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Editorial Office:
Department of Forensic Medicine
Faculty of Medicine
University of Peradeniya
Sri Lanka

Websites: **med.pdn.ac.lk**
sljfmsl.sljol.info

E-mail: **sljfmsl@med.pdn.ac.lk**
Tel: **+94 812 388083**

Cover Design & Typesetting: **Ms. Vinodani Dharmasena**

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EDITORIAL

BRIDGING GENERATIONS: EVOLVING FORENSIC EDUCATION IN THE DIGITAL AGE

Chathula Ushari Wickramasinghe

Forensic medicine is the bridge between medicine and law, where medical expertise supports the legal system in the pursuit of justice. Although often viewed as a specialised discipline, all doctors bear medicolegal responsibilities, making forensic medicine an integral component of both undergraduate and postgraduate medical training. It nurtures responsible medical professionals with curiosity, keen attention to detail, and sound analytical skills, enabling them to formulate scientifically valid opinions[1].

Generation Z (Gen Z), born between 1995 and 2012, comprises a large proportion of today's medical undergraduate population. Unlike previous generations, they have grown up with uninterrupted internet connections, fast access to information, and predominantly virtual interactions. This has made Gen Z fluent and dependent on technology, with a higher preference for self-directed, digitally orientated learning. On the other hand, this has also resulted in shorter attention spans, reduced capacity for passive learning, and challenges in interpersonal skills and social interactions[2]. This emphasises the timely need, as medical educators, to reconsider whether current pedagogical methods meet the needs of the evolving learner.

Forensic education for medical undergraduates in Sri Lanka is delivered through bedside teaching, autopsy observations, case discussions, court/crime scene visits, and lectures. While over the years it has been effective, students' exposure may be limited in some instances by time constraints, case availability, health and safety concerns, and legal/ethical restrictions.

Students may witness only a fragment of a medicolegal case, without appreciating the full process, from clinical/autopsy examination to giving expert evidence at court[3]. However, the digital shift in medical education and the introduction of virtual simulation, augmented reality, and blended learning create the opportunity to rethink ways forensic medicine can be taught to engage and inspire.

Technology-enhanced learning offers several possibilities:
Virtual and augmented reality may be used to simulate crime scenes, autopsies, sexual assault examinations, and mass disaster response, allowing safe exposure to situations that are ethically sensitive or logistically restrictive.

Digital repositories and online case libraries can be used to broaden clinical exposure beyond what is achievable during a short forensic medicine rotation while offering a wide range of rare cases, postmortem vignettes, specimen archives, and interactive case narratives.

Blended learning platforms can allow students to follow a medicolegal case from the beginning to the end without being physically present throughout. Students may gain a deeper understanding of the subject when they can witness the full process, from evidence collection, analysis, and interpretation to formulating a sound medicolegal opinion.

Self-paced modules and flipped classrooms will be well-suited for the independent learning style of Gen Z. They can further minimise the time spent on didactic lectures and create

opportunities for in-depth case discussions and skill-based learning[4].

While these opportunities appear enticing and cutting-edge, progress must be strategic, considering the ethical and practical restrictions. Could the increased digitalisation adversely affect an already socially awkward student population? Will it further distance them from the humane aspect of death, trauma, and suffering, which are central to a well-rounded, compassionate medical professional? Creating digital case repositories and online museums may raise concerns about confidentiality, consent, and copyright issues. Resource inequality across institutions may lead to uneven learning experiences, prompting the question of how feasible and fair digital transformation truly is in Sri Lanka.
As we move forward in this digital age, the goal should be not to replace traditional teaching but to enhance and complement it. Evolving forensic education for Gen Z must be undertaken with careful and responsible planning[5]. Technology should uplift and support forensic medicine education by giving students access to a wide array of forensic cases, keeping them interested and eager to learn, while preserving the human touch, empathy, and ethics that are core values in forensic medicine.

With technology infiltrating every aspect of life, its impact on medical education is unavoidable. The challenge before us, then, is not just to teach *with* technology but to teach *through* it, without losing the human story at the heart of every medicolegal case.

CONFLICTS OF INTEREST

The author declared no conflicts of interest.

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Chathula Ushari Wickramasinghe

chathula_wick@yahoo.com
ORCID iD: https://orcid.org/0000-0002-9737-1283

Department of Forensic Medicine,
Faculty of Medicine,
University of Peradeniya,
Peradeniya, Sri Lanka

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ORIGINAL ARTICLE

UNLOCKING FORENSIC INSIGHTS THROUGH DENTAL RECORDS: A SURVEY OF DENTAL RECORD KEEPING AMONG PRACTITIONERS IN TAMIL NADU, INDIA

Franklin R[1]*, Rajalekshmi MP[1], George B[1], Angelin D[1], Mary SJ[1], Parthiban N[2]
[1]*Department of Oral Pathology, Sree Mookambika Institute of Dental Sciences, Kulasekharam, Kanniyakumari District, Tamil Nadu, India*
[2]*Sree Mookambika Institute of Dental Sciences, Kulasekharam, Kanniyakumari District, Tamil Nadu, India*

ABSTRACT

Introduction: Forensic dentistry plays a major role in the identification of individuals who cannot be identified visually by other means. Dental records are crucial in the identification of victims in mass disasters. Many previous studies indicate that people are often unaware of how to maintain dental records. Hence, the present study was intended to explore the forensic relevance of dental records in Tamil Nadu, India.

Methods: A cross-sectional study was conducted among 250 randomly selected general dental practitioners from Tamil Nadu. Data were collected through a structured questionnaire via a Google form. The questionnaire addressed information on dental documentation, data keeping, and forensic awareness. Based on the responses, data was collected, and the percentages were computed for the conclusion.

Results: Out of 225 respondents, the majority were male, aged 28-45 years, with 3-20 years of work experience. While most maintain dental records and use digital radiographs, only a few maintain them digitally and obtain written consent from the patients for their treatment. Prosthetic documentation is limited, with less than half of them recording implant serial numbers and using denture markings. Forensic awareness was low, with only a minimal number having formal forensic training, and many reporting inadequate undergraduate education in the field.

Conclusions: Most practising dentists remain under-digitalised in dental record maintenance, highlighting the need for standardised protocols to enhance patient care, data management, and forensic readiness. These improvements can be achieved through focused education and training, empowering dental professionals to contribute more effectively as forensic experts.

Corresponding Author: Franklin R
franklinbds.smiley@gmail.com
ORCID iD: https://orcid.org/0009-0009-3955-6608

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INTRODUCTION

Forensic odontology is the practice of applying the knowledge of dentistry in criminal investigations and legal proceedings[1]. Forensic odontologists do casework for human identification, age estimation, sex determination, and crime investigation using the principles of forensic science and forensic odontology[2].

Person identification is important for initiating criminal investigations and confirming the identity of deceased individuals. It ensures the proper conduct of legal and social procedures, including burial and the settlement of related matters. Accurate identification is a great relief to the family members and an act of justice to the persons who have been missing for a very long time.

A dental record is a patient's dental health report that is kept by the dentist, containing all subjective and objective information. This file comprises the patient's chief complaint, the history of illness and associated systemic illnesses, clinical examination, dental charts, diagnosis, investigations, treatment plan, the treatment done, and notes on subsequent follow-up(s)[3].

It is legal to maintain dental records in many developed nations, but in developing countries like India, it is not a standard protocol in practice. In addition to this, many dentists in the country are unaware, and many do not maintain it, and most often, the ones that have been maintained are substandard.

Various disasters like earthquakes, tsunamis, air crashes, train accidents, terrorism, homicides, and suicide bombings across the world take away thousands of lives, leaving a large number of unidentified victims.

Forensic odontology plays a vital role in the identification of victims, and the dental records serve as important antemortem records. This method of identification has high accuracy, as teeth are unique and can withstand many types of physical and chemical reactions[4].

In this regard, the need of the hour is to know the present status of dental record maintenance among general dental practitioners (GDPs). Hence, this study was conducted to evaluate the status of the practice of dental record-keeping at GDPs of Tamil Nadu, India.

OBJECTIVES

This study aimed to evaluate the status of dental record-keeping practices among GDPs in Tamil Nadu, emphasising their forensic and medicolegal relevance.

METHODS

A cross-sectional survey was done among dentists practising in the private sector in Tamil Nadu to gauge their awareness, knowledge, and attitudes on the state of dental records and their maintenance patterns. This study was conducted over a period of 6 months from November 2024 to April 2025. A list of registered dentists from the Indian Dental Association's Tamil Nadu chapter was acquired to randomly select the study sample. In each district, eight to ten dentists were chosen by random sampling. For the study, a total of 250 dentists were included. GDPs with either a Bachelor of Dental Surgery (BDS) or Master of Dental Surgery (MDS) degree with a valid Tamil Nadu Dental Council Registration and a minimum of 3 years of dental practice in their own setup were included in the study.

A web-based electronic survey was done using Google Forms, distributed through WhatsApp and email. Ethical clearance was obtained from the Institutional Human Ethics Committee of Sree Mookambika Institute of Dental Sciences, Kulasekharam, Kanniyakumari District, Tamil Nadu, India (SMIDS/IHEC No. 05, Protocol No. 40/2024).

The questionnaire was a 40-item structured questionnaire consisting of 3 sections, in accordance with the AAPD (American Academy of Paediatric Dentistry) guidelines[5,1,4,6]. To validate the questionnaire, a pilot study was done on 10% of the study sample (25 dentists). Section A consisted of questions on demographic details of GDPs, and Section B on dental documentation and data keeping, including the method of obtaining dental data from patients. Section C consisted of questions on the forensic awareness of the practising dentist. It also tested the knowledge and attitudes towards dental records in forensic odontology.

The collected data were entered into MS Excel. Descriptive statistical analysis was used to summarise the findings in the study. Each response's percentage was computed, and conclusions were derived from the data. The results were then tabulated and interpreted to draw relevant conclusions.

RESULTS

A response rate of over 90% was observed, with a total of 225 dentists participating in the study.

General information about study participants

The majority of respondents (65.5%) were males in the 28–45-year age group, with 3–20 years of work experience.

Private dental colleges accounted for 72.4% of all practising dentists, followed by deemed universities (17.2%) and government dental colleges (10.3%). While 62.1% of dentists possessed a BDS degree, the remaining 37.9% had pursued an MDS.

Information on general documentation

Among GDPs, 96.6% start a dental file for all new patients, whereas 3.4% of them do not maintain any dental records. Out of all the dental practitioners who maintain dental records, 65.5% of them record them manually in preprinted forms, with the remaining dentists recording them manually on blank paper and digitally using computer software, in decreasing frequency (Fig. 1).

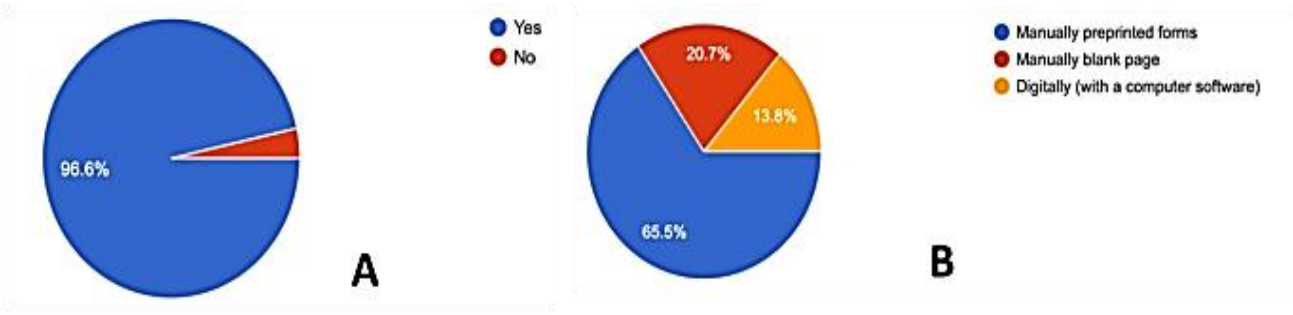

Figure 1: Responses to the question "Do you start a dental file for all new patients?" (A) and "How do you record the case history?" (B)

Among GDPs who maintain dental records, 96.6% collect demographic information, such as name and address, during every new patient registration. Among these, 93.1% of the GDPs collect contact information like phone numbers and email addresses. Medical history is collected by 75.9% of GDPs through a structured process involving patient interviews, a review of existing medical records, and the use of questionnaires. Additionally, 89.7% of GDPs document a "medical alert" in the patient's record, while 10.3% do not. Regarding extraoral examination, 72.4% of GDPs record any asymmetric features observed on the face.

Information on oral health status and treatment records:

Most dentists maintain thorough clinical records. They typically document whether teeth are present or missing and commonly use the Fédération Dentaire Internationale (FDI) system for identifying teeth. Records of restorative procedures, such as fillings, are usually included. Many dentists also note each patient's periodontal condition, as well as their molar relationship and malocclusion status (Fig. 2).

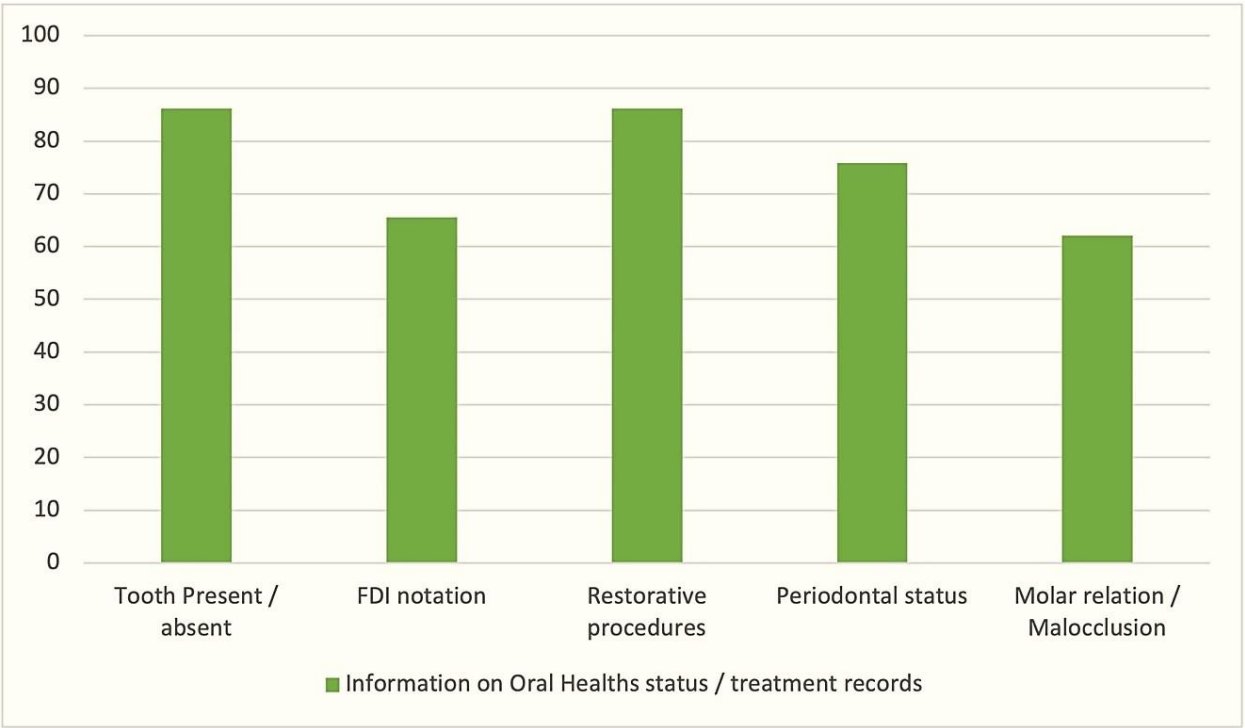

Figure 2: Information on oral health status and treatment records

Dental prosthetic record

Dentists document all details of prosthetic work, such as crowns, veneers, Fixed Partial Dentures (FPD), Removable Partial Dentures (RPD), Complete Dentures (CD), and implants, in 89.7% of cases, whereas 10.3% do not mention this information. Before treatment, 72.4% of dentists make study casts or take photographs, while 27.6% do not. Only 51.7% of dentists mention the serial number of the implant used, and 20.7% of dentists use personalised denture markings in their prosthetic work (Table 1).

Table 1: Dental prosthetic record

Question	Option	Percentage
Do you write all the details of the prosthetic work (crowns, veneers, FPD, RPD, CD, implants) done in the oral cavity?	Yes	89.7
	No	10.3
Do you take study casts/photographs before treatment?	Yes	72.4
	No	27.6
Do you mention the serial number of implants used?	Yes	51.7
	No	48.3
Do you mention the personalised denture markings used in your prosthetic work?	Yes	20.7
	No	79.3

Radiographic record

For routine dental examinations, 86.2% of dentists use digital Radiovisiographs (RVG), while 13.8% rely on conventional X-rays. Only 17.2% of dentists do Orthopantomograms (OPG) for all patients, and only 13.8% of dentists think OPGs are needed for all patients seeking treatment.

The findings of the radiographs are mentioned in the case history form by 79.3% of all dentists. Regarding record keeping of patients' radiographs, 69.5% of dentists retain the radiograph as a soft copy, 13.8% retain the radiograph as a hard copy, and 17.2% hand over the radiographs to the patients (Fig. 3).

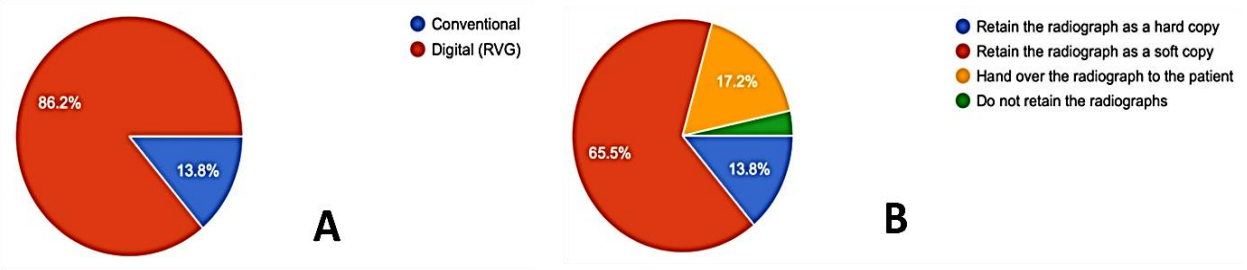

Figure 3: Responses to the question "What type of radiograph do you take?" (A) and "What do you do with the patients' radiographs after the treatment?" (B)

Consent and preservation of dental records

The proposed treatment plan is mentioned in the dental record by 96% of dentists. Only 69% get the signature for the financial agreement. Written consent for dental procedures is obtained by 69% of dentists, and 31% don't get written consent. The prescribed medication is recorded in the patient file by 75.9% of dentists, while 82.8% document detailed information about the treatment performed in the patient record.

Regarding dental record storage, 72.4% of dentists use paper files, 13.18% use electronic health records, and 13.8% use secure online storage. The patient records are stored for >5 years by 51.7% of dentists, 3 to 5 years by 27.6%, for 1-3 years by 13.8%, and for 1 year by 6.9%. Only 79.3% of dentists update patient details on subsequent dental visits. Only 6.9% of dentists share patient details with external parties like insurance companies or researchers (Fig. 4).

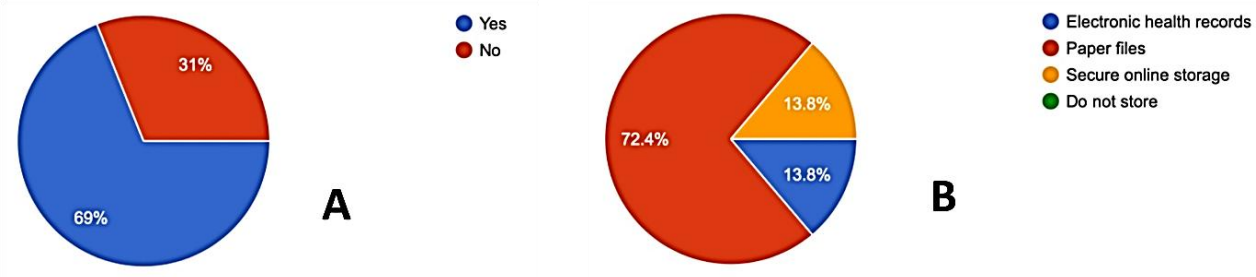

Figure 4: Responses to the question (A)-"Is written consent, signed by the patient, a standard part of your practice protocol?" and (B)-"Where are dental records typically stored?"

Forensic awareness

The last section was the questions on the forensic awareness of the practising dentist, testing their knowledge and attitude towards dental records in forensic odontology. The responses are shown in Table 2.

Table 2: Percentage distribution of various components in forensic awareness

Question	Option	Percentage
Do you think a tooth/teeth can be used to estimate the age of an individual?	Yes	93.1
	No	6.9
Can DNA be extracted from the teeth?	Yes	79.3
	No	20.7
Do you regularly note and record dental anomalies?	Yes	86.2
	No	13.8
Are you aware of denture markings, such as electronic bar codes or QR codes, being incorporated in RPD/CD?	Yes	51.7
	No	48.3
Your knowledge level/awareness about forensic dentistry is	Adequate	27.6
	Inadequate	13.8
	Average	58.6
Do you think there is adequate teaching about forensic odontology at the undergraduate level?	Yes	37.9
	No	62.1
Have you undergone any formal training in the field of forensic dentistry?	Yes	10.3
	No	89.7
Have you handled any forensic dentistry-related cases before?	Yes	10.3
	No	89.7
Are you aware that you can be an expert witness in court to present forensic dental evidence?	Yes	62.1
	No	37.9
Are you aware of the "Interpol antemortem" form of dental records?	Yes	69.0
	No	31.0

DISCUSSION

Dental records serve as an antemortem tool in forensic identification. Being important, several dentists and legal professionals are still unaware of such dental records, which may help in the identification of unknown persons[3].

In our study, 96.6% of GDPs maintained a dental record for every new patient, which aligns with the study by Wadhwani S, with 87% of GDPs maintaining dental records and only 31% of them filling in all the details in the dental record[7].

Even though the rate of maintenance is high, the method of documentation varies significantly. The majority of the dentists (65.5%) used manually preprinted forms, followed by blank papers and digital aids. This finding is consistent with the study by Sarode et al., where the majority of the GDPs use manually preprinted forms, followed by blank paper and digital software for record keeping.[3] This high dependence on manual methods raises concern about long-term storage of data, their retrieval,

and accessibility. This issue can be better addressed with the implementation of Electronic Health Records (EHR).

Oral health status and treatment documentation showed a mixed trend. 86.2% recorded the teeth present, followed by 62.1% recording the molar relation or malocclusion. Periodontal status was noted by only 75.9% of respondents. This trend suggests a gap in detailed oral health evaluation, which may be due to a lack of time in busy practice, focusing only on patient needs.

In our study, prosthetic treatment records were maintained by 89.7% of GDPs, whereas only 72.4% take study casts or photographs before treatment. Only 20.7% of GDPs used personalised denture markings, and just 51.7% documented implant series. These findings are in line with the study by Asteka et al., where most of the GDPs maintain a record of their prosthetic work and document implant serial number[6]. It is concerning that these prosthetic records and photographs taken during treatment may be crucial for future reference, legal documentation, and personal identification.

In radiographic records, 86.2% of dentists use digital radiographs, which is a promising trend facilitating better image quality, storage, and sharing. However, only 17.2% refer patients for OPG routinely, and just 13.8% believe OPGs are necessary for all patients. This reflects the selective use of OPG imaging, which may miss out on many hidden diagnoses. While 69.5% of dentists prefer to store radiographs as soft copies, 79.3% record the findings in the case history, which is a good indicator of integrated diagnostics. These findings are in common with Kaur et al., where 70% of GDPs use digital radiographs, and they (52.5%) retain them as soft copies, mentioning all the radiographic findings (81.9%) in the case sheet[8].

Informed written consent from a patient indicates that the patient has been provided with clear and understandable information about the dental procedures, allowing the patient to make a voluntary decision. Although 96% record a treatment plan, only 69% obtain a financial agreement signature and formal written consent. In light of this significant legal and ethical gap, it highlights the need to raise awareness about obtaining proper informed consent to protect both patients and practitioners.

Record storage practices among GDPs remain highly manual, with 72.4% using paper files and only 13.8% using a secure online system. This indicates a minimal transition towards the modern digital world. The use of electronic health records is an advancement of information and communication technology worldwide. It is widely used among dental professionals in developed countries, but in developing countries like India, the awareness and its use are minimal[9]. According to a study from Australia, electronic health records were observed to provide more complete information than manual dental records[10]. In developing countries, there are many challenges to implementing these electronic dental records, as most dental clinics are small, and they don't want to invest a lot of money in their setup. They may also face ethical issues such as privacy breaches when proper security measures are not applied[11].

According to the Revised Dentists (Code of Ethics) Regulation – 2014, a "dental record must be maintained for a minimum period of three years by the dentist from the date of onset of the treatment"[12]. This retention of dental records varied in our study, with just half (51.7%) of the GDPs maintaining them for 3–5 years.

The one important factor to consider lies in the forensic awareness. 93.1% of the respondents were aware that teeth can be used to estimate age, and 79.3% acknowledged the possibility of DNA extraction from the tooth. This high level of awareness appears to be theoretical. Despite the known importance of dental anomalies in personal identification, not all respondents regularly document dental anomalies in their dental records. This detailed documentation of dental anomalies is essential for antemortem and postmortem comparison. Any setback may compromise forensic identification in the case of unidentified remains.

The knowledge of forensic odontology seems to be average in 58.6% of the respondents, with the majority of the GDPs lacking confidence in forensic applications. This low self-confidence

may lead to under-detecting potential forensic implications or an unwillingness to participate in legal investigations when called upon. A striking 62.1% of GDPs reported in the survey that forensic odontology was inadequately taught during the BDS degree course, and only a few (10.3%) had received any formal training. This indicates a massive failure in the undergraduate teaching curriculum. This branch of dentistry is often treated as an elective topic in dental education, although there lies an importance in victim identification, medicolegal cases, mass disasters, etc. This lack of teaching may limit general dental practitioners in fulfilling their role as forensic experts.

The findings of our study were supported by a study conducted by Ishee et al., which demonstrated a lack of knowledge, awareness, and practice of forensic dentistry amongst dentists of Punjab. This can only be improved by taking necessary steps to incorporate forensic odontology training into the undergraduate curriculum and creating more exposure from a varied practical point of view[13].

Dental hard tissues are a unique tool in the forensic process whenever visual identification is not practical. While 96.6% of GDPs maintain dental records, most of them depend on manual documentation with limited use of either digital tools or EHRs. There is a reasonable awareness of forensic concepts among GDPs, but practical training and confidence in forensic odontology remain low. The gap must be improved with digital adoption, standardised record keeping among GDPs, and integration of forensic training into the dental curriculum[14].

LIMITATIONS

As the data were self-reported via an online survey, responses may be subject to social desirability bias. The study is limited to Tamil Nadu and may not reflect practices across India.

CONCLUSIONS

Dental records play a vital role in forensic identification and legal proceedings. Although it is important to maintain dental records, there exists a considerable variability in consistency, accuracy, and duration of retention of dental records. Having a good record-keeping practice is essential for quality patient care and may aid in forensic cases of unidentified bodies, mass disasters, or medico-legal issues. To bridge this gap, there is a need for continuing dental education programmes, forensic workshops, implementation of record-keeping systems etc. By following this, GDPs can contribute to public health and law enforcement by unlocking the power of dental records in forensic science.

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None

CONFLICTS OF INTEREST

The authors declared no conflicts of interest.

ETHICAL ISSUES

Ethical approval has been obtained from the Institutional Human Ethics Committee of Sree Mookambika Institute of Dental Sciences, Kulasekharam, Kanniyakumari District, Tamil Nadu, India (SMIDS/IHEC No. 05, Protocol No. 40/2024).

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None

AUTHOR CONTRIBUTIONS

RF: Conception and design of the work; revising the draft critically for important intellectual content; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. **MPR:** Conception and design of the work; revising the draft critically for important intellectual content; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. **BG:** Analysis and interpretation of data for the work; drafting the work; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are

appropriately investigated and resolved. **DA:** Analysis and interpretation of data for the work; drafting the work; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. **SJM:** Analysis and interpretation of data for the work; drafting the work; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. **NP:** Conception and design of the work; drafting the work; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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CASE REPORT

A SINGLE-CENTRE EXPERIENCE IN THE APPLICATION OF Y-STR ANALYSIS IN CHALLENGING FORENSIC CASEWORK: A CASE SERIES

Rathnayake RWRK*, Marasinghe E, Perera LRT, De Silva PG
Government Analyst's Department, Pelawatta, Sri Lanka

ABSTRACT

Short Tandem Repeats (STRs) are the gold standard for human identification. They are widely used in crime scene investigations, kinship testing, and disaster victim identification due to their high polymorphism, sensitivity, and discrimination power. Combining autosomal STRs and sex-specific STRs (Y-STRs and X-STRs) across multiple tissues yields highly informative genetic profiles. Y-STRs, which target Y-chromosome DNA inherited through the paternal line, are particularly useful when autosomal STR profiling is inconclusive. They enhance the reliability of the results in establishing patrilineal relationships of male offspring, paternal kinship testing, and missing persons and disaster victim identification, as well as in sexual assault cases when male DNA is masked by female DNA. In complex mixtures involving male victims or multiple male contributors, autosomal DNA profiling is challenging. Even with advanced probabilistic genotyping software, separating these mixtures can still be limited due to issues like template imbalance, low quantity DNA, or degraded DNA. In such cases, Y-STR analysis serves as a valuable complementary tool. However, Y-STRs cannot distinguish between closely related males within the same paternal lineage, limiting their individualising power. Their evidentiary value also depends on robust population-specific databases for accurate statistical interpretation. This review outlines the practical uses and limitations of Y-STR analysis, emphasising the importance of population databases such as the Y Chromosome Haplotype Reference Database in evaluating evidentiary weight.

Based on these considerations, this study highlights the effectiveness of the PowerPlex® Y23 system, as a valuable complementary tool to autosomal STRs, particularly in challenging cases where autosomal results alone are insufficient.

Keywords: *Autosomal STR; PowerPlex® Y23 System; sex-specific STR; short tandem repeats*

Corresponding Author: Rathnayake RWRK
kalharigad@hotmail.com
https://orcid.org/0009-0000-9639-3889

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INTRODUCTION

DNA fragment analysis is an essential tool in forensic investigations and plays a critical role in supporting the judicial system, contributing to both civil and criminal proceedings. Various technological approaches to DNA fragment analysis are employed for different forensic applications. Short Tandem Repeats (STRs) are considered the gold standard in human identification due to their high level of polymorphism, sensitivity, speed, and strong discriminatory capability. STR markers are repeating sequences of nucleotides located within the non-coding regions of the human

genome. STR analysis has proven to be extremely valuable in various applications, such as parentage testing, kinship determination, locating missing persons, and identifying disaster victims. It also plays a crucial role in forensic investigations, such as analysing DNA from biological evidence found at crime scenes, on related items, or on victims' bodies[1].

The combination of autosomal STRs with sex-specific STR genetic markers, across various evidentiary items and bodily fluids, provides highly informative genetic information. Autosomes are inherited from both parents by all offspring through the process of recombination. Daughters inherit one X chromosome from each parent, allowing recombination between them, whereas sons inherit their single X chromosome from the mother and a Y chromosome from the father, so no X recombination occurs in sons[2]. Y-STR in the male-specific region of the human Y chromosome is commonly used in forensic DNA analysis, especially in cases where conventional autosomal DNA profiling is not informative. These Y-STR haplotypes are inherited as a unit from father to son, and this distinct pattern of inheritance makes them useful for tracing paternal lineages across generations and for male-specific DNA analysis[3].

The Y-STR analysis is used in resolving paternity disputes involving male offspring, as well as in other paternal kinship testing and in the identification of missing persons and disaster victims. Y-STR haplotyping, when used in crime scene investigations, can eliminate male suspects, establish the paternal lineage of male offenders, reveal multiple male contributors within a DNA sample, and provide valuable leads for identifying unknown males involved in a crime[4].

In conventional STR testing in sexual assault cases, the presence of excess female DNA can mask male DNA, potentially leading to incomplete or undetectable male STR profiles. Y-STR genotyping effectively isolates the male component in such complex female–male DNA mixtures. Additionally, in mixtures involving multiple unrelated male contributors, such as in gang-related cases, Y-STR analysis can separate the male lineages, as each contributor has a unique Y-STR pattern. This is useful when autosomal STRs may overlap, and individual contributor profiles are difficult to resolve. However, since Y-STRs do not undergo recombination and are passed from father to son, they cannot distinguish between paternally related males, such as brothers or father and son, who will share identical Y-STR profiles[5,6,7].

This article presents three cases that had analytical challenges due to complex DNA mixtures and the lack of one biological parent for comparison, highlighting the importance of including additional autosomal and sex-determining markers to enhance the reliability of the results.

CASE HISTORIES

Case 1

A case involving the sexual assault of a mentally disabled girl was reported, with the identity of the perpetrator(s) unknown. Two garments worn by the victim at the time of the incident, along with blood samples from the victim and a suspect, were submitted to the Government Analyst's Department's DNA laboratory through a court order.

Case 2

A case of sexual assault of a female by an unidentified individual was reported. A vaginal swab and blood samples from the victim and the suspect were submitted through a court order.

Case 3

A severely decomposed body was found hanging under suspicious circumstances in a jungle area near the deceased's residence. An individual had identified the body as his son's based on personal belongings and accessories found with the remains. No other close relatives were available to confirm the relationship. A piece of the humerus bone from the deceased and a blood sample from the alleged father were submitted through a court order for identification.

DNA extraction and analysis were carried out on all submitted samples following standard guidelines and operational procedures (Fig. 1).

For the interpretation of the obtained DNA profiles, the laboratory followed established guidelines to assess whether the DNA typing results were suitable for comparison.

Y-STR analysis gives lineage-specific information, and its interpretation is based on haplotype frequency estimates derived from population databases such as the Y Chromosome Haplotype Reference Database (YHRD). However, YHRD statistics are not presently incorporated into the reports.

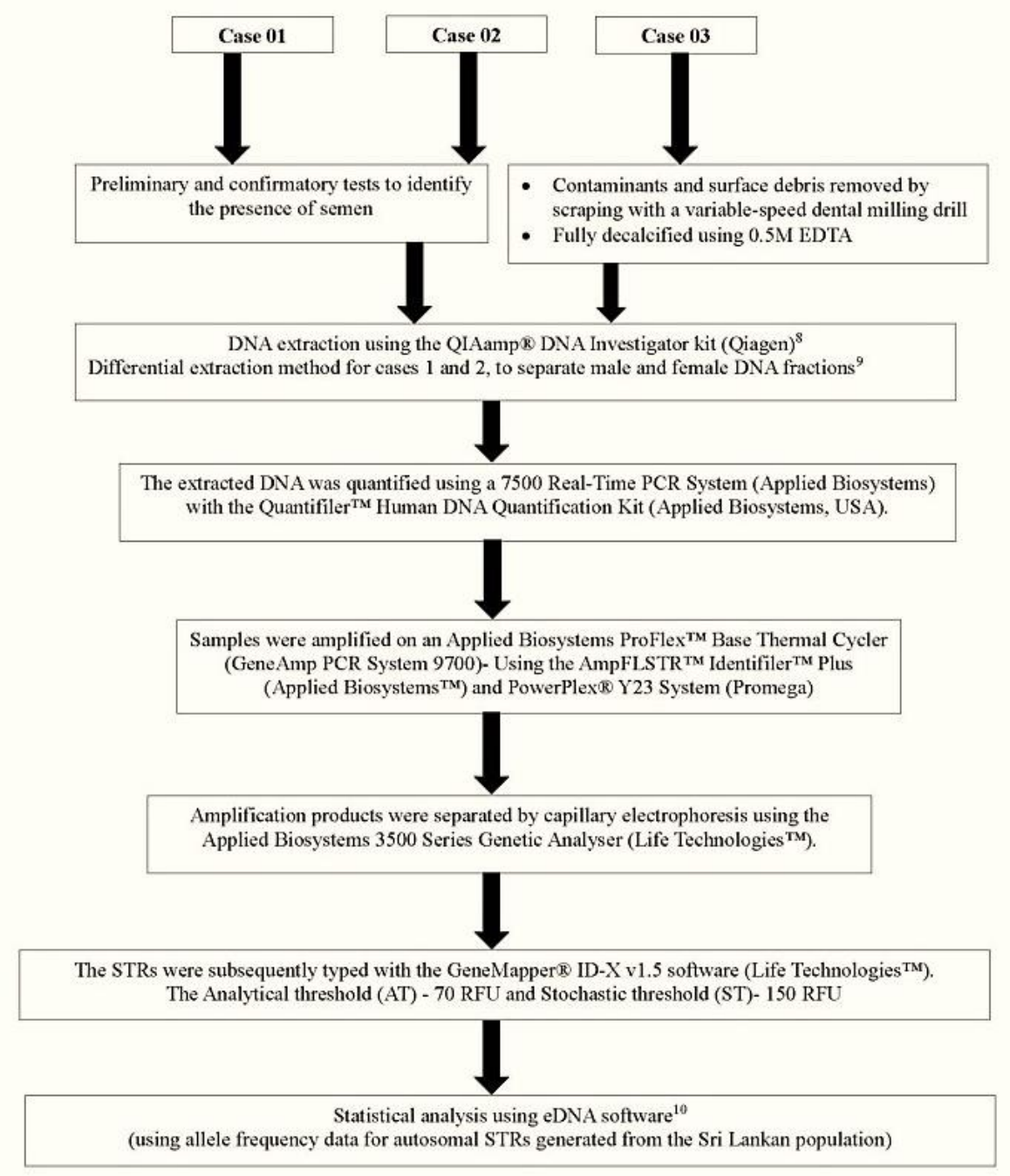

Figure 1: Flow chart showing the process of DNA analysis in the submitted samples

RESULTS INTERPRETATION

Preliminary and confirmatory tests were performed on samples from cases 1 and 2 before DNA extraction, to identify the presence of semen, which were positive in only garment-2 and vaginal swabs. The following DNA profiles were generated for autosomal STR amplification on garment-2 and the two blood samples from case 1 (Table 1). In the male fraction of garment-2, the bolded alleles matched the suspect's bolded alleles at each locus.

Table 1: Autosomal DNA profiles for garment-2 and blood samples of the victim and suspect, generated using Identifiler™ Plus

STR Locus	Garment-2 (Male fraction)	Garment-2 (Female fraction)	Blood sample (Victim)	Blood sample (Suspect)
D8S1179	**10,**11,**16**	10,11	10,11	**10,16**
D21S11	29,30,**32.2,33.2**	29,30	29,30	**32.2,33.2**
D7S820	11,**12**	11,12	11,12	**12,12**
CSF1PO	**11,12**	11,11	11,11	**11,12**
D3S1358	**15,16**,18	15,18	15,18	**15,16**
TH01	**9**	9,9	9,9	**9,9**
D13S317	8,**11**,14	8,14	8,14	**11,11**
D16S539	**10**,11,12,**14**	11,12	11,12	**10,14**
D2S1338	**18**,23,24,**26**	23,24	23,24	**18,26**
D19S433	**13,13.2,**14	13,14	13,14	**13,13.2**
vWA	**14,**17,**19**	17,19	17,19	**14,19**
TPOX	8,**9,11**	8,8	8,8	**9,11**
D18S51	**14,16**,19	14,19	14,19	**14,16**
D5S818	**10,12,**13	13,13	13,13	**10,12**
FGA	**22,23**,24	24,24	24,24	**22,23**
Amell	XY	X	X	XY

Interpretation: *STR profiles of the male and female fractions of Garment-2 were compared with blood samples from the victim and suspect. The male fraction showed alleles from both individuals, indicating a DNA mixture, while the female fraction matched the victim.*

Given the peak imbalance in the initial mixture, Y-STR analysis was also performed to provide additional support (Table 2).

Table 2: Y-STR profiles for garment-2 and suspect's blood sample, generated using PowerPlex® Y23

STR Locus	Garment-2	Blood sample (Suspect)
DYS576	18	18
DYS389 I	12	12
DYS448	20	20
DYS389 II	28	28
DYS19	16	16
DYS391	10	10
DYS481	26	26
DYS549	12	12
DYS533	10	10
DYS438	11	11
DYS437	16	16
DYS570	20	20
DYS635	22	22
DYS390	26	26
DYS439	13	13
DYS392	13	13
DYS643	11	11
DYS393	12	12
DYS458	18	18
DYS385	14,19	14,19
DYS456	15	15
YGATAH4	11	11

Interpretation: *The Y-STR profile from Garment-2 and the suspect's blood were identical, indicating the male DNA on the garment is consistent with originating from the suspect or a paternal relative.*

In Case study 2, the vaginal swab collected from the victim produced a mixed DNA profile in the autosomal STR analysis. Table 3 summarises the DNA profiles obtained from the vaginal swab and the blood samples from both the victim and the suspect, using autosomal STR amplification. In the female fraction of the vaginal swab, the bolded alleles matched the victim's bolded alleles at each locus.

Table 3: Autosomal DNA profiles for vaginal swab and blood samples of the victim and suspect, generated using Identifiler™ Plus

STR Locus	Vaginal swab (Female fraction)	Blood sample (Victim)	Blood sample (Suspect)
D8S1179	10,**11,12**,14	**11,12**	11,15
D21S11	29,30,**31.2,32.2**	**31.2,32.2**	30,33.2
D7S820	**8,10**,11	**8,10**	10,11
CSF1PO	**12**	**12,12**	11,11
D3S1358	15,**16,17**	**16,17**	17,17
TH01	**7,9.3**,10	**7,9.3**	6,9.3
D13S317	**8,**10,**11**,12	**8,11**	9,11
D16S539	**9**,12,**13**	**9,13**	11,13
D2S1338	18,**22,24**	**22,24**	18,19
D19S433	12,13,**14,16**	**14,16**	14,15.2
vWA	14,16,**17,18**	**17,18**	18,19
TPOX	8,**11**	**11,11**	9,11
D18S51	**14,15**	**14,15**	15,17
D5S818	10,**11,13**	**11,13**	13,13
FGA	**20,22**,23,25	**20,22**	19,24
Amell	XY	X	XY

Interpretation: *The vaginal swab profile shows a mixture of alleles from at least two contributors. The victim's alleles are present, and other alleles not corresponding to the suspect suggest the presence of DNA from another individual.*

Table 4 presents the Y-STR DNA profile obtained from the vaginal swab and the suspect's blood sample.

Table 4: Y-STR profiles details for vaginal swab and suspect's blood sample, generated using PowerPlex® Y23

STR Locus	Vaginal swab	Blood sample (Suspect)
DYS576	18	17
DYS389 I	12	12
DYS448	19	19
DYS389 II	29	29
DYS19	14	15
DYS391	10	10
DYS481	23	26
DYS549	13	12
DYS533	13	12
DYS438	10	11
DYS437	15	14
DYS570	16	18
DYS635	24	22
DYS390	22	23
DYS439	12	12
DYS392	14	14
DYS643	10	11
DYS393	11	14
DYS458	15	16
DYS385	12,16	13,16
DYS456	15	15
YGATAH4	12	12

Interpretation: *The Y-STR profile obtained from the vaginal swab does not fully match the suspect's profile. Several loci show allele differences, indicating that the male DNA in the vaginal swab is not consistent with originating from the suspect or his paternal lineage.*

In Case study 3, autosomal STR DNA profiles were successfully obtained from both the bone and blood sample of the individual presented as the deceased's father. Table 5 displays the autosomal STR DNA profiles obtained from both samples. The bolded alleles show that the alleged father shares one matching allele with the bone sample at each locus.

Table 5: Autosomal DNA profiles for bone sample and blood sample of alleged father, generated using Identifiler™ Plus

STR Locus	Bone sample	Blood sample (Alleged father)
D8S1179	**9**,14	**9**,15
D21S11	**30**,32.2	29,**30**
D7S820	**11**,12	**11**,11
CSF1PO	**10,11**	**10,11**
D3S1358	16,**17**	**17**,17
TH01	7,**9.3**	6,**9.3**
D13S317	**8**,8	**8**,8
D16S539	**10**,11	**10,**12
D2S1338	**17**,18	**17**,26
D19S433	**13**,13	**13**,14
vWA	17,**18**	16,**18**
TPOX	**8**,11	**8**,10
D18S51	**11**,17	**11**,14
D5S818	**10,12**	**10,12**
FGA	**21**,23	**21**,27
Amel	XY	XY

Interpretation: *The bone sample shows a single-source autosomal STR profile, with one allele at each locus matching the alleged father's blood sample, consistent with a possible paternal relationship.*

According to our DNA laboratory's internal quality assurance procedures, Y-STR testing or Argus X-12 and PowerPlex Fusion analysis are employed when one parent is unavailable. In this case, PowerPlex Fusion analysis did not provide interpretable results. To address this limitation, Y-STR analysis was carried out to provide additional confirmation of the paternal lineage. The resulting Y-STR profile details are illustrated in Table 6.

Table 6: Y-STR profiles for the bone sample and the blood sample from the alleged father, generated using PowerPlex® Y23

STR Locus	Bone sample	Blood sample (Alleged father)
DYS576	17	17
DYS389 I	12	12
DYS448	18	18
DYS389 II	28	28
DYS19	15	15
DYS391	10	10
DYS481	21	21
DYS549	13	13
DYS533	12	12
DYS438	10	10
DYS437	16	16
DYS570	15	15
DYS635	23	23
DYS390	24	24
DYS439	10	10
DYS392	11	11
DYS643	8	8
DYS393	13	13
DYS458	16	16
DYS385	16,17	16,17
DYS456	16	16
YGATAH4	11	11

Interpretation: *The Y-STR profile from the bone sample matches the alleged father's blood sample across all tested loci. This indicates that the male DNA in the bone sample is consistent with the alleged father or a paternal relative.*

DISCUSSION

Y-STR analysis has become an essential tool in forensic science, with the development of high-resolution multiplex systems like Power Plex Y-23, which have enhanced discriminatory power. Although Y-STRs cannot replace autosomal STRs for individual identification, their uniparental inheritance and male specificity make them highly valuable in forensic investigations. They are particularly useful for detecting male contributors in complex DNA mixtures, including female-dominated samples or cases with allele dropout or peak imbalance. Additionally, Y-STRs are important for paternity testing without a

maternal reference and in complex kinship analysis where autosomal data alone may be insufficient.

According to international guidelines, when a mixture can be partially or fully deconvoluted, single-source statistical methods should be applied to the identifiable contributor(s). In contrast, when the mixture cannot be deconvoluted, and the contributors cannot be distinguished, a single statistical calculation should be performed for the entire mixed profile [11]. However, in complex mixtures involving multiple contributors with a high degree of allele overlapping, peak imbalance, low template, or degraded DNA, producing reliable statistics can be challenging. In such scenarios, probabilistic genotyping tools can assist in interpretation, but the results should be considered cautiously[12]. In our case, probabilistic genotyping tools are not presently incorporated. Instead, mixture deconvolution was performed using GeneMapper ID-X Software v1.5, the standard software supplied with the genetic analyser.

Differential extraction performed on garment-2 resulted in two DNA profiles; the female fraction matched the victim's profile, while the male fraction gave a mixed DNA profile for autosomal STR analysis. The DNA results for the male fraction from garment-2 showed the presence of multiple contributors, with at least two individuals involved. The mixture showed a significant peak imbalance between major and minor contributors. However, the analysis using GeneMapper ID-X Software v1.5, the standard software provided with the genetic analyser, revealed that the major component matched the suspect's DNA profile, while the minor component corresponded to the victim's profile.
The Y-STR analysis of garment-2 showed a single male DNA profile, as Y-STRs only detect male DNA and not female DNA. These findings suggest that the specific area of garment-2 contained DNA from both male and female contributors. While the presence of male DNA is initially indicated by amelogenin typing during autosomal STR analysis, Y-STR profiling added additional discriminatory power by generating a male-specific haplotype. This allowed for a direct comparison between the male component of the mixture with the suspect's reference profile, especially when the male DNA was present in very small amounts or mixed together with female DNA. It provides lineage-specific information that is critical in mixed or low-template samples where autosomal data may be insufficient for individualisation.

Differential extraction performed on the vaginal swab yielded no DNA profile in the male fraction, while the female fraction produced a mixed DNA profile for autosomal STR analysis. Similar to Case study 1, the DNA results indicated a mixed profile, involving at least two contributors with significant peak imbalances. Mixture deconvolution was performed using GeneMapper ID-X Software v1.5, and the major component matched the victim's profile, while the minor component was not consistent with the suspect's DNA profile. Although some autosomal STR loci like D8S1179, CSF1PO, and TH01 showed alleles inconsistent with the suspect's genotype, the overall autosomal profile represented a mixture containing alleles that could originate from multiple contributors. Because of the mixture's complexity and the possibility of allele dropout or peak imbalance, a definitive exclusion could not be made using autosomal data alone. Therefore, Y-STR analysis was performed to obtain a male-specific profile, enabling a more definitive comparison to the suspect. The Y-STR profile provided additional evidence for exclusion, showing mismatches at multiple Y-STR loci compared to the suspect's haplotype.

The Y-STR results from the vaginal swab showed a single male contributor, whereas the autosomal STR profile was a mixture, indicating DNA from both male and female contributors. Furthermore, the Y-STR profile from the vaginal swab did not match the Y-STR profile of the suspect. The Y-STR results obtained using the PowerPlex® Y23 System further confirmed the absence of the suspect's male DNA in the vaginal swab, thereby supporting the exclusion of the suspect from DNA analysis.

For case 3, autosomal STR DNA profiles were successfully obtained from both the bone sample and the blood sample of the deceased's father. A trio test, which includes DNA analysis of the father, mother, and child, was the ideal approach for paternity testing. However, the mother of the

deceased was not available for testing, and the only close relative present was his father. The autosomal STR analysis showed that half of the alleles at each STR locus in the deceased matched those of the alleged father. A statistical analysis to evaluate the probability of a paternal relationship was conducted using the eDNA 2.3 software package. The analysis produced a Combined Relationship Index (CRI) of 99.999%, indicating that the alleged father has a 99.999% likelihood of being the biological father of the deceased.

However, in rare cases, by coincidence, an unrelated random person could also share one allele at each locus with the child. In this case, the analysis was performed without the mother's sample; the possibility of a coincidental match cannot be entirely ruled out.

The Y-chromosome haplotypes of the deceased's bone sample and the blood sample of the father matched across all tested Y-STR loci, strongly suggesting that they share a common paternal lineage. Therefore, the autosomal STR results have a high statistical probability, providing strong evidence that the tested male is the biological father of the missing individual. In addition, the Y-STR analysis offered complementary confirmation of the paternal lineage.

However, the routine use of Y-STRs in casework remains limited due to their inability to identify a person rather than the paternal lineage and provide the same degree of confidence in identifying a specific individual as autosomal DNA. These limitations include the inability to distinguish between close paternal relatives, lower discriminatory power compared to autosomal STRs, and difficulties in interpreting mixed male profiles when contributors share the same Y-haplotype.

Although Y-STRs have been well established in the international literature, published data documenting their forensic applications are limited in this region. This study adds contextual novelty by demonstrating how internationally recognised forensic tools can be effectively applied in local casework, particularly in challenging scenarios such as complex male DNA mixtures and kinship testing in the absence of maternal references.

The absence of population frequency data for the observed Y-haplotypes may limit the ability to assign strong statistical weight to the findings. Nevertheless, for future consideration, the inclusion of YHRD statistics for male-specific markers should be accompanied by proper understanding and awareness within the judiciary system. This would help clarify how the combination of Y-STR haplotype with autosomal STRs can provide stronger and more comprehensive interpretation in forensic investigations.

Y-STR analysis provides information specific to paternal lineage, with interpretation relying on haplotype frequency estimates obtained from population databases like YHRD. When applying YHRD, the level of discrimination is not as strong as that provided by autosomal STR analysis[13]. Legal professionals may sometimes interpret these results incorrectly due to limited familiarity with the scientific differences. Therefore, YHRD statistics are not presently incorporated into the reports. In the future, the application of Y-STR statistics will be considered following thorough discussions with forensic science experts and legal professionals.

CONCLUSION

The outcomes of these three case studies highlight the importance of using Y-chromosome lineage markers as a supportive tool for establishing paternal relationships, particularly in cases where autosomal STRs provide inconclusive results.

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None.

CONFLICTS OF INTEREST

The authors declared no conflicts of interest.

ETHICAL ISSUES

All DNA samples and associated data were anonymized prior to analysis, meaning that any personal identifiers were removed or replaced with unique codes to ensure that individuals could not be directly or indirectly identified.

SOURCES OF SUPPORT

None

AUTHOR CONTRIBUTIONS

RWRKR: Design of the work; the acquisition; analysis and interpretation of data for the work; Drafting the work and revising it critically for important intellectual content; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. **EM:** Analysis and interpretation of data for the work; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. **LRTP:** Analysis and interpretation of data for the work; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. **PGS:** Design of the work; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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CASE REPORT

FATAL DRUG-INDUCED NEUTROPENIA WITH NECROTISING GASTRITIS AND SEPSIS: A CASE REPORT

Jayasinghe HM*, Warushahennadi J
Department of Forensic Medicine, Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka

ABSTRACT

Azathioprine is an immunosuppressant widely used in the management of autoimmune and inflammatory disorders, including dermatological conditions. Despite its clinical usefulness, it is associated with a wide range of adverse effects, the most significant of which is bone marrow suppression. Necrotising gastritis is a rare and life-threatening condition where predisposing factors include immunosuppression.

This case report describes a fatal necrotising gastritis following immunosuppression due to azathioprine therapy. The clinical presentation, laboratory investigations, ultrasound scan evidence, and the autopsy findings were supportive of gastrointestinal sepsis. This fatal outcome highlights the importance of haematological monitoring, clinical vigilance, and prompt recognition of early signs of drug toxicity or infection.

Keywords: *Azathioprine, immunosuppression; necrotising gastritis*

Corresponding Author: Jayasinghe HM
maduwass90@gmail.com
ORCID iD: https://orcid.org/0009-0001-4358-2579

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INTRODUCTION

Azathioprine is a purine analogue and an immunosuppressant, widely used in the management of autoimmune and inflammatory disorders, including dermatological, rheumatological, and gastrointestinal conditions[1,2]. It acts by inhibiting DNA and RNA synthesis, resulting in decreased proliferation of rapidly dividing cells[1]. Despite its clinical usefulness, azathioprine is associated with a wide range of adverse effects, the most significant of which is bone marrow suppression[1,2], which may manifest as leukopenia, thrombocytopenia, or pancytopenia, directly correlating with the patient's vulnerability to infections. Severe or rapidly progressive neutropenia may predispose patients to fulminant and fatal infections.

Necrotising gastritis is a rare and life-threatening condition characterised by widespread inflammation, mucosal necrosis, and bacterial invasion of the gastric wall. Although the pathogenesis is unclear, the predisposing factors include immunosuppression and treatment with cytotoxic drugs[3]. It typically presents with severe abdominal pain, vomiting, haematemesis, and systemic signs of sepsis. Prompt diagnosis and early surgical intervention are critical in preventing fatal complications[3,4]. The development of necrotising gastritis in the context of drug-induced neutropenia is

exceedingly uncommon, with a limited number of cases reported in the medical literature[5].

We present a fatal case of necrotising gastritis in a previously healthy elderly male following azathioprine-induced neutropenia. This case underscores the importance of timely haematologic monitoring during immunosuppressive therapy and awareness of rare gastrointestinal complications while on immunosuppressive agents[4].

CASE HISTORY

A 64-year-old male presented to a dermatologist with a recently developed rash over the upper limbs. He had no significant past medical history, took no regular medications, and was not known to have any immunological or haematological disorders. He was prescribed azathioprine 25 mg twice daily. The full blood count before starting treatment was within normal limits (total white cell count - 7000/µl, neutrophils - 44%).

Over the subsequent three weeks, the patient experienced clinical improvement of his cutaneous symptoms, with complete resolution of the rash. However, he began to notice significant hair loss during this period, although no severe systemic symptoms were recorded. Further investigations revealed marked leukopenia (3150/µl). The azathioprine therapy was discontinued, and an alternative treatment regimen was prescribed.

Despite the new treatment regimen, the patient presented to the hospital with progressive neutropenia (total white cell count - 1870/µl, neutrophils - 11.5%), high-grade fever, severe epigastric pain, and repeated episodes of profuse vomiting. He was clinically diagnosed with neutropenic sepsis, and broad-spectrum intravenous antibiotics and aggressive supportive therapy were initiated. However, he succumbed two days later.

Inward laboratory investigations showed further progression of leucopenia with critically low neutrophil counts (total white cell count - 370/µl, neutrophils - 11%). Platelet counts (281000/µl) and the haemoglobin levels (12 g/dl) were within normal limits. C-reactive protein was 289 mg/l. The ultrasound scan of the abdomen revealed thickening of the gastric and lower oesophageal walls.

An autopsy was performed. The external examination revealed diffusely thinned scalp hair that was easily detachable. No significant rash was observed. The examination of the gastrointestinal tract revealed markedly thickened and congested gastric mucosa with widespread areas of extensive necrosis (Figure 1). Both lungs showed pulmonary oedema with fatty degeneration of the liver. The other internal organs were unremarkable.

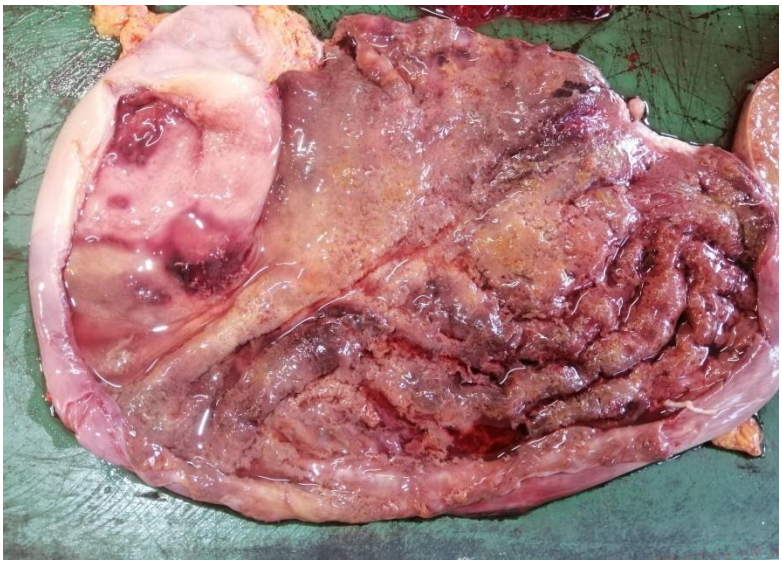

Figure 1: Specimen of the stomach demonstrating the congested and oedematous mucosa with multiple areas of necrosis.

Histopathological examination of the gastric mucosa revealed ulceration and necrosis (Figures 2 and 3), accompanied by extensive vascular congestion (Figure 4). Bacterial colonies were observed deep within the gastric mucosa (Figure 5), indicating severe infectious infiltration. There was widespread necrosis with a lack of accompanying inflammatory cellular response (Figures 2 and 3), consistent with profound neutropenia.

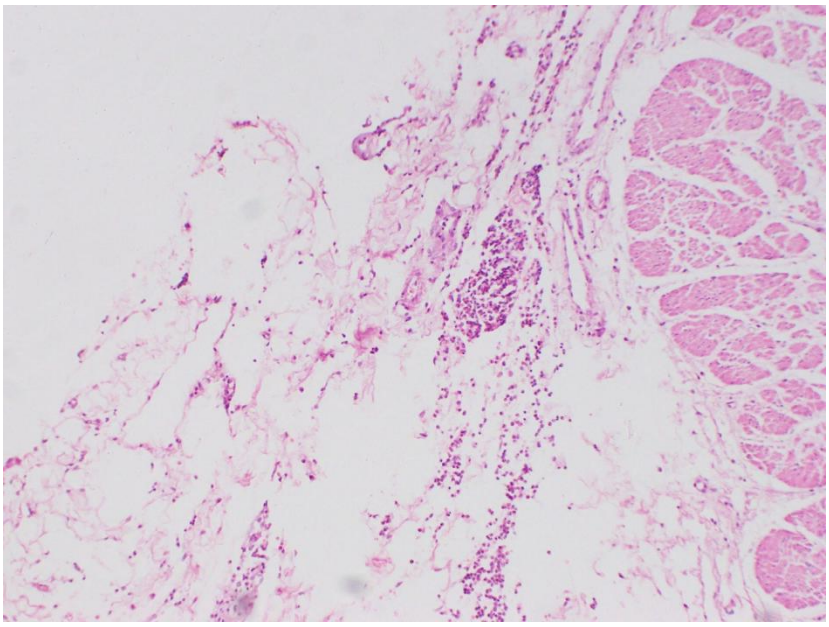

Figure 2: The surface epithelium is significantly absent, extensive tissue destruction involved beyond the superficial mucosa, with loss of normal gastric architecture. Scattered neutrophil infiltration noted (H&E x10)

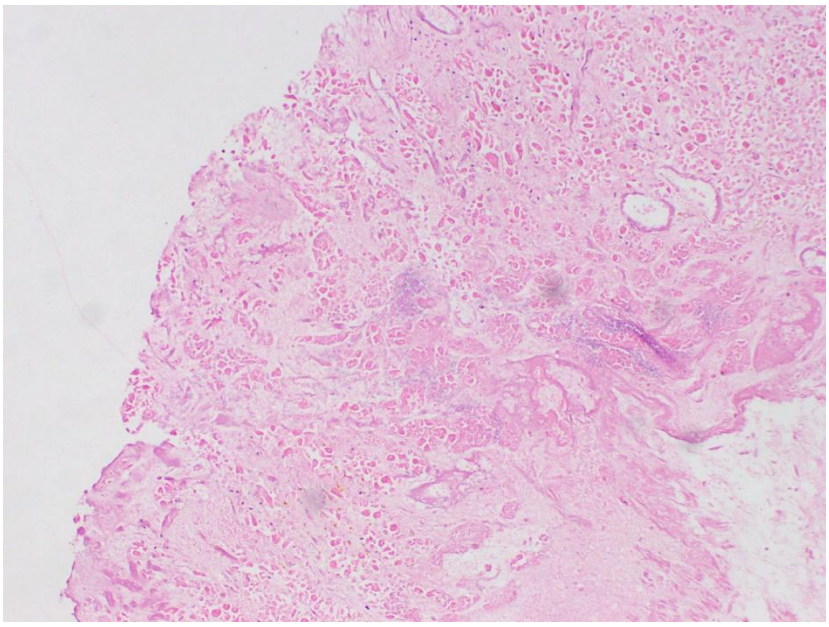

Figure 3: The gastric mucosa is largely replaced by homogeneous eosinophilic necrotic tissue and few neutrophil infiltrations with loss of normal architecture (H&E x10)

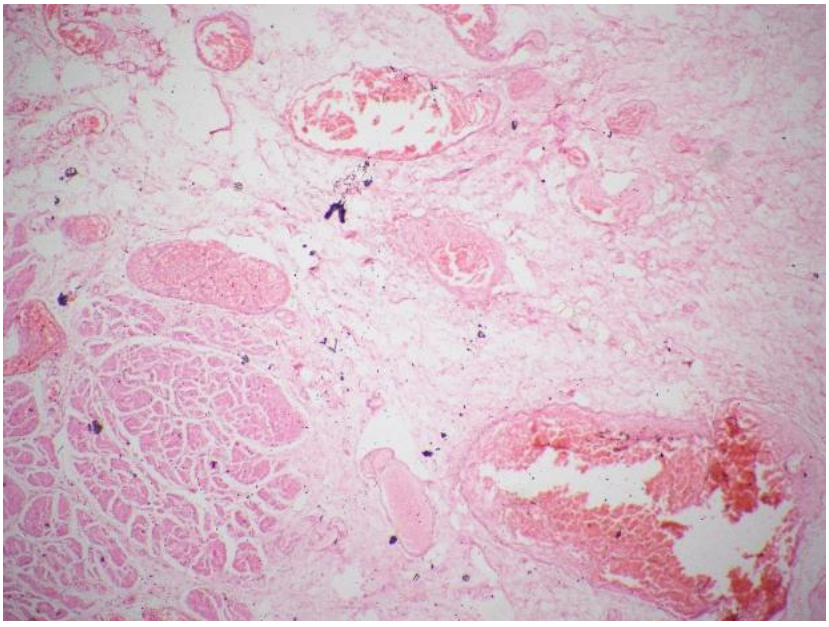

Figure 4: Micrograph of the gastric mucosa demonstrating extensive vascular congestion (H&E x10)

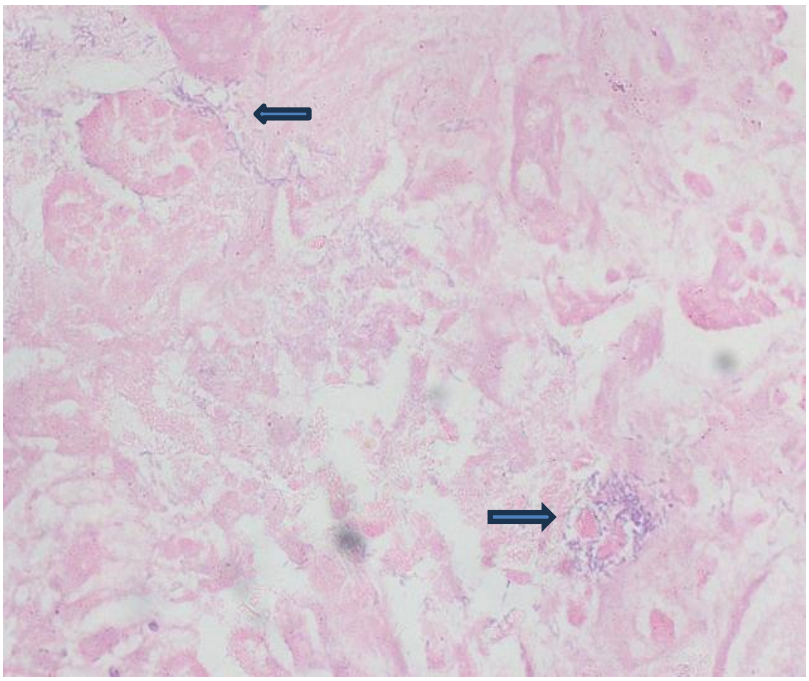

Figure 5: Micrographs of the gastric mucosa demonstrating bacterial colonies (arrows) deep within the gastric mucosa, with a few neutrophils infiltrating the necrotic tissue (H&E x10).

Considering the clinical history, laboratory investigations, and autopsy findings, the cause of death was concluded to be sepsis due to necrotising gastritis in a patient who was recently on azathioprine therapy.

DISCUSSION

This case report describes a fatal necrotising gastritis following immunosuppression due to azathioprine therapy. Azathioprine is metabolised through several pathways, including the **thiopurine methyltransferase (TPMT)** enzyme system[7]. Deficiencies or reduced activity in these pathways significantly increase the risk of drug-induced myelotoxicity[1,6]. The most serious side effect of azathioprine is bone marrow suppression, which may be life-threatening[6]. The deceased presented with alopecia following azathioprine therapy, which is an early warning sign of marrow toxicity[6]. The neutropenia progressed despite discontinuation of the drug. The rapid progression to agranulocytosis created an immunologically vulnerable state, predisposing the patient to fulminant bacterial invasion.

The clinical presentation, laboratory investigations, and the ultrasound evidence were supportive of gastrointestinal sepsis. During neutropenia, the gastrointestinal mucosa becomes susceptible to translocation of enteric organisms, often leading to severe, rapidly progressive infections[8]. In this case, autopsy examination revealed extensive mucosal necrosis, thickened and congested gastric folds, and necrotic debris, consistent with necrotising **gastritis.** Histology confirmed deep mucosal destruction with bacterial colonies penetrating the tissue and a lack of inflammatory cells, a hallmark of profound neutropenia. The lack of an inflammatory response reflects the inability of the host to mount a defence response, resulting in widespread bacterial proliferation and mucosal necrosis.

CONCLUSION

This case underscores a rare complication of azathioprine, which has precipitated severe bone marrow suppression leading to aggressive infections. The fatal outcome in this instance highlights the importance of assessing **baseline full blood counts** and TPMT levels where available before safe therapy initiation. Identifying individuals with low TPMT enzymatic activity, which is not routinely done in Sri Lanka, allows clinicians to adjust dosing or select alternative medications, thereby reducing the likelihood of severe adverse outcomes. After initiating the treatment, it is important to continue haematological monitoring, be clinically vigilant, and promptly recognise early signs of drug toxicity.

This case serves as a reminder that even widely used immunosuppressive agents can lead to life-threatening consequences, reinforcing the need for strict monitoring protocols when prescribing azathioprine. The clinicians must ensure

structured surveillance and provide clear patient education to enable timely medical intervention.

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CONFLICTS OF INTEREST

The authors declared no conflicts of interest.

ETHICAL ISSUES

The presented autopsy case was conducted for medico-legal purposes, and this information is used for academic purposes, according to the institutional guidelines.

SOURCES OF SUPPORT

None.

AUTHOR CONTRIBUTIONS

HMJ: Conception and design of the work; the acquisition, analysis, and interpretation of data for the work; drafting the work; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. **JW:** Conception and design of the work; the acquisition, analysis, and interpretation of data for the work; revising the work critically for important intellectual content; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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CASE REPORT

HIGHLIGHTING FIRE RISKS ASSOCIATED WITH STORAGE OF WATER TREATMENT CHEMICALS: A CASE STUDY

Fernando NIK*, Dahanayake LAWK, Fernando WAR
Explosive & Fire Investigation Section, Government Analyst's Department, Pelawatta, Sri Lanka

ABSTRACT

This article presents a case study of a major accidental warehouse fire in Sri Lanka involving the storage of incompatible water treatment chemicals. The warehouse stored excess calcium hypochlorite alongside some incompatible chemical substances, such as trichloroisocyanuric acid (TCCA) (**CAS 87-90-1)**, polyaluminium chloride (PAC) or aluminum chlorohydrate (**CAS 1327-41-9**), linear alkylbenzene sulfonic acid (LABSA) (**CAS 27176-87-0)**, sodium hydrosulfite (disodium dithionite) (**CAS 7775-14-6)**. As a powerful oxidizer, calcium hypochlorite is highly susceptible to spontaneous exothermic decomposition when exposed to heat, moisture, contaminants, or incompatible substances. In addition to heat, this process continuously releases large quantities of oxygen and corrosive chlorine, leading to immediate spontaneous ignition of the bulk stock and nearby combustible materials and posing a risk of violent physical explosions of confined containers or spaces. This investigation highlights such a critical fire and explosion risk associated with storing calcium hypochlorite and reveals the catastrophic consequences of poor adherence to safety regulations. Key safety recommendations derived from this incident are provided to minimise future risks during the storage and transportation of this hazardous chemical, emphasising proper handling protocols, material segregation, protective packaging, and mandatory safety training.

Keywords: *Calcium hypochlorite; fire investigation; spontaneous decomposition; water treatment chemicals*

Corresponding Author: Fernando NIK
indu.fernandogad@gmail.com
ORCID iD: https://orcid.org/0009-0005-6594-3469

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INTRODUCTION

Calcium hypochlorite is a powerful oxidizing chemical that is produced and shipped internationally in large quantities, commonly to purify drinking water and sanitize water in swimming pools. While effective, it possesses significant fire and explosion risks under improper storage conditions due to its tendency to undergo spontaneous exothermic decomposition or thermal runaway[1]. Although this hazard is not unique to calcium hypochlorite, the material is particularly sensitive to elevated temperatures, contamination, moisture, and incompatible substances, which can accelerate its decomposition. During decomposition, calcium hypochlorite can release oxygen, chlorine, and other byproducts[2,3,4]. Once spontaneous decomposition starts by any means, if the heat

generated cannot dissipate quickly enough, the temperature of the substance will rise[5]. This higher temperature may accelerate the decomposition by generating more heat. Therefore, it can spread through a bulk stock instantly until it is entirely consumed. The oxygen released by the exothermic decomposition can spontaneously ignite nearby combustible materials with extreme intensity[6]. Further, the buildup of pressure caused by releasing gases and heat in confined containers can cause violent physical explosions. Nevertheless, due to poor adherence to good safety practices, it was reported that a considerable number of fires, particularly on cargo ships transporting calcium hypochlorite and in storage warehouses, have resulted in significant loss of life and property[7]. Even though these types of disasters are not common, the forensic investigation of such incidents is crucial to identify root causes to minimise fire and explosion risks associated with the storage and transportation of these hazardous water treatment chemicals.

This article is a case study of the forensic investigation of a major accidental warehouse fire in Sri Lanka that occurred in August 2023, where calcium hypochlorite was stored alongside several incompatible chemicals, including trichloroisocyanuric acid (TCCA) (**CAS 87-90-1)**, polyaluminium chloride (PAC) or aluminum chlorohydrate, (**CAS 1327-41-9**), linear alkylbenzene sulfonic acid (LABSA) (**CAS 27176-87-0)**, sodium hydrosulfite (disodium dithionite) (**CAS 7775-14-6).**

The cause of the fire was determined to be an exothermic oxidizing reaction that occurred when calcium hypochlorite came into contact with an incompatible substance. The most hazardous combination was likely to be calcium hypochlorite and disodium dithionite, which can undergo a vigorous redox reaction in which hypochlorite rapidly oxidizes dithionite, releasing significant heat that can lead to spontaneous ignition.

Key forensic observations included fire pattern analysis, structural damage assessment, exploded chemical containers, and evidence of chemical incompatibility.

Additionally, this case study suggests some key safety recommendations to minimise fire risks during the storage and transportation of calcium hypochlorite. These include proper handling, packaging, storage procedures, safety precautions, regular training and awareness, as well as adhering to guidelines enacted by regulatory bodies.

CASE HISTORY

This case focuses on a catastrophic fire involving water treatment chemicals reported at an industrial zone warehouse in Sri Lanka in August 2023. Although no fatalities were reported, the fire resulted in corrosive chlorine gas emissions that polluted the air across a significant residential area for several weeks.

According to the witness statement, an explosion was seen with a flash of light, and flames were seen coming through the gaps in the corrugated metal front entrance door of the warehouse, localised to the middle area towards the back wall of the building. All the doors had been closed, and the weather was dry, with no documented lightning or rain events.

Despite strong efforts by the police and fire department, the fire was not put out until about noon the following day; however, they were successful in containing it and preventing it's spread to surrounding buildings.

According to the statement given by the quality assurance manager of the company, various chemicals were stored on wooden pallets on the floor of the building. These mainly included plastic barrels of different quantities of calcium hypochlorite in two common commercial strengths (35% and 70% available chlorine). According to the product's details, the inert ingredients and byproducts found within the bulk calcium hypochlorite also included calcium hydroxide ($Ca(OH)_2$), calcium chloride ($CaCl_2$),sodium chloride (NaCl), and water. Additionally, sodium carbonate, PAC, sodium hydrosulfite, LABSA, and TCCA were also in the warehouse.

As a safety measure, approximately a 1 m gap was maintained between different highly reactive

calcium hypochlorite piles, and sodium carbonate packages were kept on the floor among those gaps as a physical barrier, as well as a neutralising agent in case of a spill.

The warehouse building had been purchased one year prior, and since then, they had been storing imported water treatment chemicals and selling them to the local market. The only renovation made was the installation of a new electrical supply for lighting a single bulb inside, which could be operated from outside. Further, he stated that there were no other electrical appliances or plug sockets inside the building.

INVESTIGATION OF THE SCENE OF THE FIRE

A team of fire investigators from the Government Analyst's Department (GAD), the sole forensic laboratory in Sri Lanka, along with a team of Scene of Crime Officers (SOCOs) in the Police Department, attended the scene three days after the fire was extinguished to determine its cause and origin.

Upon arrival, the team found the scene under the protection of company security personnel.

The warehouse building comprised a rectangular-shaped hall measuring approximately 43 m x 16 m with 3 m high cement-plastered brick walls and asbestos roofing supported by a metallic truss structure adjoining the slightly damaged front office part (Figure 1). The main entrance door was made of corrugated metal sheeting, which was consistent with the overall structural design. Some of the roof's metal support bars were deformed, while the asbestos roofing sheets were missing in a large portion of the building (in the left and middle areas). Since asbestos is chemically inert and non-combustible, this suggests the roofing was either forcefully dislodged or collapsed by multiple factors, such as the buildup of gas pressure, thermal stresses of aggressive reactions, and the impact of ruptured metallic barrels and their fragments during the fire (Figure 2).

Figure 1: Outer view of the burnt warehouse, built attached to the office part (slightly damaged front office part indicated by the red arrow)

Figure 2: Close-up structural views of the burnt warehouse: (A) Main entrance door forced-open by the firefighters and metallic grill structure on the front wall (yellow arrow); (B) Inner view opposite the main entrance and metallic grill structure on the back wall (orange arrow); (C) Burnt forklift machine kept at the left front side; (D) Severely damaged inner structure in the right direction.

A 75-cm-tall metallic grill structure between the roof and the walls provided ventilation. Evidence indicated that all the doors, including the main entrance, were closed when the fire occurred (Figure 2). Further, no fire alarms or any fire prevention systems were present inside the building.

The fire severely damaged the warehouse, and the upper levels of surrounding buildings were also affected, though with considerably less damage (Figure 3).

Figure 3: Hut built facing the warehouse, used to store empty containers, indicated by the red arrows.

Except for sodium carbonate packages, which were piled up in a few locations within the hall (Figure 4), all other chemical packages were completely burnt to floor level, leaving a layer of porous white solid residue.

The investigating team noted the smell of chlorine gas and detected heat emanating from the porous white solid substance, likely due to ongoing reactions of buried calcium hypochlorite remnants. Additional heat generation could also result from the hydration of the calcium chloride formed within the mass residue on the floor.

From the burnt debris remaining in the store, the maximum level of burning was in the middle area closer to the back wall, which indicated an area of intense burning of the stored material here, compared to others, which had about 75 cm – 1 m deep layers of residues, especially in the vicinity of the front door. Outer polythene packages of sodium carbonate, located in two areas adjacent to the severely burnt area, were also burnt. Partially burnt and unburnt packages were found in the far-right corner of the building, next to the severely burnt area, as highlighted in Figure 4. This intensely burnt area is believed to be where the fire originated, based on witness statements, physical evidence of the distorted metal roofing, the damaged cement-plastered wall, and the chemicals stored in this location and surrounding areas, according to the information given by the Quality Assurance Manager, as shown in Figure 5.

Figure 4: Sites where sodium carbonate packages were stacked (red circles) and the intensely burnt area (yellow arrow), site where the 35% calcium hypochlorite was stacked (blue arrow).

Figure 5: Sites where the highly reactive chemicals were placed (yellow circles).
(A – plastic cans of 90% TCCA, B – metal drums of $Na_2S_2O_4$).

Based on the background information and scene observations, the cause of the fire incident was concluded to be a vigorous reaction between the strong oxidizer, calcium hypochlorite, and incompatible substances stored nearby. While TCCA and LABSA were present as incompatible chemicals, disodium dithionite posed the most severe incompatibility, as it is a strong reducing agent capable of undergoing vigorous redox reactions with oxidisers.

DISCUSSION

Some substances possess significant fire risks due to their inherent chemical nature. Those substances may be flammable, reactive, or have a potential for decomposition and auto-ignition. Therefore, additional protocols for safe handling, storage, and transportation procedures are required for such dangerous substances.

Any substance with the potential for self-accelerating decomposition under thermal runaway conditions is particularly dangerous. When such a substance or chemical compound is stored improperly with incompatible chemicals or combustible materials, decomposition reactions will accelerate. Furthermore, the released heat and gaseous products can cause a fire or intensify an existing fire. Therefore, a comprehensive understanding of the chemistry involved, including chemical identifiers, reaction pathways, and mechanistic insights, is critical for proper risk management.

Calcium hypochlorite is a powerful oxidizing material in this category. It can decompose to produce heat and various products, including oxygen (O_2), chlorine (Cl_2), calcium chloride ($CaCl_2$), calcium chlorate ($Ca(ClO_3)_2$), calcium oxide (CaO), water (H_2O), and hydrogen chloride (HCl) fumes, etc., depending on one or more contributing factors such as temperature, moisture, impurities, and certain incompatible materials[1,2].

Conversely, disodium dithionite is a strong reducing agent that can react violently and uncontrollably upon contact with calcium hypochlorite or other incompatible materials through a rapid exothermic redox reaction, generating sufficient heat to ignite nearby combustible materials.

If the heat generated from these exothermic reactions cannot dissipate quickly enough, the temperature may increase rapidly, accelerating the decomposition of the hypochlorite or dithionite itself. This heat may initiate a self-accelerated thermal runaway process that can lead to a catastrophic fire by intensifying with the liberation of oxygen and chlorine gas[3,4]. Therefore, accidental mixing of calcium hypochlorite and disodium dithionite or any other incompatible substances must be strictly avoided by storing them in separate, isolated areas in completely dry, airtight containers at ambient room temperature.

During the manufacturing process, the introduction of impurities or moisture can initiate a slow decomposition reaction. However, this reaction may accelerate over time due to heat accumulation, potentially occurring during handling, packaging, transportation, and storage[3,5].

Furthermore, localised temperature elevations may occur due to accidental wetting or introduction of water in small quantities, especially in cleaning, using wet containers and tools, or even in a humid environment. Consequently, these localised temperature elevations may also lead to exothermic decomposition runaway reactions[5].

Once ignited by any means, a calcium hypochlorite fire can propagate uncontrollably through the entire bulk storage until the material is fully consumed[6]. This is because the continuous liberation of oxygen and chlorine sustains and intensifies the growth of the fire or can also cause other nearby materials to autoignite[1,6]. Therefore, firefighting measures are particularly difficult, and boundary cooling is the practical response to mitigate fire spread[3].

HYPOTHESIS MADE

The fire likely originated due to a chemical reaction of calcium hypochlorite interaction with incompatible substances.

OTHER CAUSES CONSIDERED

Deliberate Fire-Setting (Arson): Ruled out based on closed and secured doors and the remaining evidence of door locks. However, clearer confirmation (e.g., closed-circuit television (CCTV) footage, tamper evidence) would strengthen this conclusion.

Electrical Faults: Eliminated due to no electrical power supply in the building at the time, as confirmed by observing a thermally affected

switch fixed to the outside wall. Further, any electrical wire or evidence related to plug sockets was not found.

Gas Leak: No internal gas supply, and the building was structurally isolated, making cross-contamination from adjacent structures unlikely.

Smoking Materials/Fireworks: None were found in or around the premises.

Accidental Water Introduction: Ruled out due to dry weather, no recent rainfall, and no active water supply. However, the possibility of moisture from packaging or cleaning cannot be entirely dismissed without further analysis.

INTERPRETATION OF THE SCENE OBSERVATIONS

According to the witness statement, the first sign of the incident was an explosion followed by a fully developed fire originating from the middle area near the back wall of the warehouse. Furthermore, according to the floor plan of the chemical stock in the warehouse provided by the Quality Assurance Manager (Figure 6), plastic cans containing 90% TCCA were stacked somewhat closer to the large number of plastic drums containing 70% and 35% calcium hypochlorite in this middle area. Furthermore, metal drums of disodium dithionite ($Na_2S_2O_4$) were also stacked closer to 35% calcium hypochlorite.

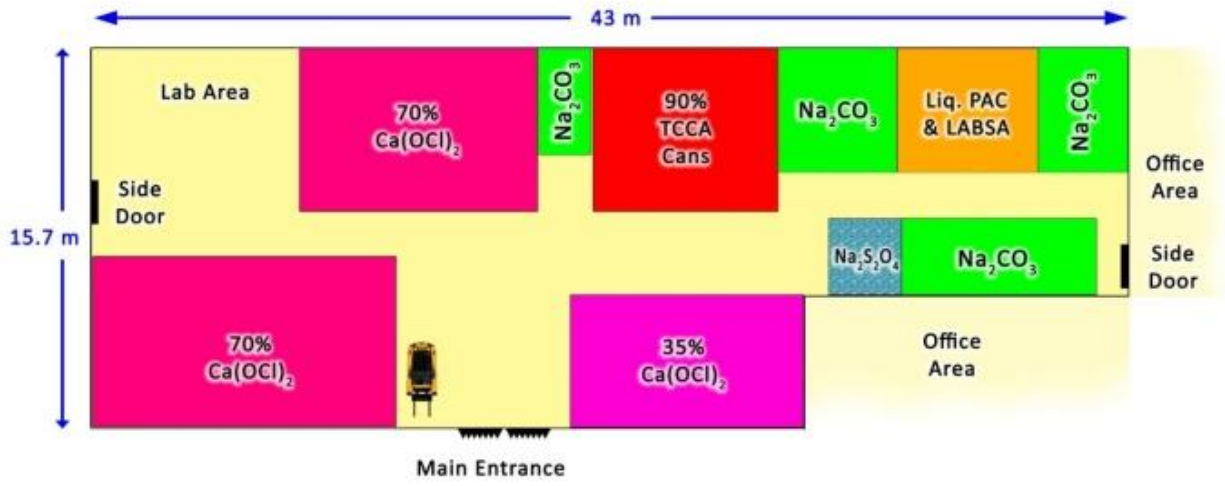

Figure 6: Schematic diagram of the warehouse showing its chemical arrangement

Upon examining the burnt debris on the floor and considering the background information, it was evident that 70% calcium hypochlorite and 35% calcium hypochlorite were stacked with other incompatible chemicals, such as 90% TCCA and disodium dithionite, in the same area of the warehouse without adequate safety measures. Additionally, contrary to the given statement, a large number of sodium carbonate packages were piled in the far-right corner of the warehouse without keeping a single bag between 35% calcium hypochlorite and disodium dithionite, or on the right side of the warehouse.

The improper co-storage of incompatible chemicals, poor packaging practices, and the lack of environmental controls could create a high-risk scenario for exothermic redox reactions, especially involving an oxidizer like calcium hypochlorite.

Such reactions would release significant amounts of heat, oxygen, and chlorine gas, contributing to a thermal runaway and eventual explosions, followed by a rapidly spreading fire inside the building[3,8,9]

CONCLUSION

Based on the physical evidence of the scene, witness accounts, and chemical storage records, the fire investigation team of the Government Analyst's Department concludes:
The fire originated due to an exothermic chemical reaction involving calcium hypochlorite, triggered by contact with an incompatible substance, possibly TCCA, $Na_2S_2O_4$, or other reactive materials. This reaction led to rapid thermal decomposition, gas generation, and an uncontrolled fire that consumed the entire building.

SAFETY RECOMMENDATIONS

- Any company should adhere to the safety guidelines provided by the manufacturer when storing and handling powerful oxidizing chemicals[10,11,12].
- The company should comply with the rules and regulations enacted by the Sri Lankan government to prevent such hazardous incidents. Additionally, relevant government authorities should implement proper monitoring plans.
- The warehouse's structural design and chemical storage arrangements should meet global safety standards, and staff should undergo frequent training conducted by qualified resource personnel[9,11,13].
- The company should install fire alarm systems and establish emergency firefighting procedures.
- Chemical storage warehouses of this nature should be constructed in remote areas, ensuring they are isolated from the public.

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CONFLICTS OF INTEREST

The authors declared no conflicts of interest.

ETHICAL ISSUES

None.

SOURCES OF SUPPORT

None

AUTHOR CONTRIBUTIONS

NIKF: Conception and design of the work; the interpretation of data for the work; drafting the work and revising it critically for important intellectual content; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. **LAWKD:** Interpretation of data for the work; drafting the work and revising it critically for important intellectual content; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. **WARF:** Interpretation of data for the work; drafting the work and revising it critically for important intellectual content; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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POINT OF VIEW

THE EPIGENETIC CLOCK CONCEPT AS AN EMERGING MOLECULAR TOOL FOR POST-MORTEM INTERVAL ESTIMATION IN FORENSIC INVESTIGATIONS

Alwis MSN[1]*, Hennayake HMAM[1], Panagoda PAOK[1], Dissanayake DMDD[1], Tennakoon TDDT[2]
[1]*School of Biomedical Sciences, Faculty of Health and Life Sciences, Coventry University, Coventry, United Kingdom*
[2]*Teaching Hospital Jaffna, Sri Lanka*

ABSTRACT

Accurate estimation of the post-mortem interval (PMI) remains a significant challenge in forensic investigations, particularly when conventional methods, such as body temperature, rigor mortis, and entomological analysis, are confounded by environmental variables or late-stage decomposition. The epigenetic clock concept, which assesses DNA methylation patterns at specific cytosine–phosphate–guanine (CpG) sites, has recently emerged as a promising molecular tool for PMI estimation. Originally developed to measure biological age in living individuals, models such as Horvath's, GrimAge, and PhenoAge have shown potential in forensic contexts due to their tissue specificity and relative post-mortem stability across biological matrices, including blood, muscle, bone, and dental pulp. However, to date, the epigenetic clock concept has not been validated for PMI estimation, limiting direct forensic application. These methylation-based tools can be tested for more objective and reproducible PMI estimates, especially during early to intermediate post-mortem intervals, and can be further enhanced by integrating with protein degradation or microbiome-based analyses.

However, the forensic application of the epigenetic clock concept faces multiple challenges. Post-mortem DNA degradation, particularly in tropical climates, compromises methylation signal integrity and limits the reliability of time-sensitive analyses. Most existing models are trained on living datasets and lack post-mortem calibration across diverse populations and decomposition conditions. Additional barriers include the high cost of sequencing technologies, infrastructural limitations in low-resource settings, and the absence of universally accepted forensic validation protocols. Ethical concerns regarding genetic data privacy and the legal admissibility of molecular evidence must also be addressed. Future studies should prioritise the development of forensic-specific adaptations of the epigenetic clock concept tailored to regional and environmental contexts, establish standardised protocols for PMI estimation, and promote interdisciplinary collaboration among forensic pathologists, molecular biologists, and legal authorities. With rigorous validation and ethical oversight, the epigenetic clock concept may significantly enhance the accuracy and objectivity of medico-legal death investigations.

Corresponding Author: Alwis MSN
sachinthanirash11@gmail.com
ORCID iD: https://orcid.org/0009-0001-7285-8036

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INTRODUCTION

The post-mortem interval (PMI), defined as the time between death and body discovery, plays a main role in medico-legal death investigations, helping a wide spectrum of legal, investigative, and humanitarian functions. From identifying decedents in missing persons cases to validating witness timelines in homicides, PMI estimation remains a cornerstone of forensic casework[1]. Despite its importance, accurate PMI determination continues to pose significant challenges due to the complex interplay of intrinsic and extrinsic factors influencing the rate and pattern of decomposition[1]. Conventional techniques, such as evaluating rigor mortis, body temperature, entomological activity, or circumstantial evidence, are often limited by subjectivity, environmental variability, or late-stage decomposition[1,2].

In recent years, the forensic sciences have moved toward molecular and tissue-based methods to overcome these limitations. Techniques involving dental pulp histology[3], cadaveric microbiome profiling[4], and RNA decay analysis[5], have shown potential, particularly in early to intermediate PMIs. However, these approaches are often constrained by sample degradation, standardisation issues, and inter-individual variability. Among these emerging tools, the epigenetic clock, biomarkers based on DNA methylation (DNAm) at specific cytosine–phosphate–guanine (CpG) sites, have gained traction due to their correlation with biological age and apparent post-mortem stability[6].

Originally developed to estimate chronological age in living individuals, epigenetic clocks such as Horvath's, PhenoAge, and GrimAge are now being explored in forensic contexts for their potential to enhance PMI estimation. These models, rooted in molecular aging biology, may provide objective, reproducible, and time-sensitive insights into tissue degradation at the cellular level, offering a promising complement to conventional forensic pathology[7]. To date, epigenetic clocks have not been validated for PMI estimation[8,9]. As forensic medicine moves toward precision-based post-mortem diagnostics, understanding the applicability and limitations of the epigenetic clock concept in real-world death investigations becomes more important.

METHODS

To develop this 'Point of View' article, a focused literature exploration was conducted using databases including PubMed/MEDLINE, ScienceDirect, and Google Scholar. The search spanned publications from 2018 to 2025, employing keywords such as "post-mortem interval estimation", "DNA methylation", "epigenetic clocks", "forensic molecular tools", and "biological aging markers". Filters were applied to prioritise peer-reviewed articles, recent advancements, and studies directly related to forensic applications. Relevant data were extracted and organised using a structured format that included study objectives, techniques used, findings, and limitations. The collected literature was critically analysed by the authors, with emphasis on emerging molecular tools and their practical integration into forensic contexts. Final interpretations were developed through consensus among the researchers, aiming to provide an evidence-informed perspective on the evolving role of epigenetic clocks in PMI estimation.

THE EPIGENETIC CLOCK CONCEPT: MECHANISMS AND BIOLOGICAL BASIS

Epigenetic clock concepts are molecular tools that estimate biological age by analysing DNAm patterns at CpG sites[10]. These clocks emphasize stable chemical modifications to DNA that affect gene expression without altering the DNA sequence, giving a quantifiable measure of cellular aging[11–13]. The concept of epigenetic age acceleration (EAA), a divergence between biological and chronological age, has been associated with disease susceptibility and environmental influences[10].

Among the most recognized models, Horvath's clock uses 353 CpG sites across various tissues, while Hannum's clock, based on 71 CpG sites in blood, has been associated with aging-related diseases[12,13]. Second-generation models such as PhenoAge and GrimAge integrate clinical biomarkers and mortality predictors, enhancing accuracy in health span and survival prediction[14].

The DNAmTL clock, which estimates telomere length from DNAm patterns, provides additional mechanistic insights into aging[14].

These epigenetic clocks have key ageing mechanisms like epigenetic drift, the random loss of DNAm fidelity with age, leading to altered gene regulation[10]. Ageing is marked by global hypomethylation and localised hypermethylation, affecting transcription, DNA repair, and cellular homeostasis[13]. These modifications, along with chromatin remodelling and histone changes, contribute to age-related functional decline[12].

For forensic purposes, the post-mortem stability of DNAm is a key advantage. Methylation signs in tissues such as blood, brain, muscle, and teeth remain sufficiently intact for analysis shortly after death[15,16]. Blood is the most commonly used tissue in epigenetic clock studies due to its accessibility and predictive accuracy[10]. Age-predictive methylation markers have also been successfully validated in buccal cells, bone, and cortical tissue, making them applicable in diverse forensic contexts[9,15,].

Despite their promise, these models may be affected by confounders such as disease, lifestyle, or environmental exposures, underscoring the need for robust, forensic-specific calibration[9]. Nevertheless, the relative stability of DNAm post-mortem, combined with the increasing accuracy of these clocks, supports their integration into forensic workflows for both age-at-death and post-mortem interval estimation.

POST-MORTEM EPIGENETICS: MEDICAL AND FORENSIC APPLICATIONS

Although epigenetic clocks have become powerful tools for estimating age in living individuals, no published studies to date have directly applied this concept to PMI estimation. This limitation must be explicitly recognised, as the core of ageing-clock research has not yet been translated into validated forensic tools for PMI. Nevertheless, research on post-mortem epigenetics provides strong evidence that several epigenetic marks remain stable after death, supporting the biological plausibility of future molecular tools for PMI estimation.

A. Stability of DNA Methylation After Death

- In rat brain tissue, global levels of 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) remain largely unchanged across PMIs up to 96 hours, indicating strong post-mortem stability of these cytosine modifications[17].
- Human, pig, and mouse brain studies have similarly demonstrated that 5mC, 5hmC, 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC) show minimal degradation for at least 72 hours post-mortem[17].
- Immunohistochemical analyses further confirm that these cytosine modifications remain preserved for up to 4 to 5 days after death, although some other epigenetic marks, particularly histone acetylation, begin to decline with prolonged PMI[17].
- Quantitative data from the same study show no significant reduction in global 5mC levels for at least three days post-mortem, reinforcing the chemical stability of methylated cytosines[17].

B. Histone Modifications and Chromatin Structure Post-Mortem

- According to the same study mentioned above, histone methylation marks (such as H3K4me3 and H3K27me3) were found to remain stable for 48–72 hours post-mortem in animal brain tissue[17].
- By contrast, histone acetylation marks (H3K9ac, H3K27ac, H4K5ac, etc.) decline more rapidly: some of these marks start to decrease within 24 hours after death[17-20].
- Chromatin immunoprecipitation experiments further support that nucleosome-bound DNA remains associated with histones in the human brain for as long as 30 hours post-mortem and that differences in histone methylation at specific gene loci can still be recovered[21].
- A forensic genetics-oriented review also argues that nucleosomes may provide structural resilience after death, protecting DNA and allowing certain

histone post-translational modifications (PTMs) to persist even in degraded samples[19].

C. *Forensic Implications and Research Gap*

These data collectively demonstrate that epigenetic features can resist decay at least during early-to-intermediate PMIs, which means it is biologically plausible to develop post-mortem molecular markers. However, the lack of any study calibrating established epigenetic ageing clocks to PMI remains a critical gap. No research has, for example, measured how clock-CpG sites change in a time-dependent manner after death. Therefore, while the epigenetic marks themselves are preserved, the clock algorithms have not been tested in post-mortem settings, and their forensic utility remains speculative[8,22,23].

Therefore, current evidence highlights the need for dedicated post-mortem epigenetic research to determine whether specific methylation signatures or adapted clock models could eventually contribute to reliable PMI estimation.

MOLECULAR ADVANTAGES AND CLINICAL CHALLENGES IN PMI ESTIMATION

A. *Molecular Advantages*

- **Oxidative Dynamics as a Forensic Signal**
  Post-mortem biochemical processes can actively reshape cytosine modifications via oxidative stress, which may themselves serve as forensic markers. In rat cerebellum, increasing post-mortem intervals (up to 540 minutes) were correlated with elevated levels of reactive oxygen species (ROS), accompanied by a significant decrease in 5mC and a concurrent increase in 5hmC[24]. This suggests that ROS-driven oxidation may drive conversion of 5mC to 5hmC, providing a mechanistic basis for developing “oxidative epigenetic clocks” that reflect time since death.

- **Stability of Epigenetic Marks Despite Post-Mortem Delay**
  Although some methylation changes may occur, other epigenetic modifications remain robust for hours to days after death. A well-controlled study on pig and mouse brain tissue, and even human neocortex, found that cytosine modifications (5mC, 5hmC, and further oxidized forms) remained stable for at least 72 hours, even under varying post-mortem conditions[17]. This resilience supports the feasibility of recovering meaningful methylation data in real forensic settings where sampling may be delayed.

- **Resilience of DNA Methylation in Forensic Samples**
  Environmental insults, such as heat, ultraviolet (UV) exposure, humidity, and pH shifts, pose a major threat to forensic DNA. However, empirical research demonstrates that methylation-specific markers can survive in dried stains despite these challenges. In blood, saliva, and menstrual-blood stains exposed to UV, high temperature, variable pH, or salt, bisulfite-PCR assays successfully detected methylation at tissue-specific CpG sites[25]. This suggests that methylation-based forensic assays may remain robust even in degraded or environmentally exposed evidence.

- **Long-Term Storage Stability**
  For forensic applications, it is important that methylation data remain reliable during storage. A longitudinal human study showed that global DNA methylation and hydroxymethylation were stable for up to 18 months when stored at –20 °C and –80 °C, even through multiple freeze–thaw cycles[26]. This provides confidence that DNA samples from forensic contexts, when stored appropriately, can preserve epigenetic information over extended periods.

B. Forensic-Translational Challenges

- **Environmental Confounders and Sample Variability**
  While some epigenetic marks are resilient, real-world environmental factors still threaten data integrity. Elevated temperature, high humidity, and substrate type (porous vs. non-porous) significantly influence DNA degradation and may bias methylation measurements[27]. For example, relative humidity may accelerate microbial growth and strand breakage, complicating the interpretation of methylation signals in crime-scene samples[28].

- **Temperature and Humidity Effects on Specific CpGs**
  Experimental work on blood samples has shown that extreme conditions alter methylation at certain loci: under 0°C or 55°C and relative humidities of 12% and 58%, the methylation level of the *TRIM59* gene exhibited a modest but statistically significant regression over time[28]. Such sensitivity implies that epigenetic PMI models must account for environmental history to avoid systematic error[28].

- **Interpretive Complexity from Oxidative Changes**
  The conversion of 5mC to 5hmC (and potentially further oxidised forms) post-mortem raises a significant interpretive challenge: How much of the observed signal reflects pre-mortem biology versus post-mortem chemical change? Without proper calibration, one risks mistaking an oxidative artefact for a reliable clock tick. This problem must be addressed through time-resolved calibration studies in controlled settings[24,29].

- **Validation and Standard Operating Procedures (SOPs)**
  To translate epigenetic markers into forensic practice, rigorous validation is essential. There is currently a lack of forensic-specific SOPs that define:

  1. Sampling protocols (how soon after death, what tissue, how preserved),
  2. Conversion methods (bisulfite vs enzymatic),
  3. Quality-control thresholds (DNA integrity, methylation signal consistency), and
  4. Inter-laboratory reproducibility[30–32].

  Without these, method variability could undermine legal admissibility.

- **Ethical and Legal Implications**
  The use of post-mortem methylation data in forensic contexts raises ethical and legal issues. For instance, how should the uncertainty inherent in methylation-based PMI estimates be reported in a court of law? Moreover, because methylation patterns may reflect ante-mortem exposures, there are concerns about privacy for the deceased and their relatives. A framework must be developed, in consultation with ethicists and legal professionals, to guide the responsible use, interpretation, and reporting of such data[23,33].

C. Future Directions

Based on the current evidence and interpretive considerations discussed in this 'Point of View' article, we recommend several key avenues for advancing the forensic application of epigenetic ageing clocks for post-mortem interval (PMI) estimation:

- Conduct longitudinal studies in cadaveric or animal models, sampling tissues at multiple PMIs to track changes in ROS levels and cytosine modifications under controlled environmental conditions.
- Utilise kinetic methylation data to train predictive "molecular clock" models that correlate oxidised epigenetic marks with PMI rather than chronological age, incorporating environmental and tissue-specific covariates.
- Investigate and validate minimally damaging DNA treatment protocols (e.g., enzymatic conversion) in forensic-type tissues to maximise DNA yield while preserving methylation information.

- Establish comprehensive SOPs encompassing sample collection, storage, DNA extraction, conversion, methylation quantification, and quality control to improve reproducibility and legal admissibility.
- Collaborate with forensic, legal, and bioethics experts to define guidelines on data privacy, consent (post-mortem), uncertainty reporting, and courtroom admissibility for methylation-based forensic evidence.

CONCLUSIONS

The epigenetic clock concept, based on DNA methylation, offers a promising molecular approach for estimating PMI. Their capacity to reflect biochemical changes at the cellular level, particularly when combined with kinetic models of oxidative methylation, provides greater precision and objectivity than many traditional PMI methods.

Nevertheless, realising their full forensic potential requires substantial research and validation effort. Time-resolved studies to map post-mortem methylation kinetics, together with the development of forensic-specific predictive models, are essential. Similarly, method optimisation (example: conversion protocols for degraded DNA) and standardised workflows must be established to ensure reproducibility, reliability, and legal admissibility.

Finally, the deployment of methylation-based PMI tools must be accompanied by robust ethical and legal frameworks. Given that methylation signatures may reflect ante-mortem exposures, issues of data privacy, consent, and uncertainty reporting cannot be ignored. Interdisciplinary collaboration among forensic scientists, molecular biologists, ethicists, and legal professionals will be critical to translate these molecular clocks into practically useful and ethically sound instruments in medico-legal death investigations.

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CONFLICTS OF INTEREST

The authors declared no conflicts of interest.

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None.

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INSTRUCTIONS TO AUTHORS

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Material received is assumed to be submitted exclusively to this journal. All papers will be peer reviewed. The editor reserves the right to amend style, correct English language, do editorial corrections where necessary, and determine priority and time of publication. When submitting papers, authors are advised to include a covering letter indicating that all authors have consented for the publication of the article in the Sri Lanka Journal of Forensic Medicine, Science & Law.

The manuscript should be prepared in accordance with the guidelines developed by the International Committee of Medical Journal Editors. The British Medical Journal, Lancet and Annals of Internal Medicine are recommended to authors as guides to style, clarity of presentation and conciseness.

The manuscripts should be submitted to the SLJFMSL on the Sri Lankan journals Online site at https://sljfmsl.sljol.info/about/submissions/."

The paper should be typeset with double spacing in Microsoft Word format. All pages should be numbered.

The title page should give the full title, running title, names of authors with qualifications, institutional affiliations, e-mail addresses and 16-digit ORCiD number of all authors. Please underline the name of the corresponding author.

The abstract should not exceed 300 words and should illustrate what was done, the main findings and conclusions. Up to five key words should be given under the summary.

The text of research papers should be divided into Introduction, Materials and Methods, Results, and Discussion. Only generic names of drugs should be given, if applicable. Abbreviations should be spelt out when first used in the text. Scientific measurements should be given in SI units. Statistical methods should be specified in the methods section and any term which is not in common usage should be referenced.

Tables and figures should be referred to in the order of appearance in the text in Arabic numerals within parentheses, e.g. (Fig. 1). Tables should have brief titles. Figures should be used only when data cannot be expressed clearly in any other form. Photographs should have a figure number and caption and be attached as jpg files or incorporated into the MS Word document.

References should be in the Vancouver style, and numbered consecutively using Arabic numerals in superscript form, in the order in which they appear in the text.

Note that the SLJFMSL requires the complete name of the journal and not its abbreviation. Where available, please include the DOI numbers of the references.

Journal article

Westaby S, Evans BJ, Ormerod O. Pulmonary artery dissection in patients with Eisenmenger's syndrome. *New England Journal of Medicine.* 2007; 356:2110-2. DOI: 10.1056/NEJMc063492

Book

Saukko P, Knight B. *Knight's forensic pathology*. 4th ed. New York (NY): CRC Press; 2016. P.402.

Chapter in a book

Blaxter PS, Farnsworth TP. Social health and class inequalities. In: Carter C, Peel JR, editors. *Equalities and inequalities in health*. 2nd ed. London: Academic Press; 1976. p. 165-78.

Report

Rowe IL, Carson NE. *Medical manpower in Victoria*. East Bentleigh (AU): Monash University, Department of Community Practice; 1981. 35 p. Report No.: 4.

Web page

Diabetes Australia. Diabetes globally [Internet]. Canberra ACT: Diabetes Australia; 2012 [updated 2012 June 15; cited 2012 Nov 5]. Available from: http://www.diabetesaustralia.com.au/en/Understanding-Diabetes/Diabetes-Globally/

Conference paper

Patrias K. Computer - compatible writing and editing. Paper presented at: Interacting with the digital environment. *46th Annual Meeting of the Council of Science Editors*; 2003 May 3-6; Pittsburgh, PA.

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Conflicts of interest

Should be stated, if any.

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3. All references have been provided correctly.
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Prof. Dinesh M.G. Fernando & Prof. K.A.S. Kodikara
Co-editors,
Sri Lanka Journal of Forensic Medicine, Science & Law
Dept. of Forensic Medicine,
Faculty of Medicine, University of Peradeniya,
Sri Lanka