturation	94	5:00
2	Denaturation	94	0:30
3	Annealing	54	1:00
4	Extension	68	1:30
5	Final Extension	68	10:00
6	Hold	4	Hold

Gelelectrophoresis

After PCR amplification, 2 µL of each PCR product was loaded onto a 1% agarose gel, stained with 1 µL of EtBr (10 mg/mL), and photographed with a gel documentation system (E-BOX, VilberLourmat, Italy).

Post-PCR Purification

PCR products were cleaned using an enzymatic method (ExoSAP) with an ExoSAP master mix prepared in a 0.6 mL microcentrifuge tube: 50 µL of 20 U/µL Exonuclease I (Catalog No. NEB M0293L) and 200 µL of 1 U/µL Shrimp Alkaline Phosphatase (Catalog No. NEB M0371). The reaction mixture was prepared by mixing the following components and incubating at 37 °C for 15 minutes, then at 80 °C for 15 minutes. The mixture contained 10 µL of the amplified PCR product and 10 µL of ExoSAP mix (step 1).

Sanger Nucleotide Sequencing

The sequencing followed a four-step procedure. The fragments were sequenced using the Nimagen Brilliant Dye™ Terminator Cycle Sequencing Kit V3.1 (BRD3-100/1000) according to the manufacturer's instructions: https://www.nimagen.com/products/Sequencing/Capillary-Electrophoresis/BrilliantDye-Terminator-Cycle-Sequencing-Kit/. The labeled products were then cleaned using the ZR-96 DNA Sequencing Clean-up Kit (Catalog No. D4053): http://www.zymoresearch.com/downloads/dl/file/id/52/d4052i.pdf. The cleaned products were then injected into the Applied Biosystems ABI 3500XL Genetic Analyzer with a 50 cm array using POP7 (https://www.thermofisher.com/order/catalog/product/4406016), and sequence data were collected. The genome sequences obtained were deposited in GenBank with accession numbers PP563681–PP563700 and PP566797–PP566875.

Statistical Analysis

Sequences were assembled using de novo assembly software (version 10) and screened against the NCBI database using the Basic Local Alignment Search Tool (BLAST: https://blast.ncbi.nlm.nih.gov/Blast.cgi). We considered sequences with >95% identity to GenBank sequences as matches for the species in question.

Results

These results focused one lectrophoretic and molecular techniques in identifying and individualizing mosquito blood meals;

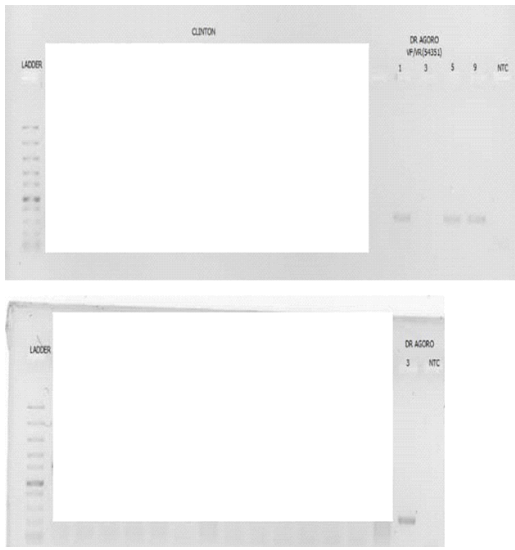


Figure 2: Ethidium bromide-stained agarose gel photograph showing host-specific cytochromeb PCR product amplified from *Anopheles* engorged and unengorged mosquitoes.

Legend

Small black boxes: DNA bands; Large White Boxes-Gel Plate

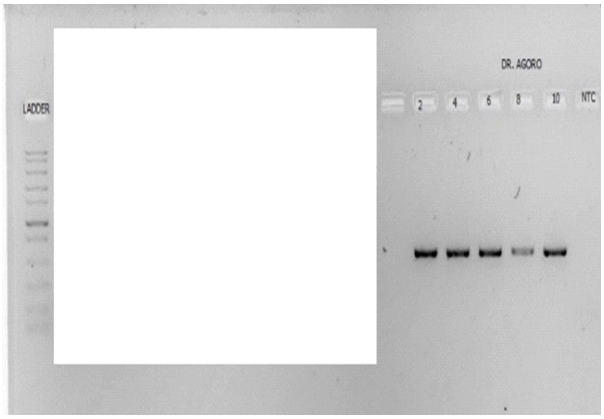


Figure 3: Ethidium bromide-stained agarose gel photograph showing host- specific cytochromeb PCR product amplified from human EDTA whole blood DNA extraction.

Editor's Note: *The authors state that Figures 2 and 3 are agarose gel images obtained following electrophoresis and represent the maximum image quality achievable with the available equipment. Attempts were made to further enhance the images; however, no meaningful improvement in clarity could be achieved without compromising the integrity of the original images. Nevertheless, the banding patterns are sufficiently clear for interpretation. Further improvement of the images is not feasible with the available equipment.*

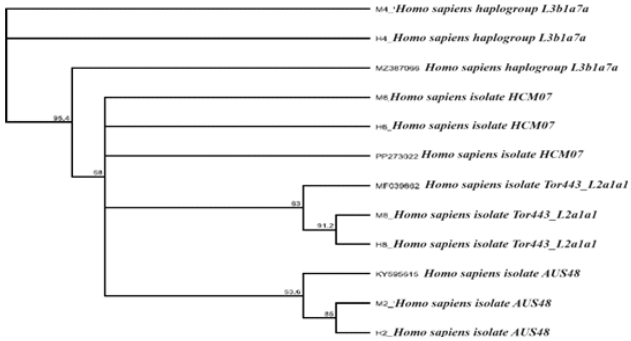


Figure 4: Phylogenetic Analysis Tree

Table 3: Sequence alignment of the Mosquito blood meal

Scientific Name	Max Score	Total Score	Query Cover	E Value	Per. Identification
Homosapiens	802	802	99%	0.0	99.54%
Homosapiens	800	800	98%	0.0	99.77%
Homosapiens	789	789	99%	0.0	99.09%
Homosapiens	795	795	99%	0.0	99.32%

Figure 2 shows an Ethidium bromide-stained agarose gel of the host-specific cytochrome b PCR product amplified from Anopheles mosquitoes, both engorged and unengorged. Control-positive products amplified from field mosquitoes are shown in lanes 1 and 3. Control-positive products amplified from engorged mosquitoes are shown in lanes 5 and 9. The outer lanes contain a 100-base-pair DNA ladder. Figure 3 shows a photograph of an Ethidium bromide-stained agarose gel of the host-specific cytochrome b PCR product amplified from human EDTA whole-blood DNA. Control-positive products amplified from whole blood are shown in lanes 2 and 4 (humans with no mosquito bite) and in lanes 6, 8, and 10 (humans bitten by study mosquitoes). The outer lanes contain a 100-base-pair DNA ladder. Figure 4 shows the mtDNA sequence obtained from the isolates that produced an exact match during a MEGA BLAST search for highly similar sequences in the NCBI non-redundant nucleotide (nr/nt) database. The 16S rRNA sequences of the isolates showed 100% similarity to those of other species. The evolutionary distances computed using the Jukes-Cantor method were consistent with the phylogenetic placement of the isolates'mt DNA within Homo sapiens mt DNA and revealed close relatedness to the Homo sapiens isolate HCM07, Homo sapiens haplogroup L3b1a7a, Homo sapiens isolate Tor443_L2a1a1, and Homo sapiens isolate AUS48. Table 3 is a sequence alignment of the mosquito blood meal after exposure to mosquito blood.

Discussion

Identifying mosquito blood meals can be valuable in forensic investigations, as it provides vital evidence linking suspects to crime scenes [8-9]. The use of mosquito blood meals to identify suspects in a crime remains controversial, with various schools of

thought and diverse perspectives [6-7]. This research team enters this discourse to broaden horizons through narratives of the female Anopheles mosquito from natural-occurrence and real-time perspectives.

The study demonstrated successful amplification using template DNA from human whole blood, mosquitoes that fed on blood from humans of known origin, and field-collected mosquitoes (Figures 2-3). In addition, the study confirmed the presence of human DNA in the mosquito blood meal by the appearance of specific bands on the gel photographs, with the observed sizes corresponding to the amplified target DNA. The intensity of the bands on the gel photograph also provided a rough estimate of the amount of human blood meal DNA in the mosquito's blood meal.

The presentation in Figure 2 revealed faint band intensity, indicating a low concentration of human DNA in the mosquito's blood meal. Field mosquitoes (lanes 1 & 3) showed successful DNA amplification. Furthermore, the engorged mosquitoes (lanes 5 & 9) also showed successful DNA amplification. In some cases, blood-meal detection in engorged mosquitoes was unsuccessful due to the amount of host DNA in the blood meal being too low for detection; the host might have been affected by the mosquito's digestive processes; the mosquito might have fed on vertebrates; or prolonged sample storage for DNA studies.

The unengorged mosquito shows no bands, indicating the absence of DNA associated with Homo sapiens. The narrative has further buttressed the possibility of extracting human DNA from a mosquito blood meal for further forensic investigations. A study by Shayna et al. [28] indicated that mosquito blood meals from Anopheles mosquitoes can be used effectively for forensic detection of host DNA and for identifying one or more hosts. The findings of this study aligned with those of several other researchers, though with differences in methodology, study location, mosquito breeding, and molecular technique employed [7-9,31].

The findings of this study could not address the use of mosquito blood meals to identify and individualize a suspect in a crime. Still, they indicated the presence of human blood that could be used to confirm the presence of humans at a crime scene, as shown in Figure 4& Table 3. The alignment and phylogenetic tree revealed Homo sapiens DNA from a mosquito blood meal. Non-engorged mosquitoes exhibited invertebrate-derived DNA.

Despite the study's laudable findings, a handful of limitations warrant further scrutiny. The major limitations are the inability to identify the alleged subject used in the study and the cumbersome procedure, as also noted by other authors [9,16]. However, these limitations could be addressed by advanced molecular tools, such as short tandem repeats (STRs), restriction fragment length polymorphism (RFLP), and emerging techniques.

Another limitation is that the study did not account for mosquito flight distance in the initial design. Perspectives on mosquito flight distance remain controversial, as several authors have noted [32]. However, this factor is highly relevant to interpreting mosquito blood meal DNA. Furthermore, the presence of mixed individuals or other primate blood complicates the interpretation of mosquito blood meals [16].

In conclusion, despite these limitations, mosquito blood meals are a viable tool in crime investigation and remain an emerging area of research.

Conclusion

The study successfully demonstrated the presence of human DNA in mosquito blood meals using molecular techniques, including DNA extraction, PCR amplification, gel electrophoresis, and Sanger nucleotide sequencing. The findings revealedthat mosquito blood meal DNA studies can identify human blood at a crime scene, thereby indicating the presence of humans in real time. However, the methods employed could not individualize thesubject's identity using mosquito blood meals. This was due to limitations of the method used. Furthermore, this study did not investigate the impact of host number, mosquito flight distance, environmental factors, or the duration after the blood meal, leaving gaps for further study.

Recommendations

The following are some recommendations resulting from the study;

1. Longitudinal studies should be conducted to determine the decay rate of human DNA in mosquito blood meals, providing valuable insight into the detection timeline.
2. Cross-validate findings by using multiple detection methods to ensure the accuracy of the results.
3. Advanced molecular tools that can identify and individualize suspects of crimes with high precision.

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References

1. Harbach RE, Besansky NJ. Mosquitoes. Current Biology. 2014; 24 (1):14-15. https://doi.org/10.1016/j.cub.2013.09.047.
2. GiatropoulosA, Papachristos D, Michaelakis A, Kapranas A, Emmanouel N. Laboratory Study on Larval Competition between Two Related Mosquito Species: Aedes (Stegomyia) albopictus and Aedes (Stegomyia) cretinus. Acta Trop. 2022; 230, doi: 10.1016/j.actatropica.2022.106389
3. Ong'wen F, Onyango PO, Bukhari T. Direct and indirect effects of predation and parasitism on the Anopheles gambiae mosquito. Parasites Vectors. 2020; 13(43). https://doi.org/10.1186/s13071-020-3915-8
4. WHO. World malaria report. Geneva: World Health Organization; 2018. https://apps.who.int/iris/bitstream/handle/10665/275867/ 9789241565653-eng.pdf?ua=1.
5. AntilS, Joon V. Biotechnology in forensic science: Advancements and applications. Journal of Forensic Science Research. 2025; 9(1):7-14. https://doi.org/10.29328/ journal.jfsr.1001073
6. Ren LP, Shang YJ, Guo YD. Progress and Application of Entomological Evidence in Forensic Science. Fa Yi Xue Za Zhi. 2021;37(3):295-294. English, Chinese. doi: 10.12116/j.issn.1004-5619.2020.401001.
7. SuwannakartP, Jeungsathapatchai K, Khuannet P. Human DNA profiling from mosquito blood meals over post-feeding intervals. Egyptian Academic Journal of Biological Sciences, a: Entomology.2024; 17 (4):57–68. https://doi.org/10.21608/ eajbsa.2024.393268
8. Suwannakart P, Jeungsathapatchai K, Khuannet P, Samsuwan J, Kitkumthorn N,Yanatatsaneejit P. Human DNA profiles from mosquito blood meal for forensicapplication in tropical country. ScienceAsia; 50 (5): 2024: ID 2024090: 1–7. doi: 10.2306/scienceasia1513-1874.2024.090
9. Ahmed AM, Alotaibi AM, Al-Qahtani WS, Tripet F, Amer SA. Forensic DNA analysis of mixed mosquito blood meals: STR profiling for human identification. Insects.2023; 14(5):467. https://doi.org/10.3390/insects14050467
10. Business Day. See how high Nigeria ranks on the 2024 crime index. https://businessday.ng/news/ article/ see-how- high-nigeria-ranks-on-2024-crime-index/ 2024.
11. Ado MD, Ahmad HA, Akorede BT. Criminal Justice System and Crime Control and Prevention in Nigeria. Journal of Political Science and Leadership Research, 2024, 10(6):24-32.DOI: 10.56201/jpslr.v10.no6.2024.pg24.37
12. Damelio, R.; Gardner, R.M. Bloodstain Pattern Analysis: With an Introduction to Crime Scene Reconstruction. 2001; (5) 1-7.https://doi.org/ 10.1201/9781420041255
13. Agoro ES, Adias CT,Ikimi CG. Use of Anopheles Mosquito Bloodmeal's Serology and Haemoglobin Genotypes in Crime Scene Forensics. Asian J. Biol. Sci. 2024, 17 (4): 574-581.
14. TzuS. Washing Away of Wrongs. Shanghai Classics Publishing House; Shanghai, China: 1981.https://muse.jhu.edu/book/52293
15. Wang J. Practical Forensic Entomology. Xi'an Jiaotong University Press; Xi'an, China: 2019.https://jglobal.jst.go.jp/en/ detail?JGLOBAL_ID=202102240054516577
16. Gomes LA, Duarte R, Lima DC, Diniz BS,Serrão ML, Labarthe N. Comparison between precipitin and ELISA tests in the bloodmeal detection of Aedes aegypti (Linnaeus) and Aedes fluviatilis (Lutz) mosquitoes experimentally fed on feline, canine and human hosts. Memorias do Instituto Oswaldo Cruz. 2001, 96:693-695.https://doi.org/10.1590/ s0074-02762001000500020
17. AlencarJ, Mello CFD, Gil-Santana HR,Giupponi APDL, Araújo AN, Lorosa ES, Silva JDS. Feeding patterns of mosquitoes (Diptera: Culicidae) in the Atlantic Forest, Rio de Janeiro, Brazil. Journal of Medical Entomology. 2015, 52(5), 783-788.https://doi.org/ 10.1093/jme/tjv098

18. Spitaleri S, Romano C, Di Luise E,Ginestra,E, Saravo L. Genotyping of human DNA recovered from mosquitoes found on a crime scene, International Congress Series, 2006, 1288,574-576.doi:10.1016/j.ics.2005.11.055

19. Gray SL, Tiedge TM, Butkus JM, Earp TJ, Lindner SE, Roy R. Determination of human identity from Anopheles stephensi mosquito blood meals using direct amplification and massively parallel sequencing. Forensic Science International: Genetics. 2020, 48:102347.https://doi.org/ 10.1016/j.fsigen.2020.102347

20. BukyyaJL, Tejasvi MA, Avinash A, Talwade P, Afroz MM, Pokala A, Neela PK, Shyamilee TK, Srisha V. DNA Profiling in Forensic Science: A Review. Glob Med Genet. 2021, 31:8(4)135-143.https://www.thieme-connect.com/products/ejournals/pdf/10.1055/s-0041-1728689.pdf

21. Reeves, L. E.; Gillett-Kaufman, J. L.; Kawahara, A. Y.; Kaufman, P. E. Barcoding blood meals: new vertebrate-specific primer sets for assigning taxonomic identities to host DNA from mosquito blood meals. PLoSNeglected Tropical Diseases, 2018, 12(8), e0006767. DOI: 10.1371/journal.pntd.0006767

22. Ren LP, Shang YJ, Guo YD. Progress and Application of Entomological Evidence in Forensic Science. Fa Yi Xue Za Zhi. 2021, 37(3):295-294.https://doi.org/10.12116/j.issn.1004-5619.2020.401001

23. Wells JD, Introna F, Jr, Di Vella G, Campobasso CP, Hayes J, Sperling FAH. Human and insect mitochondrial DNA analysis from maggots. J Forensic Science. 2001;46(3):685–687.PMID: 11373008.

24. Kavitha R,Nazni WA, Tan TC, Lee HL,Azirun MS. Review of forensically important entomological specimens collected from human cadavers in Malaysia (2005-2010). Journal of Forensic Legal Medicine. 2013, 20(5):480-2.DOI: 10.1016/j.jflm.2013.03.007

25. Joseph I, Mathew DG, Sathyan P, Vargheese G. The use of insects in forensic investigations: An overview on the scope of forensic entomology. Journal of Forensic Dental Science. 2011, 3(2):89-91.DOI: https://doi.org/10.4103/0975-1475.92142

26. Agoro ES, Eric E. Concept of Dried Seminal Biochemical Parameters in Substantiating Male Infidelity. Asian J. Biol. Sci. 2023, 16 (3): 229-233.https://doi.org/10.3923/ajbs.2023.229.233

27. Wankasi MM, Agoro ES, Ikimi CG. Vitreous Humour Biochemical Parameters as Indicators for Corroborating Acute Sniper (Dichlorvos) Induced Death . Journal of Forensic Toxicology and Pharmacology. 2020, (2)168. DOI: 10.37532/jftp.2020

28. Shayna LG,Teresa MT, Joann MB, Tyler JE, Scott EL, ReenaR. Determination of human identity from Anopheles stephensi mosquito blood meals using direct amplification and massively parallel sequencing. Forensic Science International: Genetics. 2020, 48:102347.doi:10.1016/j.fsigen.2020.102347

29. Kumar A, Kashyap A. DNA Sample Collection and Packaging: Chain of Custody. In: Kumar, A., Goswami, G.K., Huffine, E. (eds) Handbook of DNA Forensic Applications and Interpretation. Springer, Singapore. 2022. https://doi.org/10.1007/978-981-19-0043-3_4

30. Kent RJ, Norris DE. Identification of mammalian blood meals in mosquitoes by a multiplexed polymerase chain reaction targeting cytochrome B. The American Journal of Tropical Medicine and Hygiene. 2005, 73(2):336-42. https://doi.org/10.4269/ajtmh.2005.73.336

31. Rabelo KCN, AlbuquerqueCMR, Tavares VB, Santos SM, Souza CA. Trace samples of human blood in mosquitoes as a forensic investigation tool. Genetics and Molecular Research. 2015, 23;14(4):14847-56.DOI: 10.4238/2015.

32. FaimanR, Yaro AS, Diallo M, Dao A, Djibril S, Sanogo ZL, Sullivan M, Krishna A,Krajacich BJ, Lehmann T. Quantifying flight aptitude variation in wild Anopheles gambiae in order to identify long-distance migrants. Malaria Journal. 2020, 22;19(1):263.https://doi.org/ 10.1186/s12936-020-03333-2.

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Psychological Autopsy in Adolescent Hanging- A Cross-Sectional Study to Find Out the Background of Adolescent Suicide

Manivel Subramani

Junior Resident, All India Institute of Medical Sciences, New Delhi.

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Abstract

Background: Adolescent suicide is a growing public health concern worldwide, with hanging being one of the most commonly employed methods. Despite its increasing incidence, limited information is available regarding the psychosocial circumstances preceding adolescent suicidal hanging, particularly in India. Psychological autopsy is a valuable investigative tool for reconstructing the biological, psychological, social, and environmental factors associated with suicide.

Aim: To identify the socio-demographic characteristics and psychosocial risk factors associated with suicidal hanging among adolescents using the psychological autopsy approach.

Materials and Methods: A cross-sectional observational study was conducted in the Department of Forensic Medicine and Toxicology, AIIMS, New Delhi, India, from October 2021 to April 2022. Fifty-nine consecutive cases of suicidal hanging among adolescents aged 10–19 years were included. Following written informed consent, psychological autopsies were performed through face-to-face interviews with close biological relatives using a semi-structured questionnaire. Information obtained from police records, crime scene investigations, and medico-legal autopsies was integrated to reconstruct the circumstances surrounding each death. Data were analysed using descriptive statistics.

Results: Late adolescents (17–19 years) constituted the majority of victims (62.7%), with females predominating (59.3%). School dropout was observed in 32.2% of cases, while 22.0% belonged to families with financial difficulties. Depressed mood (15.3%), loss of interest in daily activities (20.3%), previous self-harming behaviour (15.3%), suicidal ideation (11.9%), suicide notes (11.9%), and substance use (17.0%) were identified in a proportion of victims. A family history of suicide was present in 8.5% of cases. Menstrual-related emotional distress (27.1%) and romantic relationship problems (8.5%) were the most frequently identified psychosocial stressors among female adolescents. Most suicides occurred at home (88.1%), with cloth serving as the most common ligature material (74.6%) and ceiling fans as the most frequent suspension point (67.8%). In 74.6% of cases, no definite precipitating stressor could be identified.

Conclusion: Adolescent suicidal hanging is a multifactorial phenomenon influenced by interacting psychological, familial, social, and environmental factors. Psychological autopsy provides valuable insights into these antecedent circumstances and facilitates the identification of modifiable risk factors that may inform targeted suicide prevention strategies, improve medico-legal investigations, and strengthen adolescent mental health interventions.

Keywords: Adolescent suicide; Psychological autopsy; Hanging; Suicide risk factors; Forensic medicine; Mental health; India.

Corresponding Author: Manivel Subramani, Junior Resident, All India Institute of Medical Sciences, New Delhi.

E-mail: Manivel95mbbs@gmail.com

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Introduction

Suicide is a major global public health problem that has profound social, psychological, and economic consequences for individuals, families, and communities. It is among the leading causes of preventable mortality. Suicide is defined as the intentional act of self-inflicted death performed with awareness of its fatal outcome [1] According to the National Crime Records Bureau (NCRB), 1,70,746 suicides were reported in India in 2024, of which 11,183 occurred among individuals younger than 18 years of age[2] These figures highlight the substantial burden of adolescent suicide and underscore the urgent need to identify modifiable risk factors for effective prevention.

The methods employed for suicide vary according to geography, culture, and demographics. Poisoning, firearm injuries, and hanging are among the most common methods worldwide. While predicting suicide at the individual level is difficult, recognising suicidal risk factors and warning signs provides opportunities for early intervention. Numerous biological, psychological, and social factors contribute to suicidal behaviour, and these differ across age groups. In older adults, risk increases with social isolation, unemployment, financial hardship, legal difficulties, personality disorders, family conflicts, living alone, low socioeconomic status, and a lack of social or religious support. By contrast, adolescents face a distinct set of developmental, psychosocial, and environmental stressors that predispose them to suicidal behaviour.

Adolescence, as defined by the World Health Organisation, is the period between 10 and 19 years of age, representing a critical stage of human development marked by rapid physical, cognitive, emotional, and social changes. It is conventionally classified into early (10–13 years), middle (14–16 years), and late adolescence (17–19 years). During this developmental period, adolescents experience increasing academic demands, parental expectations, peer pressure, interpersonal conflicts, identity formation, and emotional instability. Concurrent neurodevelopmental changes, particularly the delayed maturation of the prefrontal cortex relative to the limbic system, contribute to impaired impulse control, heightened emotional reactivity, and increased risk-taking behaviour. Consequently, adolescents are particularly vulnerable to suicidal behaviour, especially when exposed to adverse psychosocial circumstances.

Hanging is defined as death resulting from constriction of the neck by a ligature, with the constricting force generated by the weight of the body. Depending on the position of the knot, hanging is classified as either typical or atypical. A wide variety of readily available materials, including ropes, clothing, scarves, and electrical wires, may serve as ligatures [3]. Owing to its accessibility, simplicity, and high lethality, hanging has emerged as one of the most common methods of suicide across many regions of the world, including India.

Psychological autopsy is a systematic retrospective investigative technique designed to reconstruct the circumstances surrounding a suicide by collecting information from multiple sources, including interviews with family members, close relatives, friends, healthcare professionals, and other relevant informants, together with a review of medical, psychiatric, police, and forensic records[4] This approach provides comprehensive insight into the deceased individual's mental state, personality, life events, interpersonal relationships, behavioural patterns, and psychosocial stressors preceding death. Consequently, psychological autopsy is widely regarded as one of the most valid and reliable methods for investigating the complex interplay of biological, psychological, social, and environmental factors associated with suicide.

The incidence of adolescent suicide is increasing, and it has significant public health implications. Yet, psychological autopsy studies focusing on adolescents are limited, especially in low- and middle-income countries such as India. Most available literature has focused on adult populations. This leaves important gaps in our understanding of the unique developmental and psychosocial factors contributing to suicide during adolescence. Exploring these factors is essential for identifying vulnerable individuals, informing policies, and developing strategies for age-specific suicide prevention.

Materials and Methods

Study Design and Setting

This cross-sectional observational study was conducted to identify the psychosocial risk factors

and socio-demographic characteristics associated with suicidal hanging among adolescents using the psychological autopsy approach. The study was conducted in the Department of Forensic Medicine and Toxicology during the study period, and all eligible cases that fulfilled the inclusion criteria were consecutively recruited.

1. **Study duration**

October 2021 to April 2022.

2. **Study location**

Department of Forensic Medicine and Toxicology, AIIMS New Delhi, India.

3. Sample selection

All eligible cases during the study period were included consecutively.

4. **Questionnaire development**

It was adapted from previous validated psychological autopsy studies.

Study Population

A total of 59 cases of adolescent suicidal hanging were included in the study, which occurred during that period.

Inclusion Criteria

- Adolescents aged 10–19 years of either sex.
- Death certified as a suicidal hanging following police investigation and a medico-legal autopsy.

Exclusion Criteria

- Individuals younger than 10 years or older than 19 years.
- Cases in which the manner of death was accidental, homicidal, or undetermined.

Psychological Autopsy

Before conducting the psychological autopsy interview, relevant information regarding the circumstances of death, including the inquest report, case history, and crime scene photographs, was obtained from the investigating police officer to establish a preliminary understanding of each case.

Following written informed consent, a psychological autopsy was conducted through face-to-face interviews with the closest available biological relative of the deceased. The interviews were conducted predominantly on the day of death or the following day. Whenever necessary, supplementary information was obtained from other family members to enhance the completeness and reliability of the collected data. All interviews were conducted by a single interviewer; therefore, inter-rater reliability assessment was not applicable. In cases where discrepancies arose between informants or between interview responses and available records, the information was reviewed in conjunction with the police investigation records, case history, and crime scene findings and the most consistent account was used for analysis.

Data were collected using a semi-structured questionnaire specifically developed for this study. The questionnaire consisted predominantly of dichotomous (Yes/No) items and was designed to obtain information on socio-demographic characteristics, educational status, family environment, previous suicidal behaviour, psychiatric and medical history, substance use, interpersonal relationships, recent life events, and other potential psychosocial stressors associated with suicide. Owing to its structured design and predominantly binary response format, the questionnaire could be administered by an interviewer without formal training in psychological autopsy interview techniques. Accordingly, no specialised interviewer training was required.

Medico-Legal Examination

A complete medico-legal autopsy was performed in all cases according to standard forensic protocols. Detailed external and internal examinations were carried out, and all relevant findings were systematically documented. Photographic documentation included the face, ligature mark, eyes, oral cavity, fingernails, post-mortem lividity, and significant internal findings. The final opinion regarding the cause of death was formulated after correlating the autopsy findings with the available investigative information.

Statistical Analysis

The collected data were entered into Microsoft Excel (Microsoft Corporation, Redmond, WA,

USA) for data management and statistical analysis. Categorical variables were summarised as frequencies and percentages.

Ethical Considerations

The study protocol was reviewed and approved by the Institutional Ethics Committee (IECPG-656/28.10.2021 dated 29.10.2021) before commencement of the study. Written informed consent was obtained from the participants before conducting the interviews. All information collected during the study was treated as strictly confidential, and the anonymity of both the deceased individuals and their family members was maintained throughout the study.

Observation & Results

A total of 59 adolescent victims of suicidal hanging were included in the study. Most victims belonged to the late adolescent age group (17–19 years), accounting for 62.7% of the cases. Female victims predominated, with a male-to-female ratio of 1:1.45. Only 3 adolescents (5.1%) were married, while 19 (32.2%) had discontinued their education before completion.

Regarding psychosocial characteristics, 13 adolescents (22.0%) belonged to families experiencing financial difficulties. A history of depressed mood during the two weeks preceding death was reported in 9 cases (15.3%), whereas 12 adolescents (20.3%) had exhibited a noticeable loss of interest in routine daily activities during the same period. Verbal expression of suicidal ideation to family members, relatives, or friends was documented in 7 cases (11.9%), and an equal proportion (11.9%) had left a suicide note.

None of the adolescents was reported to have experienced significant interpersonal problems with friends or at their workplace. A history of substance use by the deceased was present in 10 cases (17.0%), while 14 adolescents (23.7%) had a family history of substance use. Histories of physical punishment by parents and verbal abuse by family members were reported in 1 (1.7%) and 2 (3.4%) cases, respectively.

No adolescent had a previously diagnosed psychiatric illness. However, a family history of psychiatric illness was reported in 2 cases (3.4%), and 5 adolescents (8.5%) had a family history of suicide among relatives. Current medical illness was documented in 2 cases (3.4%), consisting of one case each of active tuberculosis and goitre.

Only one adolescent (1.7%) had a documented history of a previous suicide attempt. In contrast, 9 adolescents (15.3%) had evidence of previous self-harming behaviour.

Most suicides occurred at the victim's residence (88.1%). Cloth was the most frequently used ligature material, observed in 44 cases (74.6%), while the ceiling fan was the most common point of suspension, used in 40 cases (67.8%).

With respect to behavioural characteristics, 7 adolescents (11.9%) were reported to have a regular interest in sports, and 46 (78.0%) were described by their family members as having good interaction and close relationships within the family.

Observed Psychosocial Stressors

The psychosocial stressors identified during the psychological autopsy are summarised below. In many cases, more than one stressor was reported; therefore, the frequencies are not mutually exclusive.

The most commonly reported stressor was menstrual-related distress, identified in 16 adolescents (27.1%), and followed by romantic relationship problems in 5 cases (8.5%). Family-related conflicts, including quarrels with family members and domestic violence by the husband, were reported in 2 cases (3.4%) each.

Academic stressors included stress related to school board examinations in 2 adolescents (3.4%), failure in pre-board examinations in 1 case (1.7%), and being reprimanded by a teacher for poor class attendance in 1 case (1.7%). Difficulty adjusting to hostel life was reported in 1 adolescent (1.7%).

One adolescent (1.7%) experienced significant psychological distress following the murder of the father and subsequent threats directed towards the deceased.

Despite detailed psychological autopsy interviews, no identifiable precipitating stressor could be established in 44 cases (74.6%).

Table 1: Observed stressor and its frequency

Observed stressor	n	%
Menstrual-related distress*	16	27.1
Romantic relationship problems	5	8.5
Quarrel with family members	2	3.4
Domestic violence by husband	2	3.4
School board examination-related stress	2	3.4
Difficulty adjusting to hostel life	1	1.7
Murder of father and subsequent threats	1	1.7
Reprimand by teacher for absenteeism	1	1.7
Failure in pre-board examination	1	1.7
No identifiable precipitating stressor	44	74.6

Discussion

The present study demonstrated that late adolescence (17–19 years) constituted the age group with the highest frequency of suicidal hanging, accounting for 62.7% of all cases. This finding is consistent with the observations of Shaw et al.[5], suggesting that vulnerability to suicide may increase during late adolescence. This developmental period is characterised by substantial biological, psychological, and social transitions, including identity formation, increasing academic expectations, interpersonal relationship challenges, and the transition to adulthood. These factors may collectively contribute to heightened emotional distress and an increased risk of suicidal behaviour.

Female adolescents comprised 59.3% of the victims, resulting in a male-to-female ratio of 1:1.45. This finding contrasts with that reported by Shaw et al.[5], who observed a predominance of male victims (71%). Such differences may reflect variations in sociocultural context, gender roles, healthcare access, help-seeking behaviour, methods of suicide, and regional epidemiological characteristics. The predominance of female victims in the present study highlights the need to better understand gender-specific psychosocial stressors and mental health needs within the local population.

Approximately 11.9% of adolescents had expressed suicidal ideation during the month preceding death. Comparable findings have been reported by Narayanan et al.[6], who found that 12 of 55 suicide victims had communicated suicidal thoughts to family members or friends before death. Similarly, Shaw et al.[5] reported evidence of verbal or written suicidal ideation in 45% of adolescent suicide cases. These findings reinforce the importance of recognising verbal expressions of hopelessness or suicidal intent as critical warning signs warranting timely clinical assessment and intervention. Improving suicide awareness among family members, teachers, and healthcare professionals may facilitate earlier recognition of adolescents at risk.

Suicide notes were identified in 11.9% of the present cases. This proportion is lower than that reported by Clerici et al.[7], who found farewell messages in 19% of adolescent suicides, Zinum and Cohen[8], who reported suicide notes in 26% of cases, and Shaw et al.[7], who documented suicide notes in 39% of adolescents. The observed variation may reflect cultural differences in communication, family dynamics, literacy, or the impulsive nature of suicidal acts among adolescents. The absence of a suicide note should therefore not be interpreted as evidence against suicidal intent.

Only one adolescent (1.7%) had a documented history of a previous suicide attempt. This prevalence is considerably lower than that reported by Narayanan et al.[6], in which 14.5% of victims had previously attempted suicide. Although previous suicide attempts are widely recognised as one of the strongest predictors of future suicide, the low prevalence observed in the present study suggests that many adolescents may progress directly to a fatal first attempt or that previous episodes of self-harm may have remained undisclosed or undocumented.

Clothing materials, including chunni, saree, towel, and muffler, was the most frequently used ligatures. Similar observations have been reported in previous psychological autopsy studies, where readily available household materials such as long handkerchiefs, soft cloth, school ties, and dressing-gown cords were commonly used for hanging.[5,7,8] The widespread availability of these materials supports the concept that many adolescent suicides are opportunistic and occur using immediately accessible means. Consequently, suicide prevention

strategies should extend beyond means restriction and focus on early identification of psychological distress, crisis intervention, and strengthening family and community support systems.

Among the 35 female adolescents, menstrual blood within the uterine cavity was identified at autopsy in 16 (45.7%) cases. This represents an objective post-mortem observation and should not be interpreted as evidence that menstruation or menstrual-related emotional distress directly precipitated the suicidal act. Although previous studies by Dogra et al. and Leenaars et al. have explored a possible association between phases of the menstrual cycle and suicidal behaviour [9,10], the available evidence remains limited and inconclusive. Furthermore, the present study did not assess menstrual cycle phase, premenstrual symptoms, hormonal status, or menstrual-related psychological distress. Therefore, no causal inference can be drawn regarding the role of menstruation as a proximal trigger for suicide. The finding is reported as an observational characteristic of the study population and should be considered hypothesis-generating. Further prospective studies incorporating detailed menstrual history, hormonal assessment, and standardised psychiatric evaluation are required to determine whether any clinically meaningful association exists between menstrual physiology and suicidal behaviour.

A notable finding of the present study was that no identifiable precipitating stressor could be established in 74.6% of cases. This observation should be interpreted with caution, as it may not necessarily indicate the absence of antecedent stressors but rather reflect the inherent limitations of psychological autopsy methodology. Information was obtained retrospectively from proxy informants who may have been affected by grief, recall bias, incomplete knowledge of the deceased's personal experiences, or social desirability bias. In the Indian sociocultural context, sensitive issues such as romantic relationships, family conflict, menstrual concerns, substance use, and psychiatric symptoms may also be underreported because of stigma, fear of social repercussions, or reluctance to disclose private family matters. Furthermore, adolescents often conceal emotional distress from family members, making certain precipitating factors inaccessible even to close relatives. Therefore, the high proportion of cases without an identifiable precipitating stressor should be interpreted as reflecting the limitations of retrospective informant-based assessment rather than confirming the absence of psychosocial antecedents. Future studies incorporating multiple informants, standardised psychological autopsy instruments, and corroborative information from medical, educational, and social records may improve the identification of antecedent stressors.

Overall, the findings of the present study reinforce that adolescent suicide is a multifactorial phenomenon resulting from a complex interaction of developmental, psychological, familial, and situational influences. Psychological autopsy provides valuable insights into these antecedent circumstances and facilitates the identification of potentially modifiable risk factors. Bhushan D et al. and Kmar V et al. also conclude that psychological autopsy is an important tool in finding out the background of suicide [11,12]. Integrating psychological autopsy findings into public health policies, school-based mental health programmes, family education, and clinical suicide risk assessment may contribute to more targeted and effective suicide prevention strategies for adolescents.

STRENGTHS

The present study has several strengths. First, it focuses exclusively on adolescent suicidal hanging, a relatively underexplored area in psychological autopsy research, particularly in the Indian context. Second, the use of the psychological autopsy approach, combined with detailed medico-legal autopsy findings and crime scene information, enabled a comprehensive assessment of the psychosocial, behavioural, and socio-demographic factors associated with suicide. Third, data were collected through face-to-face interviews with close biological relatives, allowing the reconstruction of events preceding death and providing valuable insights into potential risk factors that are often unavailable from routine medico-legal records. Finally, the study contributes region-specific evidence that may assist clinicians, forensic experts, educators, and policymakers in developing targeted suicide prevention strategies for adolescents.

LIMITATIONS

The study has several limitations. The relatively small sample size and single-centre design may limit the generalizability of the findings. Psychological autopsy relied primarily on interviews with surviving family members and was therefore susceptible to recall, informant, and social desirability biases. The cross-sectional observational design precluded causal inferences between identified risk factors and suicide. Psychiatric disorders were not confirmed using standardised diagnostic instruments, potentially resulting in under recognition of underlying mental illness. In addition, retrospective assessment of psychosocial variables may have been influenced by respondents' perceptions following bereavement. Certain potentially relevant variables, including the exact timing of the suicidal act, detailed educational attainment, parental education and occupation, living arrangements, and prior medical or psychiatric contact, were not consistently available from proxy interviews or official records and therefore could not be analysed.

Despite these limitations, the study provides important insights into the psychosocial circumstances surrounding adolescent suicidal hanging and identifies potentially modifiable factors that may inform future research and suicide prevention strategies.

Conclusion

The present study demonstrates that adolescent suicide by hanging is a multifactorial phenomenon influenced by a complex interaction of socio-demographic, psychological, familial, and environmental factors. A considerable proportion of the victims was school dropouts, belonged to economically disadvantaged families, and had a history of substance use or exposure to substance use within the family. Common precipitating stressors included academic pressure, school-related problems, romantic relationship difficulties, marital conflict, and family disputes, although no identifiable trigger could be established in many cases.

Several potential warning signs and risk factors were identified, including suicidal ideation, previous self-harming behaviour or suicide attempts, a family history of suicide, persistent sadness, loss of interest in daily activities, menstrual-related emotional distress among female adolescents, and substance use. These findings emphasise the importance of early recognition of psychological distress and timely intervention among vulnerable adolescents.

Most suicides occurred within the home environment, often when family members were occupied elsewhere, and the ligatures used were predominantly clothing and ropes that were readily available in households. These observations highlight the impulsive nature of many adolescent suicides and the challenges associated with restricting access to commonly available means.

Importantly, many adolescents who died by suicide had not exhibited obvious warning signs before their death. Nevertheless, any expression of suicidal thoughts, behavioural changes, or emotional distress should be regarded as clinically significant and should prompt immediate psychological assessment and appropriate intervention. Strengthening school-based mental health programmes, improving family awareness, enhancing early identification of at-risk adolescents, and ensuring timely access to mental health services are essential components of effective suicide prevention strategies.

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References

1. Palacio C, García J, Diago J, Zapata C, Lopez G, Ortiz J, Lopez M. Identification of suicide risk factors in Medellin, Colombia: a case-control study of psychological autopsy in a developing country. Archives of Suicide Research. 2007 Jun 14;11(3):297-308.
2. https://data.opencity.in/dataset/ 70aabcb9-d126-492b-8a84-316f87f31598/resource/ 70edd502-a455-41e6-adc6-d5bb8402874f/ download/adsi-2024chapter-2 suicides-excludingfarmercauses.pdf Accessed 3rd August 2026
3. Shakya KN, Bhatta A, Bhandari B. A case report on attempted suicide by hanging of an adolescent girl. Journal of Kathmandu Medical College. 2017;6(2):78-80.
4. Beskow J, Runeson B, Åsgård U. Psychological autopsies: methods and ethics. Suicide and Life-Threatening Behaviour. 1990 Dec;20(4):307-20.

5. Shaw D, Fernandes JR, Rao C. Suicide in children and adolescents: a 10-year retrospective review. The American journal of forensic medicine and pathology. 2005 Dec 1;26(4):309-15.
6. Narayanan KK, MuthukumarDuraiappa PT. Psycho-Social Factors in Adolescent Suicides: A Psychological Autopsy-Based Study. Indian Journal of Forensic Medicine & Toxicology. 2020 Jul 30;14(3):402-6.
7. Clerici CA, Gentile G, Marchesi M, Muccino E, Veneroni L, Zoja R. Two decades of adolescent suicides assessed at Milan University's medicolegal unit: Epidemiology, forensic pathology, and psychopathology. Journal of forensic and legal medicine. 2016 Jan 1;37:15-21.
8. Zainum K, Cohen MC. Suicide patterns in children and adolescents: a review from a pediatric institution in England. Forensic science, medicine, and pathology. 2017 Jun;13(2):115-22.
9. Leenaars AA, Dogra TD, Girdhar S, Dattagupta S, Leenaars L. Menstruation and suicide: a histopathological study. Crisis. 2009 Jul;30(4):202-7.
10. Dogra TD, Leenaars AA, Raintji R, Lalwani S, Girdhar S, Wenckstern S, Lester D. Menstruation and suicide: an exploratory study. Psychological reports. 2007 Oct;101(2):430-4.
11. Bhushan D, Yadav J, Rozatkar AR, Moirangthem S, Arora A. The psychological autopsy: An overview of its utility and methodology. Journal of neurosciences in rural practice. 2023 Aug 16;14(3):447.
12. Kumar V, Saha S, Kumar G, Chhetri D, Maitra R, Prasad R. A Study on Psychological Autopsy of Suicidal Cases of North Bengal Region.Prof. SK Dhattarwal. 2024 Jul;18(3):175.

Original Article

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Forensic Discrimination of Salt Water Drowning Using Vitreous Lipid and Protein Analysis

Eni-yimini Solomon Agoro[1], Kelechi Uzoma Akataobi[2], Cosmas Sopuruchi Agim[2]

[1]Department of Biochemistry, Federal University Otuoke, Bayelsa State, Nigeria. [2]Department of Medical Biochemistry, College of Medicine, Abia State University, Uturu, Abia State

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Abstract

Objectives: The use of autopsy in the discrimination of post-mortem saltwater and true saltwater drowning is handicapped due to the non-overt histological presentation. This has created a need for reproducible, accurate, and inexpensive discriminatory markers.

Methods: This is an experimental animal study that involved using albino rabbits, categorized into four groups of ten (10) rabbits each: the control (strangled) death (CD), the chloroform death (CFD), the postmortem drowned (disguised) death (PDD), and the saltwater drowned death (SDD). Vitreous humour was extracted from the eyes of the rabbits using the Coe method and transported to the laboratory for the estimation of vitreous proteins and lipids. The vitreous biochemical parameters were analyzed using WHO-approved methods, and the data were analyzed in SPSS (Version 18-20) using One-Way ANOVA (Post Hoc LSD) with a level of significance set at 0.05.
Results: The study revealed a significant increase ($P<0.05$) in vitreous protein and globulin concentrations in CFD and PDD compared with CD. The vitreous albumin/globulin ratio significantly decreased ($P<0.05$) in the PDD group when compared to the control. In contrast, vitreous high-density lipoprotein (HDL) decreased significantly ($P<0.05$) in the SDD group. In contrast, vitreous low-density lipoprotein (LDL) concentration increased in the CFD group compared to the CD group.

Conclusion: The vitreous parameter distortions presented above could serve as a potential adjunctive marker for the investigation of death due to saltwater drowning. Further replicative studies of drowning with different parameters and human models (accidental drowning) could advance this discourse.

Keywords: Forensics, drowning, vitreous, saltwater, lipids, proteins, post-mortem.

Introduction

Drowning is a type of violent asphyxia or respiratory dysfunction from submersion/immersion in liquid resulting in death[1,2]. In 2019, roughly 236,000 people died from drowning, thereby causing it to be the third leading cause of unintentional

Corresponding Author: Eni-yimini Solomon Agoro, Department of Biochemistry, Federal University Otuoke, Bayelsa State, Nigeria.

E-mail: agoroes@fuotuoke.edu.ng

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death globally, trailing traffic injuries and falls[3]. These statistics span all types of water bodies, as 90% of drowning cases occur in freshwater, such as rivers and pools, while the remaining cases are split between brackish and saltwater[4].

In lower-income countries like Nigeria, cases of drowning and fatalities caused by drowning are underreported, and data collection is limited[5]. Many low-income countries in Africa have the highest rates of drowning, with incidence rates calculated from population-based studies across 15 different countries (Egypt, Ethiopia, Kenya, Uganda, Tanzania, Malawi, Zimbabwe, South Africa, Nigeria, Ghana, Burkina Faso, Seychelles, Guinea, Cote d'Ivoire, and the Gambia) ranging from 0.33 per 100,000 population to 502 per 100,000 population.

Saltwater or seawater is water from a sea or ocean. On average, seawater in the world's oceans has a salinity of about 3.5% (35 g/L, 35 ppt, 600 mM). This means that every kilogram (roughly one liter by volume) of seawater has approximately 35 grams (1.2 oz) of dissolved salts (predominantly sodium (Na^+) and chloride (Cl^-) ions)[6].

Vitreous humor is a transparent, colourless, gelatinous mass that fills the space between the eye's lens and the retina lining the back of the eye of humans and other vertebrates[7]. The post-mortem chemical composition of vitreous humour is rarely affected by fermentation, autolysis, and putrefaction[8]. Similarly, the effect of sex and aging is negligible[9]. These attributes make vitreous humour a choice fluid for forensic studies and investigations. The application of vitreous humour in crime abounds in the literature[10-11].

The discriminatory molecular traffic in the vitreous humor makes it useful in forensic and medical toxicology. This creates a pattern that could be exploited to explain biochemical processes associated with systemic phenomena. Macromolecules such as proteins and lipids are ubiquitous substances in the body and are used to explain biochemical processes because of their central role in metabolism. Proteins and lipids, including total protein, albumin, globulin, cholesterol, and triacylglycerol, were selected as the vitreous parameters for this study. Vitreous proteins and lipids have been implicated as discriminatory markers in freshwater drowning investigations, making them choice parameters in this study[12].

Incidents of drowning abound in Bayelsa State, Nigeria, due to its vast water bodies[13]. Drowning cases are mainly categorized as accidental, leaving out the components of suicidal and homicidal drowning[14]. Furthermore, autopsy, as a gold standard for death investigation, is handicapped in effectively discriminating between drowning and a post-mortem drowned death correctly. The water body associated with drowning also plays a crucial role in drowning investigations. Studies on vitreous proteins and lipids have been conducted using freshwater and brackish water, but there is a dearth of literature on saltwater[15-16]. The research question examines the suitability of vitreous proteins and lipids for discriminating between true and disguised saltwater drowning.

The biochemical presentation of saltwater drowning may differ from that of freshwater or brackish drowning due to cascading effects, including hypoxia, suffocation, altered respiratory chemistry, muscular contractions, respiratory alterations, and excessive water intake. These events can alter biochemical parameters, which could serve as a hallmark for distinguishing saltwater drowning from freshwater and brackish water drowning.

Thus, the study aims to investigate the effects of drowning on the concentrations of vitreous lipids and proteins, using saltwater as a case study. The resultant findings could be utilized as a discriminant to differentiate death due truly to drowning from that of postmortem submersion (disguised drowning) in saltwater. It could also serve as a comparative literature on the chemistry of death across different bodies of water.

Materials and Methods

Study Location

The saltwater for the study was harvested from the Brass River near the Twon Brass River axis in Bayelsa State. The artificial drowning and vitreous collection component of the study was conducted at the Department of Biochemistry, Federal University Otuoke, Bayelsa State. Similarly, the water and biochemical analyses were carried out at Eni-Yimini

Laboratories (eL) Limited inYenezue Gene, Epie, Yenagoa, Bayelsa State. The Albino rabbits used for the experiment were obtained from the Department of Biochemistry's animal house at the University of Port Harcourt, Rivers State. The research lasted six months, from February to August 2023.

Research Designs/Sample Population

The research design structure was adopted from[17]. It is typically a quantitative experimental design, a scientific method for establishing cause-and-effect relationships among the groups that comprise the study.

Mead's resource equation was used to determine the sample size[18]. The study comprised four treatment groups (T4), with 10 animals per group, for a total of 40 animals (N=40). Statisticians have used Mead's resource for decades, and it is suitable for complex biological experiments with several factors and treatments.

The rabbits were divided into four groups using systematic random sampling: the control (strangled) death (CD), the chloroform death (CFD), the postmortem drowned (disguised) death (PDD), and the saltwater drowned death (SDD). The CD group was made up of rabbits that were strangled to death, and then vitreous was extracted for the study as a control and baseline. Similarly, the CFD group was exposed to chloroform in an enclosed desiccator. The PDD group was strangled to death and then introduced into a pool of salt water, whereas the SDD died of salt water drowning. Theanimals were exposed to the same sample diet and control measures, and were subsequently randomly assigned to the groups as stated above before the experiment.

Selection Criteria

The rabbits used were all healthy and active, as confirmed by the university's veterinary doctor. Rabbits showing signs or symptoms of illness or inactivity were excluded. The age range was 7-8 months, and the weight range was 1.5-2 kg.

Ethical Approval

The Directorate of Research and Quality Assurance and the Department of Biochemistry at the Federal University Otuoke, Bayelsa State, granted ethical approval. The Animal Welfare Act of 1985 of the United States of America and Institutional Animal Care and Use Committee (IACUC) protocols were strictly followedto guide animal care and handling. The study followed the ARRIVE guidelines for reporting results[19].

Collection of Samples

a. Vitreous Humour

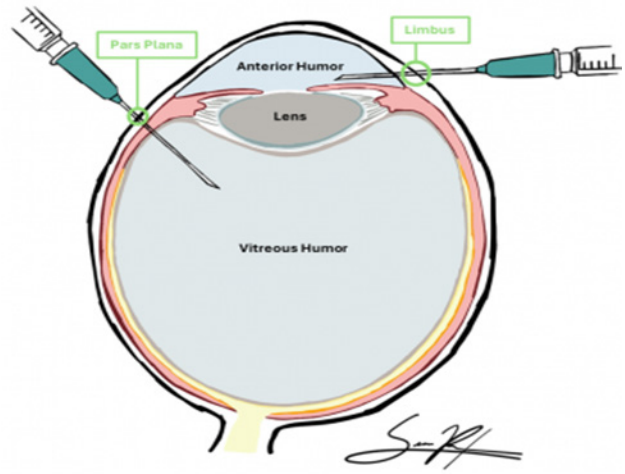


Figure 1: Vitreous Humour Collection

Adapted from https://eyewiki.aao.org/Liquid_Biopsy_in_Ocular_Oncology

This study adopted Coe's method for collecting vitreous humor[20], as shown in Figure 1.Using a 5 mL syringe and a needle, a scleral puncture was made on the lateral canthus, and the total extractable vitreous humor was aspirated from the eye carefully. Vitreous humour is crystal clear and transparent; hence, turbid or bloody samples were not used for the laboratory analysis to avoid contamination or infiltration of biochemical parameters from sources other than the vitreous. The samples were collected within 10 hours postmortem into plastic containers and transported on a cool chain to the laboratory for protein and lipid analysis.

The postmortem interval was standardized by ensuring samples were collected within the experiment's set time (10 hours), though negligible variations in seconds were recorded. The 10-hour window was used to capture the onset of decomposition, such as autolysis and putrefaction.

Laboratory Analysis

Vitreous total protein and albumin concentrations were measured using Randox kits (United Kingdom). Vitreous globulin concentration and the albumin/globulin ratio were calculated mathematically[21]. Similarly, vitreous total cholesterol, triacylglycerol, and high-density lipoprotein (HDL) concentrations were estimated using Agape diagnostic reagents (Switzerland). The low-density lipoprotein (LDL) was estimated mathematically[22].Randox quality control 1 and 2 were used for the validation and calibration to ensure accuracy and precision of the vitreous results.

Statistical Analysis

One-way ANOVA (Post hoc LSD) was the chosen statistical tool, computed in the Statistical Package for the Social Sciences (SPSS) program (SPSS Inc., Chicago, IL, USA; Version 18-21). The confidence interval level was pegged at ≤ 0.05.

Results

Table 1: Mean protein concentrations of study parameters in various death models

Parameters	CD n=10	CFD n=10	PDD n=10	SDD n=10	F-Value	P- Value
Total protein (g/L)	5.01 ± 1.50	13.12 ± 4.95[a]	13.18 ± 5.45[a]	8.98 ± 4.26[b,c]	1.30	0.34
Albumin (g/L)	0.92 ± 0.08	1.64 ± 0.40	1.33 ± 0.28	1.11 ± 0.31	1.33	0.33
Globulin (g/L)	4.09 ± 1.48	11.57 ± 3.56[a]	11.85 ± 4.21[a]	7.87 ± 3.45	1.42	0.34
Albumin/Globulin Ratio	0.22± 0.08	0.14± 0.04	0.11± 0.06[a]	0.14± 0.33	0.99	0.27

Legend: CD-Control Death (a); CFD- Chloroform Death (b); PDD- Postmortem Drowned Death (c); SDD-SaltwaterDrowned Death (d).

Symbols: a: P < 0.05 vs b, c, & d; b: P < 0.05 vs Group c&d; c: P < 0.05 vs Group d.

Data are expressed as mean ± SD; Significant at 0.05 Confidence (p < 0.05)

±- Plus-Minus Sign

Superscript indicates significant value.

n indicates the number of animals used.

Table 2: Mean lipid concentrations of study parameters in various death models

Parameters	CD n=10	CFD n=10	PDD n=10	SDD n=10	F-Value	P-Value
TC (mmol/L)	0.534 ± 0.180	1.054 ± 0.332	0.683 ± 0.172	0.605 ± 0.409	1.894	0.209
TG (mmol/L)	0.389 ± 0.161	0.706 ± 0.212	0.397 ± 0.146	0.354 ± 0.196	1.795	0.226
HDL (mmol/L)	0.389 ± 0.129	0.368 ± 0.161	0.559 ± 0.129	0.049±0.010[a,b,c]	0.802	0.527
LDL (mmol/L)	0.331 ± 0.151	0.701 ± 0.280[a]	0.477 ± 0.182[b]	0.428 ± 0.281[b]	1.382	0.017

Legend:TC: total cholesterol; TG: triglyceride; HDL: high-density lipoprotein; LDL: low-density lipoprotein.

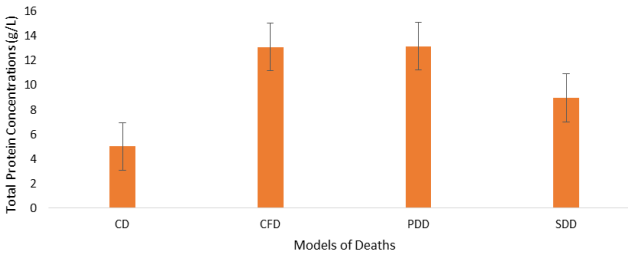


Figure 1: Total protein concentrations in various death models

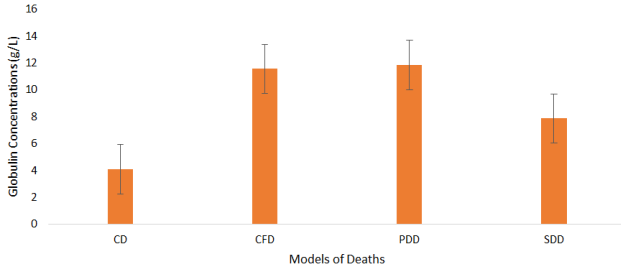


Figure 2: Globulin concentrations in various death models

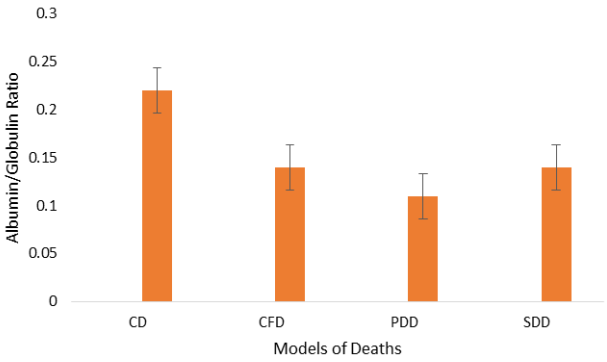


Figure 3: Albumin/Globulin ratio in various death models

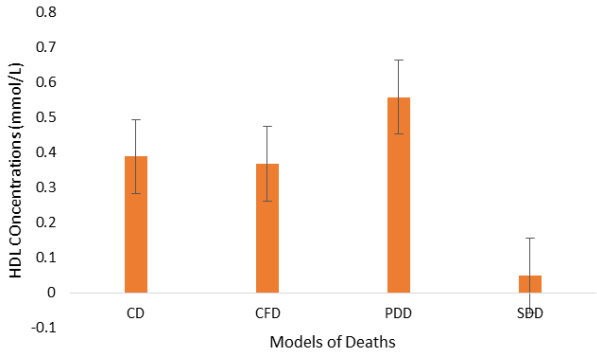


Figure 4: HDL concentrations in various death models

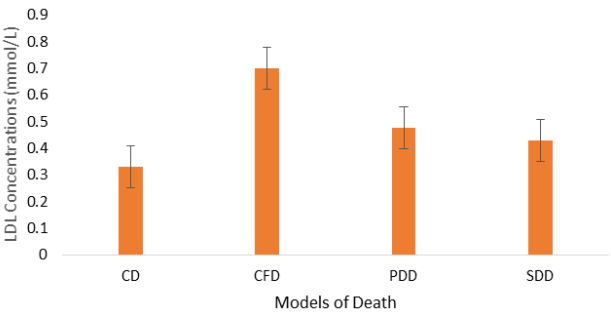


Figure 5: LDL concentrations in various death models

The study shows a significant increase (P<0.05) in vitreous total protein and globulin concentrations in CFD and PDD compared with CD and SDD. The vitreous albumin/globulin ratios significantly decreased (P<0.05) in the PDD group compared with the control group (Table 1, Figures 1 and 2), whereas the albumin/globulin ratio decreased. In contrast, vitreous HDL decreased significantly (P<0.05) in the SDD group compared with the other groups. In contrast, vitreous LDL concentration was higher in the CFD group, comparable to the other studied groups (Table 2, Figures 4 and 5).

Discussion

The study revealed a significant increase in vitreous total protein and globulin concentrations in the CFD and PDD groups compared with the CD group (Table 1, Figures 1-2). Similarly, the vitreous albumin/globulin (a/g) ratio significantly decreased in the PDD group compared with the CD group (Figure 3). The increase in total vitreous protein concentration observed in the PDD group could be attributed to the collapse of the vitreous membrane, thereby disrupting the restrictive molecular traffic. Unlike in the SDD group, restricted molecular traffic may have persisted longer, as the postmortem interval was shorter than in the PDD group. In contrast, the increase in vitreous protein in the CFD group could be due to the lipid-penetrating capacity of the toxic gases, leading to membrane collapse and subsequent increased molecular traffic. The findings of this study concurred with those of Agoro *et al.*[16] on the application of vitreous proteins in the discrimination of postmortem drowning in freshwater. However, they differed from those of brackish water[15]. These differences may be attributed to salt concentration, which is crucial in osmotic and oncotic pressure.

An increase in globulin concentration is associated with acute inflammation, chronic disease, or immune-mediated diseases[23]. The observed increase in vitreous globulin concentration in the CFD group could be immune-mediated due to chloroform, as it is alien to the body; however, that in the PDD group is unknown. The decrease in vitreous a/g ratio in the PDD group could be due to the increased concentration of vitreous globulin. This could serve as a discriminatory hallmark for the investigation of postmortem saltwater drowning. This finding is consistent with that of Agoro *et al.*[16].

Furthermore, the study revealed a significant decrease in vitreous HDL concentration in the SDD group compared with the CD and PDD groups (Table 2, Figure 4). The decrease could be attributed to myocardial infarction. A myocardial infarction (MI) or heart attack happens when there is a blockage that prevents oxygen-rich blood from getting to the heart. Low HDL concentrationis an independent risk factor for myocardial infarction[24-25]. A study conducted by Chen *et al.*[26] has established a strong correlation between drowning and MI. Therefore, a decrease

in vitreous HDL concentration may be linked to MI episodes associated with drowning, especially given the high viscosity of saltwater.

Additionally, the increase in vitreous LDL concentration was significantly greater in the CFD group compared to the other groups (Table 2, Figure 5). The increase observed could be due to the acute exposure to a high concentration of chloroform, which may have precipitated a heart attack in the CFD[27].

The non-significant concentrations of the other vitreous proteins and lipid parameters observed in the study could guide interpretation in cases of a significant increase. This suggests that drowning does not have an overt effect on those parameters, and an increase could be suspicious of other types of deaths, disguised as drowned or chloroform deaths.

Forensic Implications

The study revealed that vitreous total protein and globulin, albumin/globulin ratios, and HDL and LDL concentrations could complement conventional methods in investigating saltwater drowning. This suggests that vitreous chemistry should be used in supporting the gold standards of autopsy and diatom testing for drowning.

The applications of vitreous parameters in the investigation of drowning are still nascent and require further validation using repeated studies using larger animals and human models.However, the potential and suitability of vitreous chemistry cannot be discarded, considering the stability and resistance to autolysis and putrefaction. Therefore, further studies in this direction, expanding the parameters and perspectives, are strongly recommended.

Conclusion

The dilemma of resolving drowned death using autopsy formed the basis of this study. The study exploited vitreous protein and lipid chemistry. The findings revealed a significant increase in vitreous protein and globulin concentrations in CFD and PDD. In contrast, the vitreous albumin/globulin ratio decreased significantly in the PDD group compared with the control. Vitreous HDL decreased significantly in the SDD group, whereas vitreous LDL increased in the CFD group compared with the CD group. The alterations observed in the study could serve as a hallmark for distinguishing postmortem salt drowning from saltwater drowning. Moreover, the vitreous pattern observed could be used to investigate chloroform-related deaths.

Limitations

Although this research is novel in the drowning ecosystem, limitations should be considered in further development and research in the field.

The study used an animal model with a limited sample size; as such, it has several limitations. Furthermore, when extrapolated to humans, it cannot be definitive unlessreplicated in humans, though empirical studies follow the route administered in this study. Application in humans will validate the use of vitreous chemistry in drowning investigation.

The study also acknowledges the absence of histopathology, toxicology, electrolyte analysis, and diagnostic performance analyses (ROC, sensitivity, specificity), which limits the evaluation of the biomarker's forensic utility. However, these limitations are part of future research to be carried out by the same authors.

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Special appreciation to my 400-level Biochemistry students of 2023 for their assistance in animal breeding, sample collection, and other ancillary activities. In addition, the staff of Eni-yimini Laboratories LTD conducted the laboratory analysis and assisted with the data analysis.

Competing interests

We declare that we have no financial or personal relationships that may have inappropriately influenced us in writing this paper.

Authors' contributions

ESA collected the samples, analyzed the data, and wrote the manuscript. KUA proofread the manuscript and other ancillary supports. CSA conducted a literature review and data collation.

References

1. Moon R, Long R. Drowning and Near-drowning. Emergency Medicine. 2002, 14: 377-386.
2. Ma WJ, Shao NP, Hao FX, Yan JX, Xiu LS, Qiao ZG et al., An analysis of risk factors of non-fatal drowning among children in rural areas of Guangdong Province, China: a case-control study. BMC Public Health 2010. https://doi.org/10.1186/1471-2458-10-156
3. Isin A, Peden AE. Assessing variations in estimates of drowning mortality in Turkey from 2013 to 2019. Arch Public Health. 2022; 80:172. https://doi.org/10.1186/s13690-022-00944
4. Juya M, Ramezani N, Peyravi G. Study of drowning in fresh and salt water. Journal of Injury & Violence Research. 2019 Jul;11(3 Suppl 1). PMCID: PMC7036150
5. Agoro, E.S., Oboma, Y.I. & Diri, G.E. (2025). Forensic Discrimination of Salt Water Drowning Using Vitreous Cardio-Renal Biochemical Parameters and Minerals. Journal of Krishna Institute of Medical Sciences University, 14(1): 1-10. https://doaj.org/article/06f5487099e148f4846b762760062693
6. Sharqawy MH, Lienhard J, Zubair SM. Thermophysical properties of seawater: a review of existing correlations and data. Desalination and Water Treatment. 2010. 16(1–3), 354–380. https://doi.org/10.5004/dwt.2010.1079
7. Agoro ES. Vitreous Humor Biochemical Indicators of Acute CO Intoxication Associated Deaths: A Review of the Literature. Arab Journal of Forensic Sciences and Forensic Medicine, 2020a. 2(1):58-61. https://doi.org/10.26735/HLAX4024
8. Agoro ES, Azuonwu O, & Abbey SD. The Forensic Application of Microbiological Quality of the Vitreous Humor on the Investigation of Acute and Chronic Carbon Monoxide Poisoning. GSL J Forensic Res. 2017a. 1(101):1-4.
9. Agoro ES, Okoye FB, Azuonwu O, Ebiere NE. The Effect of Age and Sex on Vitreous Humour Chemistry and Postmortem Interval (PMI). Indian Journal of Forensic Medicine and Toxicology. 2017 b. 2 (2), 173-177.
10. Ikimi CG, Bekinbo, EC Onuoha C. Diagnostic Potentials of Postmortem Vitreous Biochemical Parameters in the Autopsy of Deaths Associated with Sniper Toxicity using Animal Models.Researchers Journal of Science and Technology 2024a, 4 (7), 27-41
11. IkimiCG, AbosedeUO, Ifenkwe JC. Thanatochemical Utility of Postmortem Vitreous Biochemical Parameters in the Discrimination of Deaths Connected with Bleach Toxicity using Animal ModelsResearchers Journal of Science and Technology 2024b, 4 (7), 10-26. https://doi.org/10.83080/rejost.vol4no7.157
12. Agoro ES, Ikimi CG, Edidiong, T. (2021). The use of vitreous renal chemistries in the discrimination of postmortem freshwater drowning. Toxicology Research and Application, 2021. 5: 1–6.
13. Ships & Ports. 4 soldiers drown in boat mishap Bayelsa State. 2016. https://shipsandports.com.ng/ 4-soldiers-drown-in- boat-mishap-in-bayelsa
14. Armstrong EJ, Erskine KL. Investigation of Drowning Deaths: A Practical Review. Acad Forensic Pathol. 2018 Mar;8(1):8-43. doi: 10.23907/2018.002. Epub 2018 Mar 7. PMID: 31240023; PMCID: PMC6474464.
15. Agoro ES, Thomas CC. Vitreous Lipid and Protein Biomarkers as Indicators of Brackish Water Drowning: A Forensic Biochemistry Study, *Nigerian Journal of Biochemistry and Molecular, Biology*, 2024; 39(4), 220-224. https://doi.org/10.4314/njbmb.v39i4.4
16. Agoro, E.S., Oboma, Y.I. & Diri, G.E. (2025). Forensic Discrimination of Salt Water Drowning Using Vitreous Cardio-Renal Biochemical Parameters and Minerals. Journal of Krishna Institute of Medical Sciences University, 14(1): 1-10. https://doaj.org/article/ 06f5487099e148f4846b762760062693
17. Adias TC, Ajugwo AO, Erhabor T, Nyenke CU. Effect of Pumpkin Extract (Telfairia occidentalis) on Routine Haematological Parameters in Acetone-Induced Oxidative Stress Albino Rats. American Journal of Food Science and Technology . 2013. 23(1): 67-69.
18. Kirkwood J, Hubrecht R. The UFAW handbook on the care and management of laboratory and other research animals. 8th edn. Wiley-Blackwell. 2010. p. 29.
19. Percie du Sert N, Bamsey I, Bate ST, Berdoy M, Clark RA, Cuthill I, Fry D, Karp NA, Macleod M, Moon L, Stanford SC & Lings B. The Experimental Design Assistant. PLoS Biol.2017. doi: 10.1371/journal.pbio.2003779
20. Coe JI. Vitreous potassium as a measure of the postmortem interval: an historical review and critical evaluation. Forensic Science International, 1989. 42: 201–213
21. Michael LB, Edward PF, Larry ES. Clinical chemistry: principles, procedures, correlations. Philadelphia: Lippincott Williams & Wilkins, 1992. p. 234-50.

22. Martins J, Steyn N, Rossouw HM, Pillay TS. Best practice for LDL-cholesterol: when and how to calculate. Journal of Clinical Pathology 2023;76:145-152.

23. Hashash JG, Koutroumpakis F, Anderson AM, Rivers CR, Hosni M, Koutroubakis IE, Ahsan M, Gkiaouraki E, Dunn MA, Schwartz M, Barrie A, Babichenko D, Tang G, Binion DG. Elevated serum globulin fraction as a biomarker of multiyear disease severity in inflammatory bowel disease. Ann Gastroenterol. 2022 Nov-Dec;35(6):609-617. doi: 10.20524/aog.2022.0748. Epub 2022 Oct 3. PMID: 36406970; PMCID: PMC9648529.

24. Ramirez A, Hu PP. Low High-Density Lipoprotein and Risk of Myocardial Infarction. Clin Med Insights Cardiol. 2015 Dec 3;9:113-7. doi: 10.4137/CMC.S26624. PMID: 26692765; PMCID: PMC4670046.

25. Bonilha I, Luchiari B, Nadruz W, Sposito AC. Very low HDL levels: clinical assessment and management. Arch Endocrinol Metab. 2023 Jan 18;67(1):3-18. doi: 10.20945/2359-3997000000585. PMID: 36651718; PMCID: PMC9983789.

26. Chen LB, Lai YC, Chen CC, Chang WH, Su YJ. Myocardial infarction after near drowning. Am J Emerg Med. 2008 Jun;26(5):635.e3-5. doi: 10.1016/j.ajem.2007.10.011. PMID: 18534314.

27. Stanciulescu LA, Scafa-Udriste A, Dorobantu M. Exploring the Association between Low-Density Lipoprotein Subfractions and Major Adverse Cardiovascular Outcomes-A Comprehensive Review. Int J Mol Sci. 2023 Apr 3;24(7):6669. doi: 10.3390/ijms24076669. PMID: 37047642; PMCID: PMC10095470.

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