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# Overkill with Scissors: A Case Report

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**Abstract**

**Introduction:** "overkilling" in Forensic Medicine refers to a specific type of homicide which is characterised by excessive amount or severity of wounds or injury. Further, stabbing is the predominant form of homicidal violence globally, resulting in both physical impairment and death.

**Case presentation:** We present a case of a 28-year-old woman who was stabbed multiple times by her husband, who had used a shawl as a ligature material and a single pair of tailoring scissors as the weapon. On external examination a total of 48 injuries were inflicted on the body- with multiple scratch abrasions and a pressure abrasion over the neck. Vital injuries were noted to hit the right lung, diaphragm, liver, kidneys, abdominal aorta, ureter, mesentery, ascending colon. The stomach contents exhibited a kerosene smell, that was later confirmed to be organophosphorus compound.

**Conclusion:** A complete autopsy is vital to determine the cause, mechanism, and manner of death and to reconstruct the events before death.

**Keywords:** Overkill; Stab wound; Scissors; Autopsy; Intimate Partner Homicide

## Introduction

Generally, stab wounds are often inflicted using knives, forks, knitting needles, screw drivers, broken bottles/glasses, pencils, picks or similar device with pointed end capable of penetrating the skin.[1] A stab wound is characterized by an injury where the wound is deeper than the length or the width on the surface on the skin. Where the depth is usually equal to or less than the length of the blade that was used in producing it.[2]

In our case a single pair of tailoring scissors were used as the weapon to inflict multiple stab wounds of varying depths and consequently varying widths. The skin wound made by closed scissors is typically shaped like a flat 'Z' or the usual impressionist sign for a flash of lightning. The offset of the two blades gives this shallow zig-zag pattern, which is unmistakable when present.[3]It is well noted that scissors tend to produce linear wounds when the blades are closed but with paired perforation wounds on opening.[4]

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Single stab wounds are not uncommon, and in some cases may even be accidental. However, multiple stab wounds are almost always homicidal until proven otherwise. Further when we consider overkill homicides; the term "overkilling" in Forensic Medicine refers to a specific type of homicide which is characterised by excessive amount or severity of wounds or injury. "Overkill is the infliction of wounds above and beyond what is required to kill the victim".[6]

## Case

A 28-year-old woman was reported to have been assaulted by her husband of 5 years, due to suspicions of her being unfaithful with multiple paramours of hers from her past. The neighbours claim to have heard loud arguing, which turned into yells for help from the victim. The neighbours were unable to intervene as the house was locked from inside, however- they reported to the police that they had seen the husband repeatedly stabbing the wife through the window. A call to the police resulted in immediate arrest and containment of the scene.

The weapons used in this case were a pair of tailoring scissors and a shawl, which were both easily available as the husband was a tailor who worked out of the home at times. The weapons were seized immediately by the police at the scene of the incident.

The case was brought for an autopsy under section 302 of the Indian Penal Code.

**At autopsy:**

External examination showed the deceased was 152cm in height, moderately built and nourished, and light brown in complexion. Clothes on the body were a green colour chudidhar top, green colour leggings, one white colour slip and pink colour underwear. The chudidhar top and slip were torn in multiple places that matched with the injuries on the body, all articles of clothing were soaked in blood.

There was postmortem staining that was faint and fixed over the back of the body. Rigor mortis had set in all over the body. The nailbeds showed pallor and faint bluish discolouration. There was dried blood stains present over the face, neck, chest, upper limbs including hands, and both plantar and dorsal aspect of feet.

A total of 48 injuries were inflicted on the body- with multiple scratch abrasions over the neck, and a pressure abrasion due to the use of a shawl as a ligature for strangulation. The right side of the neck showed three parallel scratch abrasions measuring 1cm x 0.2cm, 1cm x 0.2cm, and 0.8cm x 0.1cm. The left side of the neck showed two parallel linear scratch abrasions measuring 2.5cm x 0.2cm and 2.5cm x 0.1cm.

The neck also had a pressure abrasion measuring 18cm x 3.5cm present over the front and sides of neck, situated over the thyroid cartilage level, 3cm below either angle of mandible, and 3.5cm below the chin.

There were 30 stab wounds, 12 puncture wounds (4 paired, 8 unpaired), and 3 incised wounds present over the thorax and abdomen. Out of the 30 stab wounds 26 were found to be fatal. The stab wounds were shaped in a zig-zag fashion (Z-shaped).

On examination of the torso there were40 injuries comprising of 29 stab wounds, 7wedge shaped single puncture wounds, 3 paired puncture wounds, and one muscle depth laceration. Of the 29 stab wounds on the torso, there were 8 on the right side of the chest, 2 on the left side of the chest, 26 injuries on the back. (Figures to demonstrate)

On internal examination and dissection of the external injuries, it was noted as follows: Neck: The tissues and strap muscles beneath the ligature mark showed extravasation of blood on the right-side measuring 5cm x 3cm, and a fracture of the lamina cartilage of the thyroid with blood effused into the surrounding tissues.

Thorax: There were 5 stab wounds piercing the right lung. The left lung was relatively spared.

There were two stab wounds that traversed the right dome of diaphragm and hit the upper surface of the right lobe of liver.

Abdomen: 13 stab wounds pierced the liver, 3 stab wounds to the kidneys (2 right and 1 left), 5 stab wounds to the right side of abdominal aorta, right ureter, right side of mesentery, and posterior aspect of ascending colon.

Other relevant internal examination findings: The brain and its membranes were noted to be intact

and pale. The heart was noted to be intact with diffuse petechial haemorrhages in the endocardium. The lungs were noted to be collapsed on both sides, with pleural cavity containing 150ml of blood and blood clots on either side. The peritoneum contained 1000ml of blood and blood clots. The stomach contained 300ml of light brown colour fluid with kerosene smell, stomach mucosa was congested. The uterus was normal in size and the cavity was empty.

For further investigation:

1. The blood and viscera were collected for chemical analysis
2. Fingernail clippings were collected
3. The weapon (tailoring scissors) examined.

Chemical analysis of blood and viscera revealed that the deceased had consumed organophosphorus compound prior to death.

The weapon sent for examination showed that it was consistent with the injuries on the body as described in the report.

The postmortem changes corroborate the police history, and the postmortem interval was 12 to 24 hours prior to autopsy procedure.

Final opinion was given as:

"Death was due to shock and haemorrhage as a result of multiple stab injuries sustained, however, the deceased had consumed substance containing organophosphorus compound prior to death".

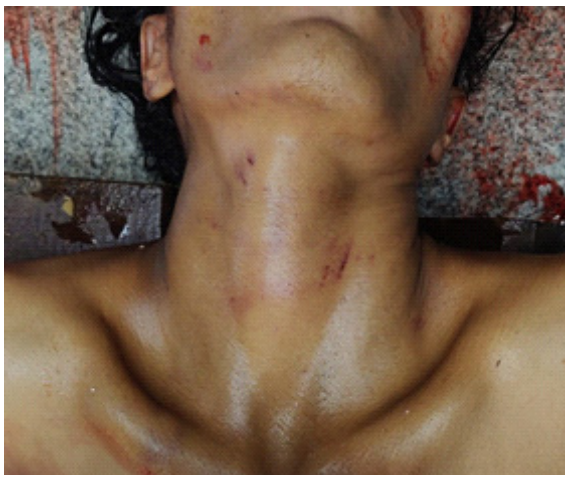


**Figure 1: Scratch abrasions present over the front and sides of neck.**

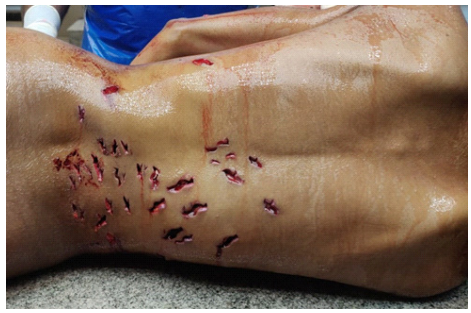


**Figure 2: Multiple Z-shaped stab wounds present over the right side and back of torso.**

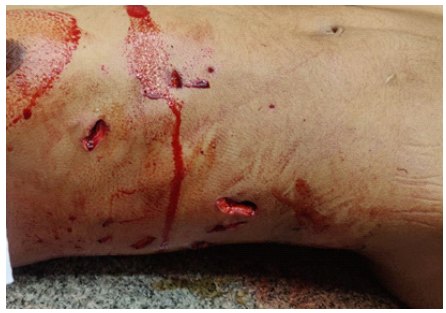


**Figure 3: Stab wounds over the right side of chest and flank**

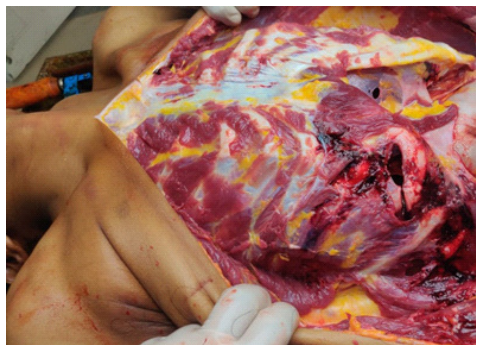


**Figure 4: Ribcage and sternum exposed, note the stab wound over the right 7-8th intercostal space, cutting the cartilaginous costochondral margin.**

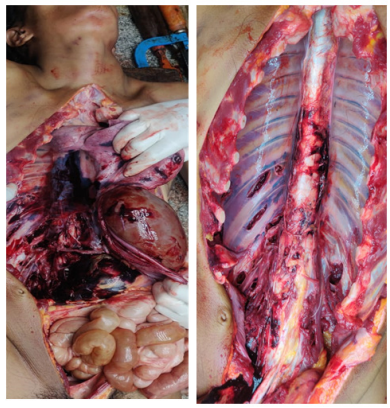


**Figure 5 & 6: Organ removal from the thoracic and abdominal cavities, to reveal haemorrhages, affected viscera and wound tracks.**

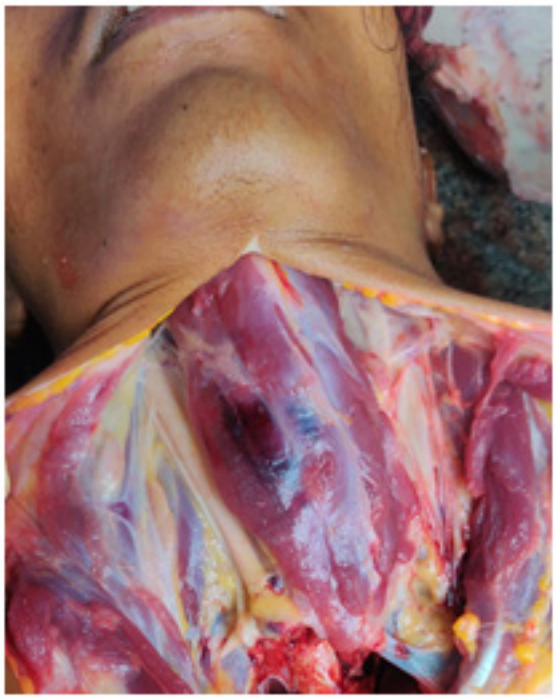
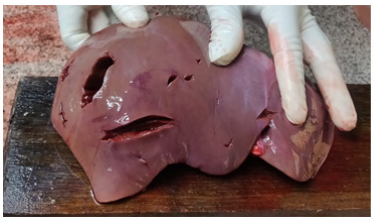


**Figure 7: Dissection of neck: Extravasation of blood into the strap muscles due to underlying thyroid cartilage fracture.**



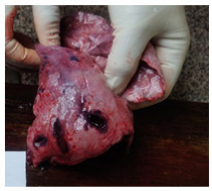


**Figure 8 & 9: Liver with multiple stab wounds present over the right lobe. Right lung with stab wounds over the back of lower lobe.**

**Figure 10: Tailoring scissors sent for weapon examination.**

## Discussion

Stabbing is the predominant form of homicidal violence globally, resulting in both physical impairment and death. In cases of overkill homicide, it is common to see multiple injuries, many of which are inflicted on the body well beyond the death of the victim.[8]

In our case it was noted that the weapons used were those that were easily available and in reach of the aggressor and further the grouping of injuries was localized in the head, neck, and chest regions, which is similar to the studies conducted by Alessandro Mauro Tavoneet al and Kopacz P et al.[9,10] The scattered evidence and use of multiple weapons indicate the disorganization, the lack of planning and the escalation of the act, and the use of multiple weapons can also signal improvisation during the crime.[7]This is also in concurrence with the study conducted by Salfati.[5]

The injuries on the body give clues to the relative positions and the manner in which the weapons were used. The majority of injuries being on the back of the body and being directed right to left, upwards and forwards- suggests the position of the assailant was behind the victim, this is if we consider a normal right-handed grip on the weapon and not an icepick grip.

The dual use of a shawl as a ligature material to both asphyxiate the victim and prevent them from moving away to defend themselves, also plays a role in the distribution pattern of the injuries. This is noted in that injuries are present on all sides of the torso. The use of more than one harmful tool is a common finding with 32.9% of cases in the study conducted by Alessandro Mauro Tavone et al.[9]

The scratch abrasions over the neck showed an active effort by the victim to release herselffrom the restraint on the neck. Further, the presence of bloodstains on the dorsal and plantar aspects of both feetindicates a standing up position of the victim and also pooling of blood on the floor from the wounds. Notably, there is no grouping of injuries on the breasts or the genital areas, which tends to be a feature seen in murders arising from infidelity or crimes of a sexual nature.[3]

It is also of note that the deceased had consumed organophosphorus compound prior to death, the exact nature and motive behind this is speculative at best. Whether the deceased had decided to end her life, or she was forced/tricked into consuming the product prior to the altercation with the assailant is unclear. The blood and all viscera sent for chemical analysis were positive for organophosphorus,

suggesting the consumption-to-death window period was not narrow. There was no suicide note or previous history of suicidal tendencies or self-harm on questioning the relatives. Another possibility discussed with the investigating officer was if the deceased had consumed poison and confessed to her husband knowing he may respond violently, in a sub-type of complex suicide- This was not pursued as there is no proofto corroborate.

The eyewitness testimonies also gave great credence to the case, as they explained the relative positions of the assailant and victim- which matches the pattern of injuries present on the body. The eyewitness information informs us that they saw the scenario wherein which the assailant was behind the victim with scissors in a closed position, holding it in his right hand as one would hold a knife and thrusting it into her back. All while holding both ends of the shawl in his left hand, the loop of which was encircled around the neck of the victim.

## Conclusions

The main duties of the forensic pathologist revolve around delivering an accurate description of the injury, this includes: its position, size, number, pattern, direction, evaluation of internal damage, and also very vitally the cause of death. Multiple injuries pose multiple questions with respect to the number of assailants, number of weapons used, varying age, size, position of the wounds all having different implications, obfuscation of wounds on top of wounds, fabricated injuries, etc.

The role of eyewitnesses can never be understated and if found to be consistent with the facts of the case, can be a powerful tool in piecing the crime together.

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**Ethical Clearance:** Not applicable as long as consent for pictures is obtained from the next of kin.

**Consent:** Has been obtained from the next of kin to publish images for academic purposes, taking care to avoid any identifiers.

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Case Study

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# Tramadol Dependence and Withdrawal Syndrome: A Case Report

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**Abstract**

Tramadol is a synthetic analgesic that appears to have two mechanisms of action: a reuptake inhibition of serotonin and noradrenaline and a weak μ-opioid receptor agonism. Even though its potential for abuse is well reported, the drug is readily available off on the market and is not regarded as a controlled substance in many nations, in contrast to the majority of other opioids. The patient in this case report developed severe tramadol dependence after taking large doses of the drug to treat his musculoskeletal pain. We suggest that It should be made a controlled substance at the administrative levels due to the possibility of dependence and severe withdrawal symptoms.

**Key Words:** Tramadol, Drug withdrawal, Seizure, Drug abuse, Drug dependence.

## Introduction

Tramadol is a synthetic analgesic that appears to have two mechanisms of action: a weak μ-opioid receptor agonism and a reuptake inhibition of serotonin and noradrenaline. It is as effective as morphine or meperidine for treating mild-to-moderate pain, but it is less effective for treating severe or chronic pain. The primary active metabolite of tramadol, o-desmethyltramadol (M1 metabolite), also has an agonistic activity at the μ-opioid receptor, albeit with a greater affinity[1]. Despite the abundance of preclinical, clinical, post marketing, and epidemiological data showing comparatively little, but not zero, misuse/dependence, concerns remain over its potential for abuse and proper regulatory classification. Tramadol is readily available off on the market and is not regarded as a controlled substance in many nations, in contrast to the majority of other opioids[2]. Tramadol withdrawal symptoms can include anxiety, paranoia, depersonalization, derealization, and auditory hallucinations in addition to the usual opiate withdrawal symptoms[3]. Opioids are frequently sold over-the-counter in India due to the country's loose drug laws, which raises the possibility of opioid abuse. However, there are very few documented cases of tramadol dependence from India[4]. Both therapeutic and toxic dosages of tramadol have been known to induce seizures. In one research, a young woman taking tramadol for migraine episodes frequently experienced withdrawal seizures as a result of frequent and excessive ingestion[5]. When tramadol was initially prescribed to treat a fractured tibia, the young male

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patient in this case study self-medicated with the medicine, which led to the development of tramadol dependence and withdrawal seizures.

## Case Report

35-Year-old male, smoker, known case of hypertension on irregular medications presented to ER at 4 pm. History of one episode of jerky movements of all four limbs associated with up rolling of eyes and tongue bite at 11.30 am. The episode lasted less than 10 mins, followed by confusion about 30 mins. No history of any trauma, fever, altered sensorium, headache, vomiting, chest pain, or any focal deficits. Patient was taken to nearby hospital from they received primary care and then referred. Patient received loading dose of Levetiracetam from the outside hospital.

During assessment in ER, airway was patent, breathing – RR –16/min, SPO2 – 96% on room air, air entry was equal bilaterally. In circulation all peripheral pulses felt normal, HR – 82/min, BP – 180/100 mmHg. Disability – GCS: 14/15 (E3V5M6), GRBS: 102 mg%, pupils bilaterally 2 mm and reactive to light. There were no bite marks or Injection marks after exposing the patient.

Patient had no known allergies, was on treatment for hypertension with Amlodipine 5 mg which he was not taking regularly. Relatives gave history of taking analgesic tablets for leg pain. He was sleeping post night duty, at that time brother noticed he is having jerky movements of limbs with up rolling of eyes and tongue bite. On detailed history elicitation patent gave history of consumption of Tramadol tablets 5-10 /day for leg pain post fracture of tibia 2 years back. Last taken 10 tablets one day prior to the onset of seizures.

On CNS examination there was no neck stiffness, cerebellar signs were negative. There were no focal neurological deficits but bilateral plantar reflex was extensor. Other system examinations were within normal limits.

MRI Brain Seizure protocol done in ER showed multiple discrete hyperintensities in deep white matter of frontal, parietal and occipital lobes which were non-specific, probable post-ictal oedema (Figure:1) The blood investigations were largely within normal limits. Haemoglobin, total white cell count, differential counts (neutrophils and lymphocytes), PCV, sodium, potassium, creatinine, calcium, magnesium, and phosphorus are all within standard reference ranges, with no major abnormalities evident (Table:1). Patient was admitted to critical care unit for further monitoring and management.

**Table 1: Results of relevant laboratory investigations.**

| Laboratory investigation | Result |
|---|---|
| Total Count (TC) | 8000cells/µL |
| Haemoglobin (Hb) | 12.3g/dL |
| Neutrophils (N) | 66% |
| Lymphocytes (L) | 22% |
| PCV | 38.7% |
| Sodium ($Na^+$) | 138mmol/L |
| Potassium ($K^+$) | 3.5mmol/L |
| Creatinine | 1.2mg/dL |
| Calcium | 8.7mg/dL |
| Magnesium | 2.41mg/dL |
| Phosphorus | 3.3mg/dL |

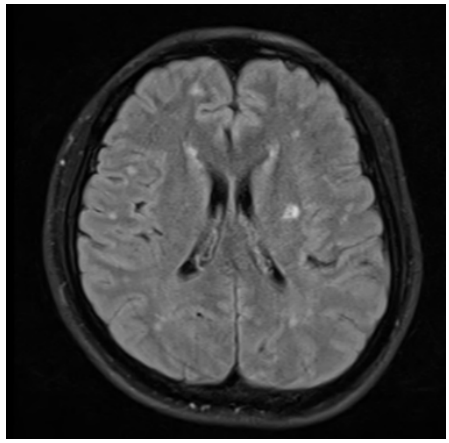

**Figure 1: MRI Brain showing multiple discrete hyperintensities.**

24 hours post admission patient became restless which gradually worsened. Patient had insomnia, tachycardia, pupils were 2 mm bilateral and reacting to light.MRI brain with contrast was performed which showed multiple small T2 FLAIR hyperintense foci in deep and periventricular white matter involving bilateral frontal and parietal lobes with involvement of bilateral semi-ovale. Which likely represent microvascular changes secondary to opioids in view of history. A CSF analysis was performed but no

abnormalities were detected. Baseline clinical opiate withdrawal scale (COWS) score was 8.

Patient was administrated with 2 doses of 0.4 mg of Naloxone and started on IV buprenorphine 300 mcg. There was slight improvement of symptoms with the treatment. Other supportive measures including sedatives continued for 2 days. Clonidine was added to the medications to control blood pressure. After a hospital course of 6 days patient got discharged from the hospital. Buprenorphine IV got replaced by Tablet 5 mg during Discharge.

## Discussion

Tramadol is a synthetic opioid that is a weak MOR agonist. Noradrenaline and serotonin reuptake inhibition also contribute to analgesia. used primarily to treat mild to moderate pain, with little success in treating severe pain. The oral bioavailability of the medication is 68%. It is metabolized in liver and excreted in urine. When taken orally, the medication begins to effect within an hour, peaks in two to three hours, and lasts for six hours in total[6].

The drug frequently causes headaches, dizziness, dry mouth, nausea, vomiting, drowsiness, and respiratory depression. In people with a predisposition, tramadol can both induce and worsen seizures. Patients using MAOIs with SSRIs may experience serotonin syndrome due to the impact on serotonin reuptake. Tramadol abuse and dependence have been documented, despite its unknown potential for abuse[7].

Inhibition of serotonin and norepinephrine reuptake is linked to neurotoxicity following tramadol exposure. The reduced seizure threshold may be explained by the strong inhibition of GABA-A receptors at high tramadol dosages. Seizures are also believed to be caused by histaminergic, dopaminergic, opioid, and GABAergic neurotransmission. At high concentrations, tramadol and its metabolite M1 inhibit GABAA receptors; at clinical dosages, they inhibit NMDA receptors[6].

There are typical and atypical signs and symptoms of tramadol withdrawal. According to their frequency, tramadol withdrawal symptoms include gastrointestinal discomfort, anxiety, bone pain, depression, diarrhoea, sleeplessness, epiphora, nausea, agitation, rhinorrhoea, and excessive sweating. Severe anxiety, panic attacks, atypical CNS symptoms like delusion, confusion, depersonalization, and paranoid thoughts (2.27% prevalence), abnormal sensory experiences like tinnitus, tingling, prickling, and numbness (4.25% prevalence), and haptic, visual, and auditory hallucinations (20% prevalence) are examples of atypical symptoms. It should be noted that atypical symptoms only occur in one out of every eight instances, while typical symptoms are typically observed during the withdrawal phase[8].

Our case had 34-year-old male with history of tramadol abuse for 3 years. He had atypical symptoms like insomnia and agitation. Yates et al. described a 29-year-old woman who initially used tramadol to manage her pain from carpal tunnel syndrome. Over the course of three years, she grew reliant on a daily dosage of 30–50 mg while abstaining from all other medications and opioids. She experienced significant withdrawal symptoms from tramadol, such as headaches, sleeplessness, vertigo, and diarrhoea[9].

Similar history of tramadol withdrawal seizure in a young female was reported in a case report in India. They added that the drug's efficacy at the opioid receptor and the drug's intricate neuropharmacology may be more responsible for the non-specific withdrawal symptoms that were reported and observed[5].

In conclusion, because tramadol acts on opioid receptors, it may cause dependence, although a slight one. As medical professionals, we should be aware of the potential for tramadol dependence and use the drug in an efficient and responsible manner. Tramadol withdrawal symptoms might range from moderate nausea to potentially fatal seizures. We have an ethical obligation to comprehend the kind of side effects that the pharmaceutical industry produces and notify drug regulatory authorities of such incidents so that appropriate scheduling and warnings can be issued.

## Conclusion

This example demonstrates how tramadol, even when administered for valid analgesic reasons, has the underappreciated potential to result in dependence and clinically substantial withdrawal.

Tramadol withdrawal may exhibit a combination of opioid and neuropsychiatric symptoms due to its dual opioid and monoaminergic actions, which could result in an incorrect diagnosis in acute care settings. Physicians should use caution when prescribing tramadol, educate patients about the possibility of dependence, and keep an eye out for increasing dosage needs or recurrent episodes of vague symptoms. To avoid difficulties, early detection, planned tapering, and suitable referral to de-addiction services are crucial. Patient safety can be enhanced and sensible analgesic usage encouraged by bolstering prescribing guidelines and raising professional understanding.

The single-patient form of this report limits its generalizability and makes it impossible to draw conclusions about prevalence or causality. Tramadol exposure could not be objectively quantified, withdrawal severity could not be standardized, and there were no long-term results after the intervention. It was not possible to thoroughly investigate potential behavioural and psychosocial factors that contribute to dependence. To further describe risk factors, clinical patterns of tramadol withdrawal, and the best preventative and treatment practices in various clinical settings, more prospective studies and multicentre observational data are required.

**Declaration:** We declare that we have no financial interests or personal conflicts that may affect the study in this article.

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Case Study

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# Venomous Remedy: A Case of Sodium Thio Sulfate and Methylene Blue Poisoning

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**Abstract**

**Introduction:** Deliberate self-poisoning by ingestion of chemicals used for agriculture, insecticides and disinfectants used in houses is a common modality of poisoning across the globe. Poisoning by tablets containing sodium thiosulfate and methylene blue used as water purifying agent (both compounds are used for treatment of cyanide poisoning) is rare. Here we report clinical profile, relevant laboratory investigations and management of a rare case of sodium thiosulfate and methylene blue poisoning (a first case of dual ingestion poisoning with above mentioned chemical and such reports are not availiable in current published literature).

**Keywords:**

## Introduction

Deliberate self-poisoning (DSP) is a significant global health problem and it is estimated that it accounts for approximately 3,70,000 deaths per year in India.[1] Although Indian data regarding prevalence and implicating agents are lacking, it is widely believed that a vast majority of DSP is attributed to organophosphate, carbamate and corrosive poisoning.[1] At times, clinicians may encounter a rare and unknown offending poison, causing therapeutic dilemma. Here we report a rare and unique case of poisoning with sodium thiosulfate (STS) and methylene blue (MB) who presented with early onset altered sensorium, seizures and respiratory distress. Paradoxically the poisoning in our patient involved two substances which by themselves are used as antidotes. While STS which enhances endogenous cyanide detoxification is used to treat mild to moderate cases of cyanide poisoning, MB is a heterogeneous aromatic compound that is approved by the Food and Drug Administration (FDA) for

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methemoglobinemia and as an antidote to cyanide poisoning. In the absence of an antidote for poisoning by these two substances, therapeutic guidance and paucity of literature regarding this, we treated the patient symptomatically to which he responded.

## Case report

Following a family dispute, a 20-year-old male consumed 100 tablets of THIO (Fig. 1) (each containing STS 0.0150 gms and MB 0.0001 gms) used for dechlorinating water. He was brought to the hospital two hours later in an agitated and confused state. The initial evaluation showed tachycardia (pulse: 176/minute), hypertension (156/88 mm Hg), tachypnea (respiratory rate: 34/minute) and the oxygen saturation at ambient air was 78%. The Glasgow Coma Score (GCS) was 13/15 (E4, V4, M5) and there was no focal neurological deficit. He was admitted to the intensive care of our hospital, two intravascular accesses were established, gastric lavage was performed followed by instillation of activated charcoal. The arterial blood gases showed metabolic and respiratory acidosis (Fig. 2) (pH: 7.23, $pCO_2$: 56 and $HCO_3$: 23.2).

Within an hour of hospitalization he was noted to have worsening sensorium with the GCS dropping to 8/15 and an episode of generalized tonic-clonic seizures. Rapid sequence intubation was concurrently performed to secure the airway. The patient was managed with Nasogastric tube insertion followed by gastric lavage with activated charcoal, benzodiazepine (lorazepam 4 mg sos) and was dilantanized (with 15 mg/kg that is approximately 1 gm sodium phenytoin over 30 mins) for seizure, broad spectrum antibiotics (ceftriaxone & clindamycin) in view of suspicion of aspiration pneumonia (respiratory suppor). Intravenous metoprolol for heart rate control, magnesium sulfate (2gm slow IV) for membrane stabilization and supportive therapy (fluids, proton pump inhibitors, anti-emetics) were also started. Post- intubation, patient was sedated with propofol and fentanyl, but patient continued to throw convulsive seizure. He was administered intravenous levetiracetam 2gm as loading dose following which seizures subsided. His urine output was adequate. Blood investigations revealed leukocytosis (TLC- 21,500/CC), Hemoglobin (Hb) 15 gm%, Hematocrit (PCV) - 44%, platelet – 2,23,000/cc, RBS- 122 mg/dl, urea/ creatinine- 16/1.0 mg/dl, sodium (Na)/potassium (k)- 142/3.6 meq/lit, Bilirubin Total/Direct – 0.4/0.1 mg/dl, SGOT/SGPT-33/43 IU/L, elevated Lactate Dehydrogenase (LDH) -1593 IU/L (Normal range of lab- 240-480 IU/L), C-Reactive Protein (CRP)- 0.5 mg/dl, Calcium – 10.4 mg/dl.

On the second day, patient remained on ventilator and under sedation but had persistent tachycardia. ABG revealed improvement in mixed acidosis. Lab parameters were suggestive of mild anemia (Hb- 12.5 gm%), rest of the metabolic and biochemical profile being within normal limit. Patient maintained good urine output. The chest radiograph was normal, ECG revealed normal sinus rhythm with sinus tachycardia (Fig. 3). He was continued on two groups of anti-epileptic drugs, antibiotics and supportive measures.

The patient was extubated on third day in view of normal vital parameters, improved sensorium and normal ABG. Patient had one spike of fever, which was managed with anti pyretics. Patient remained restless with emotional outbursts for which psychiatrist opinion was sought. He was started on oral feeding and Foley's catheter was removed. Thereafter he recovered well and revealed that he had consumed tablets with suicidal intention. All legal protocols were completed as per the hospital policy guidelines. He was later diagnosed as a case of schizoaffective disorder by the psychiatrist and was started on antipsychotic drugs.

## Discussion

Self-poisoning is one of the oldest methods for committing or attempting suicide. Suicide attempts among young adults, especially in the age group of 21–30 years, may be due to unemployment, break up in the family support system, failure of love relations, an individual's job stress, difficulty in coping with some immediate situation, impulsive behaviors, etc. In any case of poisoning, the mortality and morbidity

relies on a number of factors such as type of poison, amount consumed, availability of medical facilities and the time of interval between intake of poison and arrival at health care facility, etc.[2]

STS is a cyanide poison antidote used along with sodium nitrite and has been conventionally used as an antidote in cyanide poisoning and as a nephron protectant during cisplatin administration.[3-4] It is also used for Calciphylaxis (also called as calcific uremic arteriopathy).[4] STS has known adverse effects gastrointestinal disturbance like nausea and vomiting, headaches, running nose and anion gap acidosis. [5]

MB is an organic chloride salt. It is a commonly used dye, which has medicinal use in view of its antimalarial, anti depressant and cardioprotective properties.[6] The intravenous MB has been approved by the FDA for management of methemoglobinemia in both pediatric and adult patients.[6] MB has off- label use in vasoplegic syndrome (generally defined as an arterial pressure <50 mm Hg, cardiac index >2.5 L /min/$m^2$ , right atrial pressure <5 mm Hg, left atrial pressure <10 mm Hg and low systemic vascular resistance <800 dyne/sec/$cm^5$), a type of distributive shock. MB increases systemic vascular resistance in epinephrine refractory cases.[7] It has been proposed that positive outcome of MB usage in vasoplegic shock is due to its blocking effect on nitric oxide synthase and guanylyl cyclase.[8] MB also finds use as an antidote for cyanide poisoning.[9] Adverse effects of MB include central nervous system-related symptoms such as Serotonin syndrome like diaphoresis, tremors, clonus (due to Monoamine oxidase inhibiting property), cardiac arrhythmia, hemolytic anemia, bluish discoloration of urine.[10]

Our patient developed tachycardia, tachypnea, hypoxemia, elevated Blood pressure and seizure. Tachycardia, elevated BP might have been due to anxiety. Tachypnea and hypoxemia occurred most likely because of aspiration pneumonitis. Occurrence of seizure and observed deranged lab parameters (drop in Hb and elevated LDH) could be attributable to interaction of active constituents of poison or direct effect of poison on nervous system and hematopoietic system respectively.

In our case, being a subzonal peripheral hospital, there was lack of facility for emergent drug level testing, and non availability of any definitive antidote for both the drugs, hence our patient was managed symptomatically with complete recovery.

Owing to the non-availability of an effective antidote of above mentioned poison to date, we emphasize early initiation of supportive management (activated charcoal, airway management, circulatory and hemodynamic support), membrane stabilizer like magnesium sulfate, intensive monitoring (laboratory and radiology) and potential role of anti epileptic drugs in decreasing the likelihood of fatal outcome.

## Conclusion

Both STS and MB have various clinical use in modern medicine. As the drug used by our patient is being used for water cleaning purpose, caution should be taken with respect to storage of these drugs. It's not clear whether symptoms in our patient are due to drug interaction or adverse effect of individual drug. We should be cautious about the presence of anoxia due to status epilepticus effective sedation.

**Statement of ethics:** The written informed consent to publish the case was taken from the patient himself

**Declaration of patient consent:** The authors certify that they have obtained all appropriate patient consent forms. In the form the patient has given his consent for his images and other clinical information to be reported in the manuscript for publication in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Case Study

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# First flight to death: A Case Report

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**Abstract**

Choking is a form of asphyxia in which the internal airways are obstructed. Most cases of choking are accidental and often involve physically compromised or intoxicated people, often eating inappropriate foods or eating too quickly. Here, we are discussing a case of infant death on his first flight to Bangalore. A normal child with no previous history of diseases became unconscious and died. The autopsy revealed that cause of death is choking due to aspiration of milk. Lack of awareness, feeding a child in a hurry, and cramped flight seating arrangements will lend itself to dangerous positioning for breastfeeding and can result in choking.

Key words: Choking; aspiration of milk; infant death; airplane; SIDS

## Introduction

Sudden infant death syndrome (SIDS) is defined as an unexplained death seen in an apparently healthy baby of less than one year and it usually happens while the baby is sleeping. Although the cause is unknown, it appears that SIDS might be associated with defects in the portion of an infant's brain that controls breathing and arousal from sleep. Besides brain defects, respiratory infection, milk aspiration and low birth weight are also the physical factors associated with SIDS[1].

Choking is a form of asphyxia in which the internal airways are obstructed. Gagging is caused by blockage of air passage by a foreign material upto pharynx and chocking is caused by blockage of air passage by a foreign material between pharynx and trachea. Gaggingmay be homicidal if a gag is placed in the mouth and/or pharynx, but most cases of choking are accidental and often involve physically compromised or intoxicated people, often eating inappropriate foods or eating too quickly. The food bolus is usually large, often too large to enter the trachea, and becomes lodged in the posterior hypopharynx, blocking the glottis and the esophagus results in gagging. In this scenario, the person is sometimes able to exhale but cannot inhale. In chocking, the obstructing bolus of food (or other object) passes into, and occludes, the trachea or bronchi.[2] Infants usually regurgitate clotted milk after a meal, and this may fall into larynx. If there

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is a struggle to breathe and removal of occluding object are unsuccessful, asphyxial changes are well marked.[3]

Infants have yet to fully develop their immune systems and are vulnerable to a lot of situations. One of them is an aeroplane/airplane. Low cabin pressure is a difficult situation even for adults, if they are suffering from respiratory system disorders like asthma.

In a study, among flight medical emergencies involving children, the median subject age was 3.5 months with 90% being younger than 2 years, the age until which children are allowed to travel sharing a seat with an adult passenger, also known as lap infants. The number of fatalities involving seemingly previously healthy children under the age of 2 years is intriguing and could indicate a vulnerable population at increased risk of death related to in-flight environmental factors, sleeping arrangements, or yet another unrecognized factor.[4]

In another study, 15.5% flight medical events are involving children, of which 14% involved lap infants.[5] In another study, the most common in-flight consultations concerned infectious disease (27%), neurological (15%), and respiratory tract (13%) emergencies. Nineteen consultations (11%) resulted in flight diversions, most commonly because of in-flight neurological (9) and respiratory tract (5) emergencies.[6]

Here we are presentinga case of death of an infant on his first flight to Bangalore and circumstances lead to his death.

## Case Report

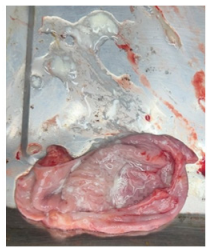
A 45 -days -old male brought dead to casualty after he became unconscious during anairplane journey. Baby was travelling to Bangalore with his mother. After take-off, child start crying and Air hostess told mother to feed the child. Child soon became unresponsive followed by vomiting of reddish white fluid after about 30 minutes of feeding the baby. When they reached the hospital, the baby was already dead. Gestational period, pregnancy, and delivery werenormal.

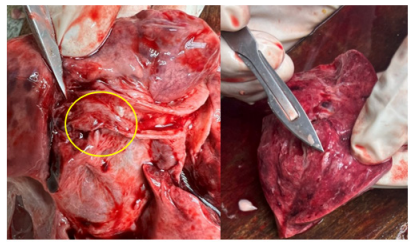
Autopsy examination findings

**External examination**

Body was that of a moderately built and nourished male baby of height 57 cm. Head circumference was 39 cm, chest circumference was 40 cm, abdominal circumference was 36 cm. The conjunctiva was congested with bluish discolouration of lips and fingernails. Rigor mortis was fully established. The postmortem staining was present over the back of trunk and was fixed. No injuries were present on the body.

White milky fluid is present in stomach (Figure 1), trachea (Figure 2) and cut section of lung (Figure 3)





Petechial hemorrhages and hemorrhagic areas were present in outer surface of lungs (Figure 4), cut section of lungs (Figure 5) and heart (Figure 6).

**Internal examination**

Reflection of scalp showed petechial hemorrhages. The brain was intact. Larynx & Trachea contained blood and white curdy particles. Mucosa was congested and oedematous. All lobes of both lungs showed hemorrhagic areas and on cut-section showed hemorrhagic areas that extruded frothy secretions and white curd like particles. The Heart showed petechial hemorrhages over the ventricles. Stomach Contains white curd like particles having no unusual smell, stomach mucosa was congested.

Tissue section of heart and lungs were sent for Histopathology examination; the report of which stated that the lungs showed features that are suggestive of pulmonary congestion and edema.

The cause of death was furnished as "Death was due to respiratory failure as a result of choking due to aspiration of milk".

## Discussion

Epidemiological trends of SIDS indicate that it occurs during post perinatal period (7-365days post-delivery).[7]

In this case, it is important to consider the circumstances that led to the death of the baby. Infants are not fit for fighting all natural conditions. They are immunocompromised and most of their systems are not well developed to thrive varying atmospheric pressure like adults. Low cabin pressure in aircraft is a difficult environment for them. It creates pressure difference in ear, resulting inearaches and crying of baby. Givinga soft-nipple pacifier or breastfeeding are usually the only options available for mother to stop crying of baby.

Flight seating arrangements will cause difficulty in proper feeding and post feeding procedures as there is no separate feeding area in most domestic flights and she would have to do it in her seatsurrounded by other passengers. The baby in this case was the first born, and the inexperience of proper feeding procedures by the mother may result in entry of milk into lungs. Another possibility is that the positioning after feeding, and low cabin pressure which may lead to regurgitation of milk and aspiration into the lungs.

During autopsy, findings like the white milky fluid present in trachea and bronchi; and extruded milk from small bronchioles in the cut-section of lungs are suggestive of choking on milk. Findings like external cyanosis features, petechial haemorrhages present on outer surface of lungs and heart, and congestion of all organs suggest that the mechanism of death is respiratory failure as a result of asphyxia. Multiple haemorrhagic areas in lung parenchyma present on cut-section shows that following occlusion, alveolar rupture happened with capillaries, leads to bleeding which resulted in hemoptysis, but the histopathological examination showed only congestion and oedema in lung tissues. Sometimes histopathological examination fails to find the conclusive findings that are clearly visible during autopsy; either because of effects of formalin or delay in examining the sample or handling of specimen by inexperienced staff resulting in choosing wrong site for grossing. Even though large hemorrhagic areas were present in the autopsy samples, histopathological examination couldnot find any hemorrhage or rupture of alveoli.

It is important to consider that even the histological evidence of leucocyte clustering around foci of gastric contents deep in the bronchi was shown by Gardner to be an early post-mortem event, not necessarily a 'vital reaction'. Almost the only definite evidence of aspiration of gastric contents is either reliable witnessed observation during life or the histological finding of an advanced 'vital reaction' with infection, necrosis and a definite inflammatory reaction. This is a relatively late change and cannot be seen where death occurred within a few hours of aspiration.[8]

Immunohistochemical staining with antibodies against human milk components, especially anti-human α-lactalbumin antibody, can detect small amounts of milk[9,10]. Non-availability of immunological examination in most of pathology departments also create difficulty in testing α-lactalbumin.

## Conclusion

Infants have a greater chance of choking due to immature body systems and other environmental factors. Feeling unsecure and flight seating arrangements will result in dangerous positioning of breastfeeding and choking. Setting a feeding room in an aircraft will give a safe area to mothers for feeding and can save a lot of precious lives. Further, travel recommendations or cabin crew training with regards to lap infants may also go a long way in preventing such cases.

It is also important is such cases that all findings are taken into consideration when forming a conclusion and overdependence on reports be avoided if there is evidence to the contrary.

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Case Study

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# Paraquat Poisoning with Negative Chemical Analysis: Forensic Value of Gross Autopsy Findings in the Pancreas

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## Abstract

**Introduction:** Paraquat is a highly toxic herbicide commonly implicated in fatal poisonings, particularly in agricultural communities. Diagnosis of paraquat poisoning poses a challenge in forensic practice due to rapid tissue distribution, metabolism, and elimination, often resulting in negative toxicological findings. In such cases, autopsy and histopathological findings become crucial in establishing the cause of death. This study highlights the forensic importance of pancreatic haemorrhage as a potential indicator in paraquat poisoning with negative chemical analysis.

**Background:** Paraquat toxicity primarily affects the lungs, kidneys, and gastrointestinal tract, leading to progressive multiorgan failure. However, pancreatic involvement is rarely reported. Additionally, instability of paraquat in biological samples often leads to negative viscera analysis, complicating medicolegal interpretation. Hence, reliance on clinical history and pathological findings becomes essential for accurate diagnosis.

**Methods:** A medicolegal autopsy was performed on a middle-aged male farmer with alleged ingestion of paraquat. Clinical history, laboratory investigations, gross autopsy findings, toxicological analysis, and histopathological examination were reviewed. Viscera, including stomach, intestine, liver, kidney, and blood, were preserved and sent for chemical analysis. Histopathological examination of major organs was conducted to identify microscopic changes.

**Results:** The patient developed severe vomiting, respiratory distress, metabolic acidosis, and acute kidney injury, leading to death on the third day following ingestion. Autopsy revealed congested lungs, haemorrhagic gastric mucosa, subcapsular hepatic haemorrhhages, fatty liver changes, and significant pancreatic haemorrhage. Toxicological analysis of viscera was negative for paraquat and other common poisons. Histopathology confirmed hepatic steatosis, acute tubular necrosis of kidneys, and pancreatic haemorrhage. Differential diagnoses including

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organophosphate poisoning, septic shock, heavy metal poisoning, and primary pancreatitis were excluded based on clinical and pathological findings.

**Conclusion:** Paraquat poisoning should be suspected in cases of rapid multiorgan failure following herbicide ingestion, even when toxicological analysis is negative. Pancreatic haemorrhage may represent a rare but important forensic indicator of systemic paraquat toxicity. This case underscores the importance of integrating clinical history, autopsy findings, and histopathological examination in establishing the cause of death when chemical analysis is inconclusive.

**Keywords:** Paraquat poisoning, Negative toxicological analysis, Pancreatic haemorrhage, Forensic autopsy, Multiorgan failure, Herbicide poisoning, Histopathological findings

## Background

Paraquat is a very toxic herbicide that is one of the major reasons of fatal poisoning in the agricultural society, especially in third world countries. Its high rate of absorption and tissue affinity particularly in the lungs, kidney and gastrointestinal tract causes multi-organ failure and progressive pulmonary fibrosis being the characteristic one. In forensic samples paraquat is instable and is rapidly metabolized thus making it difficult to detect. The adverse toxicological outcomes tend to make the medicolegal investigations rather difficult, which requires relying on the clinical history, autopsy, and histopathological data. By highlighting the diagnostic complexity of paraquat poisoning and pancreatic haemorrhage as an unexplored manifestation of systemic toxicity, the case suggests research value to the forensic literature by informing the integrative approach to diagnosis in failure of chemical analyses.

## Case Presentation

Middle aged man farmer reported to the emergency department with acute respiratory distress and severe vomiting. According to family members, he had deliberately ingested a herbicide which was suspected to be paraquat several hours before. His past medical history did not record any significant illnesses and neither was the social or family history remarkable in contributory factors. On observation, he was found to have yellowish scleral discolouration, bluish nail beds and reddish brown, parchmentized lips. No extrinsic traumas. His condition could not be supported; despite the intensive supportive care, fluids, and monitoring, his health condition deteriorated quickly, and his respiratory distress and the metabolic acidosis worsened. He suffered acute kidney disease and died on day three after taking it. A medicolegal autopsy of the case was requested because of the suspicious character of the ingestion.

### Investigations

Medicolegal autopsy was carried out. On the external inspection, there was some yellowish scleral discoloration, bluish nail-beds, and parchmentized lip lesions. The thorax contained 150 mL of a straw-coloured pleural fluid, and filled lungs (right: 810 g, left: 410 g). The abdomen showed a reddish gastric fluid of abnormal odour, bleeding gastric mucosa, and subcapsular hepatic haemorrhages and changes in fatty liver. On cut section, significant haemorrhage of the pancreas was found and it looked soft and lobulated (Figures B, C). Viscera (stomach, small intestine, liver, gall bladder, kidneys and blood) was stored to be used in toxicological studies and this was found negative of common toxins such as paraquat. Histopathological changes proved the presence of hepatic steatosis, acute tubular necrosis in the kidney and pancreatic haemorrhage. Figures A, B and C depict subcapsular liver haemorrhages, pancreatic haemorrhage respectively. Figure D is a suspected bottle of paraquat (original, created by author).

### Differential Diagnosis

Other toxic ingestions (e.g. organophosphates, heavy metals) and non-toxic causes of multiorgan failure were part of the differential diagnosis (e.g. septic shock or acute pancreatitis). Toxicological screening was negative, excluding organophosphate poisoning as the patient did not have cholinergic symptoms. The clinical presentation was less likely to cause heavy metal poisoning since Mees lines were not found. Septic shock was eliminated because of the lack of fever or foci. Acute pancreatitis was also taken into consideration but ruled as

secondary because pancreatic haemorrhage was in line with systemic paraquat toxicity, not with primary pancreatic disease. The blood investigations at admission were Haemoglobin 14.7g/dl, Total leucocyte count 27200mm$^3$, Creatinine 4.81mg/dl, Potassium 4.12mmol/L, APTT 35.7. Clinical history, acute progressive deterioration, and autopsy (haemorrhagic pancreas, congested lungs, and acute tubular necrosis) were highly pointing toward paraquat poisoning.

**Treatment**

The patient was provided with supportive care in the intensive care unit which included intravenous fluid, oxygen therapy and observation of metabolic acidosis and renal function. No particular antidote to paraquat was used, since there is none. Haemodialysis was taken into consideration and not started because of the rapid clinical deterioration. The treatment was aimed at stabilizing the patient, but the multiorgan failure went on and the patient died.

**Outcome and Follow-Up**

On the third day after the ingestion, the patient died, which was a result of metabolic acidosis and acute renal failure, which is characteristic of paraquat toxicity. Follow up data were not available because the patient had passed away. The autopsy and histopathological results proved to be important in proving the cause of death which was backed by family witness that he ingested herbicides. The patient did not survive making it impossible to apply any form of surveillance or long-term monitoring. It was reported to the legal authorities, and the findings indicated paraquat poisoning despite negative.

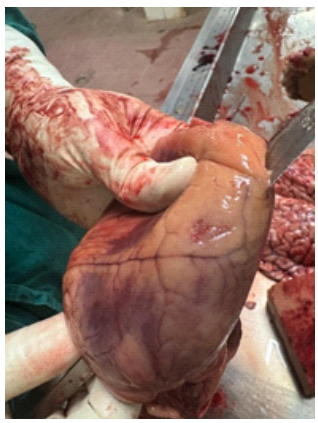

Figure A

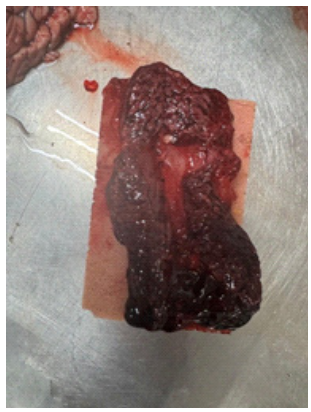

Figure B

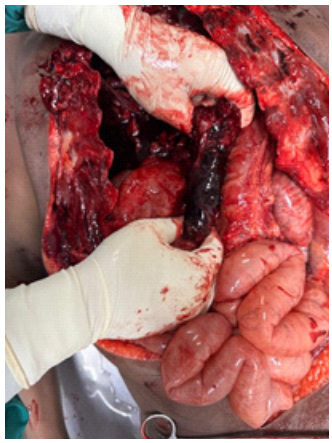

Figure C


Figure D

## Discussion

Paraquat poisoning poses a forensic problem because it rapidly accumulates and breaks down in the tissues which in most cases leads to negative toxicological results as the case is. The mechanism of Paraquat is that this drug causes reactive oxygen species, oxidative stress, cell necrosis, and multiorgan failure especially in lungs, kidney, and gastrointestinal tract [1,7]. In this case, pancreatic haemorrhage, which was a seldom-reported observation, was probably caused by vascular injury at the systemic level by oxidative stress [6]. Other similar cases have documented gastrointestinal erosions, acute tubular necrosis, and lung congestion whereas pancreatic involvement is less frequently reported [4,6]. The recent guidelines are focused on the importance of early decontamination and supportive care but there is still a low survival rate [2,10]. The negative toxicological results indicates that the usage of specialized tests (e.g., HPLC-MS or ELISA) and adequate storage of the sample is necessary to identify paraquat [5,12]. Under circumstances where chemical analysis is not able to confirm diagnosis, forensic pathologists should incorporate clinical and circumstantial evidence with pathological evidence.

Learning points/take home messages

Poisoning with paraquat should be considered in a situation where there is a rapid multiorgan failure after ingesting a herbicide that is negative in toxicological studies.

Pancreatic haemorrhage can be a new forensic indicator of systemic paraquat toxicity.

The extensive autopsy and histopathological analysis are essential to diagnosis where chemical analysis is not conclusive.

Specialized toxicological techniques (e.g., HPLC-MS, ELISA) and storage of the sample in favour of the detection of paraquat are required.

Medicolegal integration by means of a synthesis of clinical history, circumstantial evidence and autopsy findings are essential to accurate diagnosis.

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Case Study

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# Unraveling the Pathology of the Rare Marburg Virus Disease Through Autopsy: A Case Report

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**Abstract**

Marburg virus disease is a rare, highly infectious, and fatal illness with limited autopsy-based studies. This case report details the autopsy findings of a woman who died of Marburg virus disease, offering valuable insights into the pathogenesis of the disease and its effects on various organ systems. The patient initially presented with fever, nausea, joint pain, and fatigue, which rapidly progressed to multiorgan failure and disseminated intravascular coagulopathy. Autopsy revealed extensive hemorrhagic manifestations, including ecchymoses, purpura, and petechiae, on both external and internal surfaces. Significant hemorrhagic effusions were observed in the body cavities, and multiple organs showed signs of congestion, hemorrhage, and edema.This case report contributes to the limited autopsy-based literature on Marburg virus disease, emphasizing the importance of considering it in the differential diagnosis of febrile illnesses in endemic areas and the need for further comprehensive autopsy studies to guide targeted interventions.

**Keywords:** Marburg virus disease; Autopsy findings; Hemorrhagic fever; Multiorgan failure; Febrile illness; Disseminated intravascular coagulation

## Background

Marburg virus disease (MVD), formerly known as Marburg hemorrhagic fever, is a severe, highly contagious, and fatal illness caused by Marburg and Ravn viruses[1]. It was initially detected in 1967 after two simultaneous outbreaks in Germany and Serbia, which were associated with laboratory work using African green monkeys imported from Uganda[2]. Human MVD infection initially results from exposure to Rousettus fruit bats, and then spreads through direct contact with the bodily fluids of infected individuals[3]. Outbreaks and sporadic cases of this rare but highly infectious disease have been reported in Africa [4]. MVD presents significant challenges for healthcare systems owing to its rapid progression, rarity, high mortality rate, and potential

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transmission[5]. Autopsy plays a crucial role in understanding the pathophysiology of MVD and its effects on different organ systems [6]. However, owing to its rarity and high rate of infectivity and mortality, autopsy is infrequently performed for MVD deaths [7]. This limitation has resulted in gaps in our knowledge regarding the correlation between clinical manifestations, disease progression, and its effects on various organ systems[8].

This case report discusses the autopsy of a 35 years old female patient who died of MVD, offering valuable insights into the pathogenesis of the disease and its effects on various organs. By analyzing the macroscopic changes observed during the post-mortem examination, we aim to enhance the current understanding of the relationship between clinical symptoms and specific organ involvement in MVD and potentially guide future diagnostic and treatment strategies. Additionally, this study addresses gaps in the literature concerning the relationship between clinical symptoms and autopsy findings in patients with Marburg Virus Disease.

## Case Presentation

### History

The Ministry of Health officially announced a nationwide state of emergency on September 27, 2024, due to the MVD outbreak, which was subsequently declared its end on December 20, 2024. On September 18, 2024, a 35-year-old female patient visited a hospital with a five-day history of fever, nausea, joint pain, and generalized fatigue, with a body temperature of 37.6 °C. She was then diagnosed with an unspecified infection and treated with Augmentin and paracetamol on an outpatient basis. The following day, she returned to the hospital with worsening of the initial symptoms, and her body temperature was 39 °C with signs of dehydration. She was admitted to the inpatient clinic with a clinical diagnosis of acute malaria and treated with IV artesunate, ceftriaxone, and paracetamol. On September 20, 2024, acalculous cholecystitis was confirmed and intravenous metronidazole treatment was initiated. The patient's liver function and complete blood count were investigated, and the results indicated a slight increase in liver enzyme levels with thrombocytopenia. Malaria-thick smears were performed, which yielded negative results. Chest radiography showed only blunt costo-diaphragmatic angles, and chest CT tomography revealed mild bilateral pleural effusion. Abdominal ultrasonography and CT tomography indicated mild ascites and acalculous acute cholecystitis.

Despite these interventions, the patient deteriorated and experienced three episodes of diarrhea. Further investigations showed deteriorating liver and kidney function and an increase in right pleural effusion. For this, a right pleural tap was performed, which resulted in draining 700 ml of fluid. On September 22, 2024, she developed hypoxia, requiring high-flow oxygen therapy, and the treatment for cholecystitis was switched to intravenous meropenem. By September 23, 2024, the patient was confused with worsening hypoxia and developed cardiac arrest. Despite two and a half hours of advanced cardiopulmonary resuscitation, the patient died. The cause of death was classified as disseminated intravascular coagulopathy (DIC) resulting from multiorgan failure due to sepsis originating from gastrointestinal tract infection.

### Autopsy findings

Ecchymoses were detected on the right lateral side of the chest wall, epigastric area, inner and back parts of the upper third of the right arm, and injection sites (Figure 1). The conjunctiva of both eyes showed yellowish discoloration and petechial hemorrhage. The oral and nasal orifices were occluded with cotton as part of postmortem care. Rigor mortis was not present on the extremities.

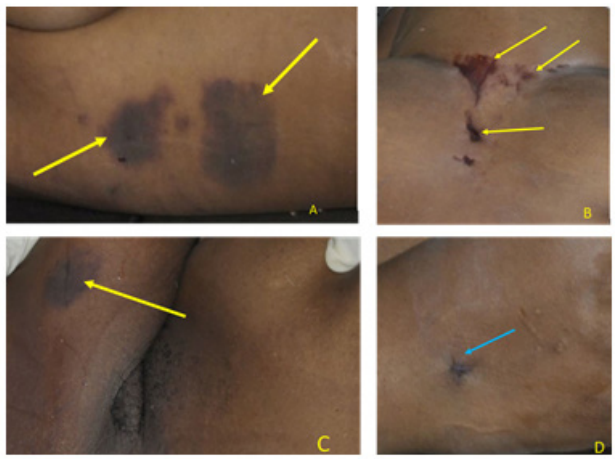


**Figure 1:Ecchymoses underneath the skin on the;**

A. Right lateral side of the chest wall (Yellow arrows).

B. Epigastric area, the site of cardiopulmonary resuscitation (yellow arrows).

C. Posterior aspect of the proximal third of the right arm (yellow arrow).

D. Sutured injection sites on the dorsum of the right hand (blue arrow).

Internal examination revealed ecchymosis, purpura, and petechia on soft tissues of the anterior neck, pharynx, larynx, esophagus, and trachea (Figure 2). The outer surface of the heart and endocardium displayed petechiae and purpura, respectively (Figure 3). The thoracic cavity contained a significant amount of blood with ecchymoses on the lower third of the inner surface of the left lateral aspect of the chest wall(Figure 4A). The pericardial and pleural cavities were effused, with large amounts of blood-tinged fluid. The lungs were heavier and showed signs of edema, bleeding, and consolidation. The peritoneal cavity was filled with a substantial amount of blood (Figure 4B). The stomach was filled with a large quantity of blood and showed hyperemia of the mucosal lining. The liver appeared pale yellow, with petechial hemorrhages on its surface and multiple internal hemorrhages. The spleen was enlarged, darker red, and friable. The gallbladder exhibited diffuse mucosal hyperemia. The pancreas and kidneys showed signs of congestion and hemorrhage. The brain appeared swollen with a grossly bloody appearance of cerebrospinal fluid. There was no evidence of injury. All the other examination findings were normal.

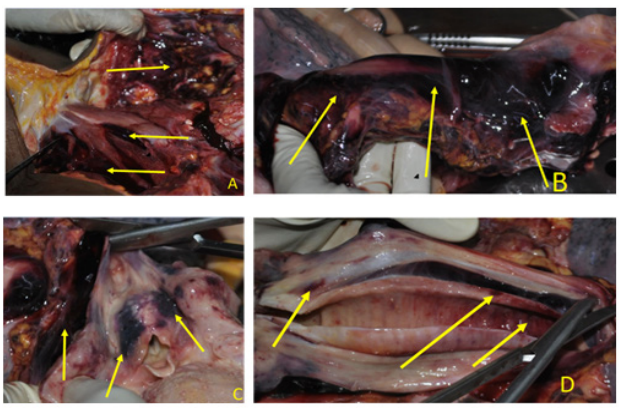


**Figure 2: Ecchymosis, purpura, and petechiae on soft tissues and organs of the neck;**

A. Reflection of the platysma muscle showing diffuse hemorrhage over the muscle compartments and subcutaneous tissues (yellow arrows).

B. Soft tissues at the posterior aspect of the esophagus and trachea showing diffuse hemorrhage (yellow arrows).

C. The hypopharynx, larynx, and soft tissues of the anterior aspect of the trachea showing hemorrhage (yellow arrows).

D. The inner and outer walls of the esophagus and trachea showing hemorrhage (yellow arrow).

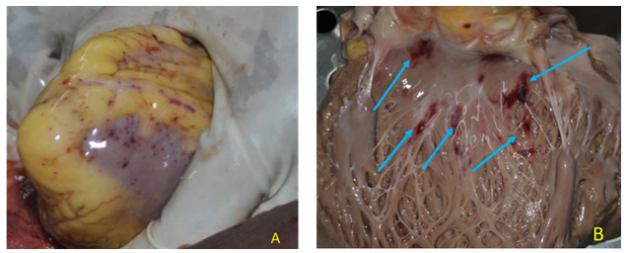


**Figure 3: Petechiae and purpura on the heart;**

A. The surface of the heart apex showing petechial hemorrhages.

B. Endocardium of the left ventricle showing purpura and petechial hemorrhages (blue arrows).

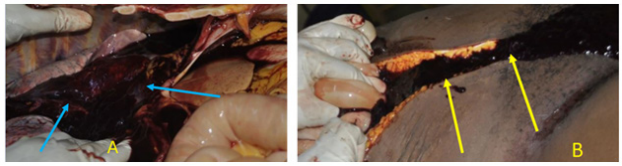


**Figure 4: Extensive hemorrhagic effusion in the body cavities;**

A. The left side of the thoracic cavity showing extensive effusion with blood after reflection of the left lung (blue arrows).

B. The peritoneal cavity showing a massive amount of blood escaping to the autopsy table through the pelvic area (yellow arrows)

Blood samples were collected to investigate Marburg virus infection, and reverse transcriptase polymerase chain reaction (RT-PCR) confirmed a positive result for Marburg virus disease. Tissue samples from the lungs, liver, brain, kidneys, and heart were collected for histopathological examination and blood, urine, gastric contents, and bile samples were collected for toxicological analysis. However, before

conducting the analysis, these samples were disposed of following biosafety protocols to minimize the risk of virus spread. The final autopsy report concluded that MVD was the underlying cause of death.

## Discussion

MVD is a rare and highly infectious disease known for its rapid progression and high fatality rate, resulting in deadly outbreaks; however, its organ-specific pathology remains underexplored.[6,9] Thorough autopsy is crucial for understanding the pathophysiology and specific organ system effects of these diseases [10]. Nevertheless, to the best of our knowledge, studies on autopsy-based MVD are limited. This study details the autopsy of a female patient who succumbed to MVD with signs of acute febrile illness and extensive spontaneous hemorrhage, offering insights into the autopsy findings of a patient with MVD, thereby enhancing our understanding of this rare disease.

MVD is primarily transmitted through direct contact with bodily fluids of an infected person, animal, or contaminated objects. Once the virus penetrates the skin or mucous membranes, it enters the bloodstream or lymphatic system by targeting monocytes, macrophages, and dendritic cells, where it begins to replicate before further spreading to hepatocytes, endothelial cells, fibroblasts, and epithelial cells[3]. Significant viral replication then takes place in the vital organs, such as the spleen, liver, and secondary lymphoid tissues, leading to a cytokine storm and compromising of the humoral immune response[5]. This dramatic immune system dysfunction causes an increase in vascular permeability, tissue damage, and DIC, leading to three phases of disease manifestations: the initial generalization phase, early organ phase, and late organ or convalescence phase[11]. In this case, the patient was initially diagnosed with an unspecified infection, which was subsequently revised to acute malaria and eventually cholecystitis, and appropriate treatment was provided for each diagnosis. Despite these interventions, the patient's clinical condition continued to deteriorate, leading to death. This underscores the difficulties in early MVD diagnosis and highlights the need to consider MVD in the differential diagnosis of febrile illnesses in endemic regions, particularly when standard treatments do not lead to improvement [12].

MVD can cause severe hemorrhage, which involves bleeding under the skin, into the body cavities and internal organs [2]. In this case, ecchymosis, purpura, and petechiae were observed in various internal organs, with extensive hemorrhagic effusion of the body cavities and ecchymosis of the external body parts. This extensive vascular leakage aligns with the widespread and severe effects of MVD on the blood clotting cascade and the vascular system [1]. MVD is a severe hemorrhagic fever with high mortality rates that affects various organ systems and causes severe bleeding, DIC, and multiorgan dysfunction [13]. Our case showed edema, bleeding, and consolidation of the lungs, suggesting acute respiratory distress syndrome, a common complication of MVD [14]. These pulmonary findings explain the patient's clinical presentation of worsening hypoxia, requiring high-flow oxygen therapy. Moreover, our case showed a pale-yellow and hemorrhagic liver along with hyperemia of the gallbladder mucosa, suggesting acute liver failure, which is typical of MVD [15]. This correlates with the slight increase in liver enzyme levels and yellowish discoloration of the conjunctiva noted in the patient's clinical history. The autopsy also revealed an enlarged, dark red, friable spleen, which indicates significant involvement of the reticuloendothelial system in the disease process. Moreover, the presence of blood in the stomach and hyperemia of the mucosal lining support the gastrointestinal symptoms experienced by the patient, including vomiting and diarrhea. The congestion and hemorrhage observed in the pancreas and kidneys further demonstrate the multiorgan involvement characteristic of MVD[10,16]. In addition, the swollen appearance of the brain with blood-tinged effusion in the ventricles suggests cerebral edema and potential neurological involvement, which could explain the patient's clinical history of confusion immediately before death.

Nonetheless, the manifestations of MVD can differ greatly among patients even during the same outbreak, although manifestations of febrile illness are commonly observed[4,17]. Kalungi et al. reported a case involving a patient with MVD who died 11 days after the onset of symptoms. Initially,

the patient exhibited symptoms of febrile illness, which later progressed to bloody stool and severe nasal bleeding and autopsy showed mild ascites and petechial hemorrhages in the subpleural areas of the lungs, with no evidence of hemorrhage in the skin or other organs[18]. This differs from our findings, which identified extensive hemorrhage in the skin, internal organs, and body cavities. The significant differences between these studies, particularly regarding the impact of MVD on internal organs and body cavities, are likely attributable to the Marburg virus strain, comorbidities, or individual immune responses[19,20]. Nonetheless, both studies shared similarities, as death was preceded by febrile illness symptoms with a similar duration from symptom onset to death, and hemorrhagic manifestations in the stomach.

This study offers essential insights into disease pathogenesis and the specific organ system effects of MVD, aiding the creation of targeted interventions. However, there are certain limitations to this study, such as the fact that the findings might not be relevant to all MVD cases, as it is a single case report, and the inability to understand the microscopic changes of the disease due to biosafety concerns of performing histopathological examinations.

In summary, this autopsy case report sheds light on the pathological features of MVD and deepens our understanding of its severe systemic characteristics. Autopsy results offer a detailed perspective on the systemic impact of MVD, aligning closely with the patient's clinical symptoms and disease progression. The extensive hemorrhagic signs, involvement of multiple organs, and vascular damage noted in this case align with the established pathophysiology of MVD and other viral hemorrhagic fever[21].

## Conclusion

This case report provides valuable insights into the autopsy findings of a patient with MVD and contributes to our understanding of this rare and highly infectious disease. The key findings include widespread ecchymoses, petechiae, and purpura on both external and internal surfacesand evidence of multiorgan involvement. This study highlights the challenges in the early diagnosis of MVD, and emphasizes the importance of considering MVD in the differential diagnosis of febrile illnesses in endemic areas.

Although this case report may not be applicable to all instances of MVD, it shares significant role in expanding the limited autopsy-based literature on MVD. Future investigations should aim to conduct more extensive autopsy studies incorporating histopathological analyses across a larger sample size to better understand the disease and guide the development of targeted interventions. Additionally, the disposal of histological and toxicological samples before analysis in line with biosafety protocols underscores the importance of following safety measures in handling MVD cases, which is vital for preventing the spread of this highly infectious disease.

**Abbreviations**

MVD- Marburg virus disease

DIC- disseminated intravascular coagulation

RT-PCR- reverse transcriptase polymerase chain reaction

**Acknowledgments:** We acknowledge the administration of the Rwanda forensic Institute for the permission to conduct this case report.

**Statements and declarations:**

**Ethical approval**: The outlined study protocol for this case report obtained ethical approvalon the date of July 20, 2025, reference number 005/2025 from the research project committees operating within the Rwanda Forensic Institute (RFI).

**Informed consent**: This study is a case report and informed consent for the study was obtained from the family of the deceased.

**Consent to Publish declaration:** Informed consent was obtained from the deceased's family in this study for the article to be published.

**Compliance with ethical standards:** The study was carried out following the ethical standards of the Declaration of Helsinki (Finland).

**Conflict of interest: Payment/servicesinfo:** no financial support was received from any organization for this study.

**Financial relationships:** there is no financial relationships at present or within the previous three years with any organizations that might have aninterestinthis study.

**Other relationships:** there isnoother relationships or activities that could appear to have influenced this study.

**Data availability:** Not applicable.

**Clinical trial number:** Not applicable.

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Original Article

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# Evaluating 7.5 M Ammonium Acetate for the Extraction of DNA from Degraded Saliva Samples: A Comprehensive Forensic Evaluation

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**Abstract**

Recovering DNA from environmentally degraded biological samples remains one of the most persistent challenges in forensic science. Saliva, though highly valuable as a source of epithelial cells, is frequently exposed to detrimental environmental factors that accelerate chemical, enzymatic, and structural DNA degradation. This study thoroughly investigates the effectiveness of a 7.5 M ammonium acetate-based extraction protocol for the recovery of usable DNA from saliva subjected to controlled UV radiation, thermal degradation, and sodium hypochlorite exposure. DNA yield and purity were assessed using NanoDrop spectrophotometry, and STR profiling was performed using the VeriFiler™ Express PCR Amplification Kit. Results demonstrated that although degradation significantly impacted nucleic acid concentration and purity, amplifiable DNA was recovered, producing partial to near-complete STR profiles. These findings indicate that ammonium acetate precipitation is a cost-effective, non-toxic alternative for resource-limited laboratories, with important implications for forensic casework involving compromised biological evidence.

**Keywords:** Forensic DNA extraction, saliva evidence, degraded DNA, ammonium acetate precipitation, STR profiling

## Introduction

Forensic samples often undergo significant degradation before being collected whether through environmental exposure, deliberate destruction, or natural biochemical decay. UV radiation induces pyrimidine dimers and conformational distortions. UV exposure can inhibit successful polymerase chain reaction (PCR) amplification, which is vital for DNA profiling [1]. Thermal exposure accelerates depurination and fragmentation, and can also affect cellular processes, leading to the breakdown of protective proteins and metabolic components. The extent of damage depends on the specific environmental conditions and the stage of the cell cycle at the time of exposure [2]. Chemical agents such as detergents can affect DNA preservation, especially when biological stains are washed before collection. These actions introduce chemical variables that influence the preservation and recoverability of DNA as it can cause base modifications and changes in DNA structure [3].

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Among the various biological materials encountered in forensic casework, saliva represents a particularly common but often overlooked form of trace evidence. It is found on cigarette butts, bottles, envelopes, bite marks, clothing, and various food items [1, 4]. Despite its often invisible nature, saliva contains abundant epithelial cells capable of yielding nuclear DNA. Its non-invasive collection makes it favorable compared to blood or tissue samples [1, 5]. However, saliva residues on surfaces are readily exposed to heat, UV light, detergents, and moisture, making reliable DNA recovery comparatively difficult.

Despite its forensic value, the reliable recovery of DNA remains challenging, particularly when conventional extraction methods are applied to degraded samples. Organic extraction, although capable of producing high-quality and high-molecular-weight DNA, is labor-intensive, time-consuming, and requires the handling of hazardous reagents such as phenol and chloroform, which pose health and environmental risks[6,7]. The Chelex method offers a rapid and cost-effective alternative, however, it produces single-strand DNA and does not effectively remove PCR inhibitors, such as haem and mucin, which can compromise downstream amplification and profiling. Consequently, its application is largely restricted to PCR-based analyses [6, 8].

Solid-phase extraction techniques, including silica column and magnetic bead-based systems, provide improved DNA purity and reproducibility. However, these methods rely on commercially available kits and specialized equipment, resulting in higher operational costs and limited accessibility in resource-constrained laboratories[1, 6].

In light of these challenges, ammonium acetate-based extraction method has gained attention as a promising alternative for forensic DNA isolation due to its ability to efficiently precipitate proteins and SDS while retaining nucleic acids in solution. Studies have demonstrated that 10 M ammonium acetate can yield high concentrations and purity of DNA from saliva samples, with reported 260/280 ratios between 1.8 and 2.0, indicating minimal protein contamination [9, 10]. Additionally, ammonium acetate forms ionic complexes with interfering metabolites, facilitating their removal and improving DNA quality [9]. While these findings highlight its effectiveness for fresh biological samples, the applicability of this method to degraded forensic evidence remains limited. Therefore, further investigation is required to evaluate its reliability for compromised evidence, which is the primary focus of this study.

## Materials and methods

This research was conducted under ethical approval granted by the University of Lincoln, United Kingdom (Ethics reference: 2025_12418).

### Sample collection

Participants provided buccal saliva samples using sterile swabs. Samples were dried, labeled, and stored under controlled conditions prior to degradation.

### Controlled degradation procedures

Three degradation conditions were designed to simulate realistic forensic environments (figure 1):

1. UV radiation for 0 (A), 30 (B), 60 (C), 120 (D) minutes in CL- 1000 Ultraviolet Crosslinker, set at 100 μJ/cm². Simulating sunlight exposure which is common at outdoor crime scenes.
2. Thermal stress via an oven of Fisherbrand at 121°C under 15 p.s.i., for 0 (A), 30 (B), 60 (C), 120 (D) minutes. Simulating exposure to fire, high-temperature environments, or enclosed vehicles.
3. Chemical exposure with sodium hypochlorite (NaClO) with 0% (A), 25% (B), 50% (C), 100% (D) concentration for 3 hours. Simulating deliberate destruction or laundering of evidence.

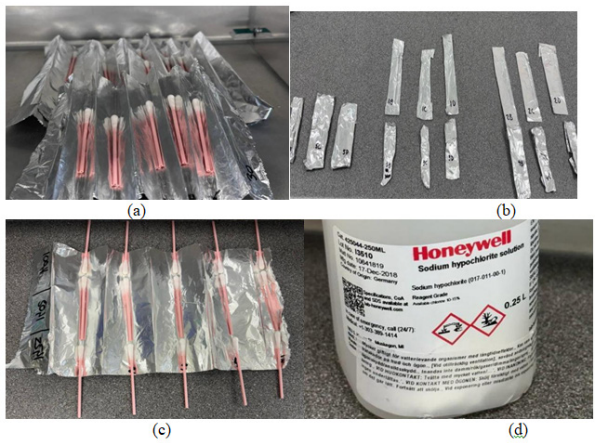



**Figure 1: Samples ready for (a) UV degradation (b) thermal degradation (c) chemical degradation (d) Sodium hypochlorite administered**

**DNA extraction using ammonium acetate**

During buffer preparation, all samples were stored in the refrigerator at 4°C to minimize further degradation. The extraction procedure was adapted from previous research in which the samples were treated with 10 M ammonium acetate [10]. To minimize further DNA degradation during sample processing, lysis was performed in 5% SDS with 50 mM Tris-HCl solution. Strong SDS denatures protein, including nucleases, and enables effective lipid solubilization and disruption of plasma membrane without aggressive mechanical disruption (vortexing) and high temperatures. Each sample received 250 µL of prepared lysis buffer.

In addition, a blank control, containing only the lysis buffer and no biological material was included to monitor for contamination. Tubes were sealed with parafilm and foil, and were incubated at room temperature under gentle rotation (20 rpm) overnight (16 hours and 40 minutes). Post-lysis, samples were centrifuged at 13,300 rpm for 5 minutes.

To the lysate, 125 µL of 7.5 M ammonium acetate was added, yielding a final concentration of 2.5 M. The role of ammonium acetate is to precipitate the proteins and SDS while leaving DNA in the solution.

Samples were refrigerated at 4°C for 15 minutes, then centrifuged at 13,300 rpm for 15 minutes. Subsequently, 1.2 mL of 80% cold ethanol was added, and tubes were centrifuged at 3,000 rpm for 60 minutes at 4°C. A primary wash using 520 µL of 70% cold ethanol was performed, followed by centrifugation at 13,300 rpm for 10 minutes. Ethanol was discharged, and the tubes were air dried in an inverted position. The DNA pellet was then resuspended in nuclease-free deionized water and vortexed gently.

**DNA quantification**

NanoDrop spectrophotometry was used to assess DNA concentration and purity via A260/280 and A260/230 ratios. These indices provided insight into protein contamination, salt carryover, and overall extraction efficiency. The blank control was also analyzed using the NanoDrop to verify the absence of measurable DNA and assess background contamination.

**STR profiling**

For the DNA profiling process, a reduced volume PCR amplification protocol was adapted and validated based on the method outlined in previous research [11]. Reduced-volume PCR was performed using VeriFiler™ Express with 5 µL total reaction volume for 26 PCR cycles, capillary electrophoresis via 3500xL Genetic Analyzer and allele calls were made using GeneMapper™ ID-X.

Accordingly, four samples from each degradation method were randomly selected, representing both higher and lower ends of DNA yield and purity to undergo DNA profiling to assess the extent of degradation and extraction efficiency. In addition to the experimental samples, Blank, PCR positive and PCR negative controls were included to monitor amplification success and detect potential contamination.

**Statistical analysis**

DNA concentration and purity (A260/280 and A260/230) were summarized using descriptive statistics. Differences in DNA purity across degradation methods (UV, thermal, and chemical) at three degradation intensities were evaluated using one-way ANOVA. Independent sample t-tests were then performed to compare each degraded group with its corresponding non-degraded control. To adjust for multiple comparisons within each degradation method, a Bonferroni correction was applied, resulting in an adjusted significance threshold of $p < 0.01$. All statistical tests were two-tailed. Statistical analyses were used to determine whether degradation significantly affected DNA purity following ammonium acetate extraction.

## Results

**DNA concentration**

Referring to figure 2, UV exposure caused a clear, time-dependent decline in DNA concentration, with prolonged irradiation (120 minutes) producing severely reduced yields due to extensive UV-induced fragmentation. Thermal degradation also led to losses in DNA concentration, although the extent varied between samples. In contrast, chemical degradation using sodium hypochlorite produced highly inconsistent DNA concentration results. Some samples showed unexpectedly elevated apparent concentrations, likely due to the release of short, degraded DNA fragments that contributed to absorbance readings rather than intact DNA. Blank reading was 0.1 ng/µL.

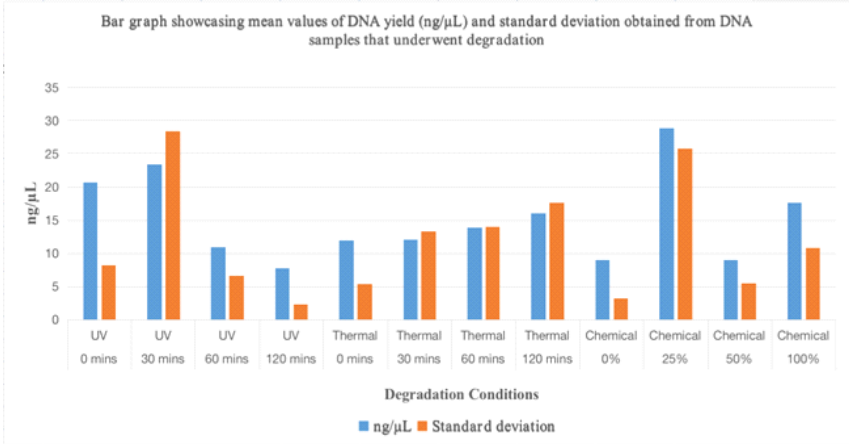



**Figure 2:Bar graph showcasing mean values of DNA yield (ng/µL) and standard deviation obtained from DNA samples that underwent degradation**

## DNA Purity

Referring to Figure 3, UV-treated samples showed a gradual increase in A260/280 ratios with prolonged exposure, rising from approximately 1.4 at baseline to around 1.7 after 120 minutes, indicating a relative reduction in protein contamination despite ongoing DNA damage. Thermal degradation produced a more pronounced increase in A260/280 values over time, with ratios approaching or exceeding 1.8 after extended heating, suggesting possible RNA contamination or changes in nucleic acid composition following heat-induced denaturation. In chemically degraded samples, A260/280 ratios also increased progressively with exposure, reaching values close to 1.8 at higher concentrations, indicating comparatively preserved purity under certain conditions. However, across all degradation models, A260/230 ratios consistently remained below 1.5, reflecting persistent salt and organic contaminant carryover, likely resulting from ammonium acetate residues and the use of a single ethanol wash step. Blank readings were - 0.44 (260/280) and 0.07 (260/230).

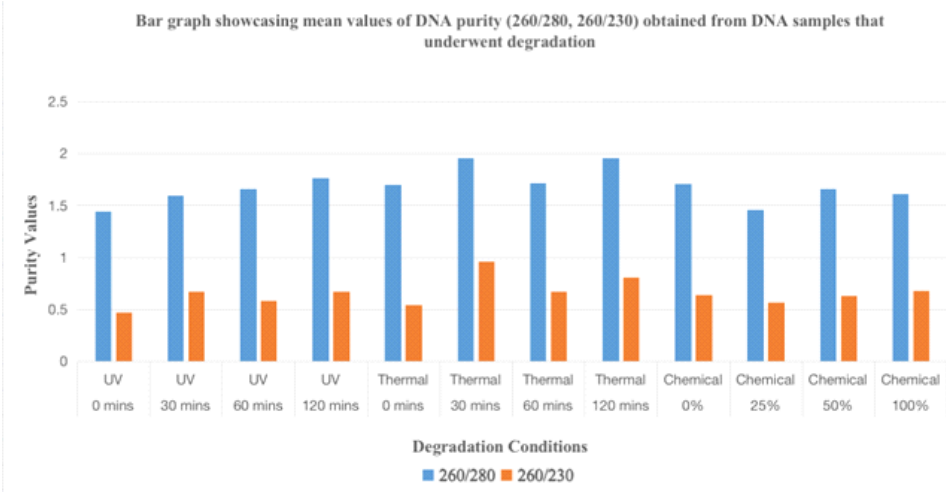



**Figure 3: Bar graph showcasing mean values of DNA purity (260/280, 260/230) obtained from DNA samples that underwent degradation**

## STR Profiling Outcomes

Control samples (0 minute or 0% concentration) produced full profiles. Referring to table 1, as degradation intensity increased, allele dropouts became frequent, particularly after UV and thermal exposure. Chemical degradation preserved profile quality slightly better, though degraded, samples still showed locus specific profiles. Some samples, like 3C-1, even after intense degradation, retained nearly complete profiles, highlighting individual sample variability and the robustness of the method. Negative controls yielded no peaks, confirming experimental validity.

**Table 1: Comparison of STR locus recovery, allelic dropout, and profile completeness in saliva samples subjected to UV, thermal, and chemical degradation**

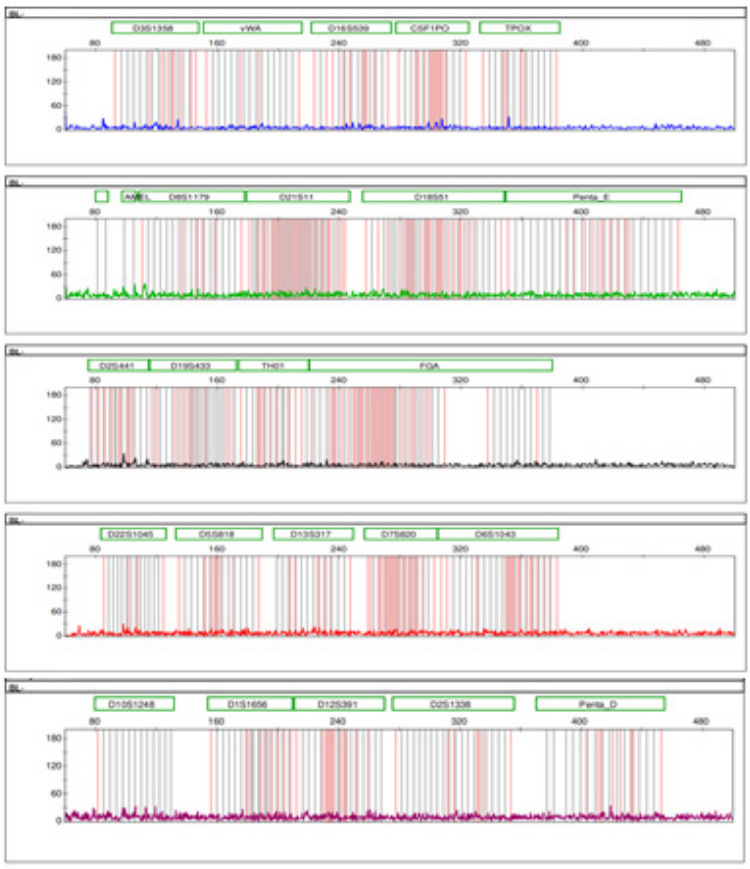
| Sample | Degradation Method | Condition (time/conc) | Total loci | Loci fully typed (n) | Allelic dropout (n) | No allele (n) | Overall profile status |
|---|---|---|---|---|---|---|---|
| 1A-3 | UV | 0 min | 22 | ≈20 | D21S11, PENTA_D | 0 | Near-full profile, minor locus dropout |
| 1D-2 | UV | 120 min | 22 | ≈17-18 | D21S11, D18S51, PENTA_E, D2S1338, PENTA_D | 0 | Partial profile, multiple dropouts |
| 1D-3 | UV | 120 min | 22 | ≈21 | PENTA_D | 0 | Near full profile |
| 1D-5 | UV | 120 min | 22 | ≈20 | TPOX , D21S11, PENTA_D | 0 | Near-full profile |
| 2A-3 | Thermal | 0 min | 22 | ≈20 | D2S1338, D21S11, PENTA_D | 0 | Partial control profile, inherent weak loci |
| 2A-4 | Thermal | 0 min | 22 | ≈19-20 | D16S539, TPOX, PENTA_E, D2S1338 | 0 | Partial profile |
| 2B-2 | Thermal | 30 min | 22 | ≈20 | D8S1179, D21S11, PENTA_E, D13S317, D6S1043 | 0 | Moderate profile loss |
| 2D-5 | Thermal | 120 min | 22 | ≈15-16 | TPOX (possible true homozygote), DS21S11, PENTA_E, D6S1043, D12S391 | D18S51, D13S317, D7S820, PENTA_D | Severely degraded, partial profile |
| 3A-2 | Chemical | 0 % | 22 | ≈18-19 | D8S1179, D21S11, PENTA_E, D13S317, D6S1043, | 0 | Near-full profile |
| 3B-2 | Chemical | 25% | 22 | ≈19-20 | D21S11, D13S317, D651043 | 0 | Near-full profile |
| 3B-5 | Chemical | 25% | 22 | ≈20 | D21S11, PENTA_E | 0 | Near-full, mild dropout |
| 3C-1 | Chemical | 100% | 22 | ≈21 | D13S317 | 0 | Near-full profile despite severe degradation |

**Statistical Results**

Overall, the statistical findings indicate that mild early degradation can affect DNA purity, but severe UV, thermal, and chemical exposure produced similar reductions across all conditions and did not surpass the corrected significance, the Bonferroni-adjusted threshold. (table 2).

**Table 2: Summary of ANOVA and independent t-test results for DNA purity (A260/280) across degradation conditions**

| Analysis Type | Degradation Condition | Comparison | p-value | Significance after Bonferroni ($p < 0.01$) |
|---|---|---|---|---|
| ANOVA | Group 1 (30 min / 25%) | UV vs Thermal vs Chemical | 0.000344 | Yes |
| ANOVA | Group 2 (60 min / 50%) | UV vs Thermal vs Chemical | 0.802 | No |
| ANOVA | Group 3 (120 min / 100%) | UV vs Thermal vs Chemical | 0.107 | No |
| t-test | UV Degradation | Control vs 30 min | 0.17 | No |
| t-test | UV Degradation | Control vs 60 min | 0.05 | No |
| t-test | UV Degradation | Control vs 120 min | 0.07 | No |
| t-test | Thermal Degradation | Control vs 30 min | 0.24 | No |
| t-test | Thermal Degradation | Control vs 60 min | 0.94 | No |
| t-test | Thermal Degradation | Control vs 120 min | 0.27 | No |
| t-test | Chemical Degradation | Control vs 25% | 0.02 | No |
| t-test | Chemical Degradation | Control vs 50% | 0.55 | No |
| t-test | Chemical Degradation | Control vs 100% | 0.30 | No |



(a)

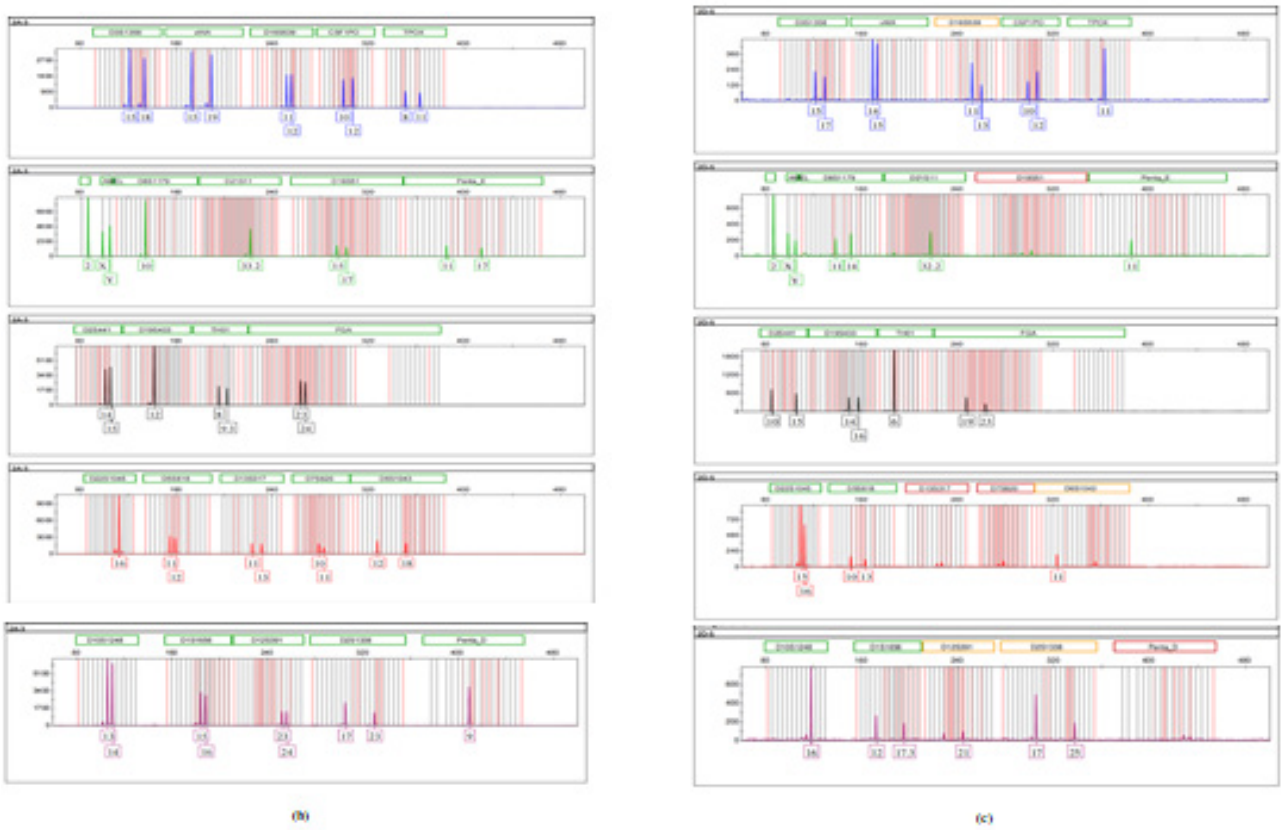


**Figure 1: Comparative electropherograms illustrating STR amplification outcomes for (a) blank, confirming absence of contamination, (b) non-degraded saliva sample, demonstrating a balanced STR profile, and (c) thermally degraded saliva sample exposed for 120 minutes, showing pronounced allelic dropout and locus loss. These figures provides a clear visual comparison of profile quality against the impact of severe thermal degradation on STR performance.**

## Discussion

### DNA degradation under simulated forensic conditions

The findings of this study confirm that ultraviolet radiation, heat, and chemical exposure adversely affect DNA integrity through mechanisms commonly encountered in forensic casework. UV exposure resulted in reduced DNA yield and partial STR profiles, reflecting both direct photochemical damage to pyrimidine bases and indirect oxidative damage mediated by reactive oxygen species. These effects are well documented in the literature and are known to interfere with polymerase activity during amplification, thereby limiting the recovery of complete genetic profiles from environmentally exposed samples [12, 13, 14]. Similarly, thermal exposure produced variable DNA yields and allelic dropout, particularly at larger loci, suggesting a combination of impaired DNA repair capacity and the accumulation of unrepaired lesions. This is consistent with previous reports indicating that heat stress disrupts key DNA damage response pathways, even where the direct induction of strand breaks remains contested [2, 15, 16].

Chemical treatment, particularly with bleach-based agents, did not lead to complete DNA loss, supporting earlier observations that DNA retained within biological fluids can exhibit a degree of resistance to chemical degradation [17]. In this study, chemically exposed saliva samples yielded interpretable STR profiles following ammonium acetate extraction, indicating that visible cleaning or chemical treatment does not necessarily eliminate forensic value. Comparable findings have been reported in studies examining sodium hypochlorite exposure, where DNA persistence was influenced by concentration and exposure time rather than the mere presence of the chemical agent [3, 18].

**Effectiveness of ammonium acetate for degraded DNA extraction**

Previous studies have shown that ammonium acetate yields high-quality DNA with minimal protein contamination. Studies [10] reported average DNA concentrations of 1.48 mg/mL from human saliva using a 10 M ammonium acetate protocol, while demonstrated recovery of high-molecular-weight DNA with consistent A260/280 ratios between 1.8 and 2.0 [9]. The mechanism underlying this efficiency lies in ammonium acetate's ability to form ionic complexes with proteins, oxidized metabolites, and secondary cellular by-products, which migrate to the organic-aqueous interface and are removed during extraction, thereby enhancing DNA purity [9].

In the present study, a modified 7.5 M ammonium acetate protocol was applied to saliva samples subjected to UV, thermal, and chemical (bleach) degradation. This molarity was selected to evaluate that 7.5 M ammonium acetate can achieve DNA yields comparable to commercial kits while reducing salt carryover and solubility issues associated with higher concentrations, which is critical for downstream STR profiling [19]. Across all degradation conditions, DNA was successfully recovered, demonstrating the robustness of the method even under extreme environmental stress.

Despite variability in purity metrics, ammonium acetate consistently enabled DNA recovery from all degraded samples. Persistently low A260/230 ratios indicated residual salt contamination, a known limitation of ammonium acetate-based protocols, which can be mitigated through additional ethanol wash steps without compromising yield [20, 21]. Importantly, ammonium acetate's volatility facilitates its removal during drying, reducing the risk of PCR inhibition compared to stronger salts and chaotropic agents [20].

These findings demonstrate that 7.5 M ammonium acetate is an effective extraction reagent for degraded forensic samples, capable of recovering usable DNA even under adverse environmental conditions. Its low cost, simplicity, and reduced inhibitor carryover highlights its forensic applicability, particularly in resource-limited settings and cases involving compromised biological evidence [9].

**Interpreting DNA profiling and statistical outcomes**

STR profiling demonstrated that the 7.5 M ammonium acetate extraction protocol reliably recovered amplifiable DNA from saliva samples subjected to UV, thermal, and chemical degradation, supporting its applicability to compromised forensic evidence. While partial to near-complete profiles were obtained across all conditions, degradation type and severity markedly influenced locus recovery. Larger loci, including D21S11 and PENTA_D, were particularly susceptible to allelic dropout and imbalance, consistent with the known vulnerability of long amplicons in fragmented DNA [22, 23]. UV exposure produced inconsistent outcomes at equivalent exposure times, reflecting the stochastic distribution of photochemical damage [24]. Thermal degradation caused more progressive and severe locus loss, likely due to fragmentation and depurination processes associated with prolonged heat exposure [15, 16]. In contrast, chemically degraded samples often retain interpretable profiles, supporting evidence that intracellular DNA may be partially shielded from hypochlorite-mediated damage [17].

Quantitative and statistical analyses highlighted the limited value of spectrophotometric measurements as predictors of STR success. NanoDrop derived concentration and purity ratios showed poor correlation with profiling performance, with low-quantity samples often yielding robust profiles and higher-yield samples exhibiting dropout, reflecting the inability of spectrophotometry to distinguish amplifiable DNA from degraded or contaminated nucleic acids [21, 25]. One-way ANOVA revealed significant differences in A260/280 ratios between degradation methods only at early exposure stages, indicating that initial degradation mechanisms influence apparent purity, whereas prolonged exposure leads to convergent damage regardless of degradation method [12, 13]. Notably, thermal degradation produced severe STR deterioration without corresponding changes in NanoDrop purity, likely due to interference from heat-altered proteins [2, 16].

Collectively, these results underscore that assessments of degraded forensic DNA must prioritize STR performance over quantitative purity metrics alone, integrating statistical trends with functional profiling outcomes to support forensic interpretation.

**Comparison with traditional methods and previous studies**

When compared with conventional forensic DNA extraction techniques, the 7.5 M ammonium acetate protocol demonstrated several practical and analytical advantages for degraded saliva samples. Phenol-chloroform extraction, although effective for recovering high-molecular-weight DNA, is labour intensive, hazardous, and involves multiple transfer steps that increase the risk of contamination and further degradation, particularly in compromised samples [6, 7]. In contrast, ammonium acetate offers a simpler and safer workflow while maintaining comparable DNA yields, as reported previously [9, 26]. Chelex-based methods, while rapid and inexpensive, retain PCR inhibitors and generate predominantly single-stranded DNA, limiting their suitability for chemically degraded samples. The successful recovery of near-complete STR profiles from bleach-treated samples in this study highlights the inhibitor removal capacity of ammonium acetate relative to Chelex, consistent with previous findings [9]. Silica-based and magnetic bead methods generally provide high purity and improved recovery of short fragments but are associated with higher costs, making them less accessible for routine or large-scale forensic analysis [27].

The findings of this study are consistent with, and extend, previous research on ammonium acetate extraction. Studies [9, 10] demonstrated high yields and purity from fresh saliva and buccal samples using higher molarities (10 M), whereas the present study shows that a reduced concentration (7.5 M) remains effective for environmentally degraded samples while minimizing salt carryover, with more than one ethanol wash step recommended [19]. As reported [28] ammonium acetate consistently produced the highest yields from fresh saliva. However, storage time significantly reduced recovery after 12 months, yield fell to 0.26 µg, reflecting the impact of prolonged cellular degradation. This aligns with the current findings that, ammonium acetate recovered usable DNA from all degradation conditions but with reduced yields in some severe cases such as in UV and thermal. Chemically degraded samples in this study exhibited comparatively higher DNA yields, consistent with the results reported [17].

## Conclusion

This study demonstrates that a 7.5 M ammonium acetate-based extraction protocol can reliably recover amplifiable DNA from saliva samples exposed to UV, thermal, and chemical degradation, addressing a key gap in forensic research focused on compromised, degraded evidence. Although DNA yield and STR profile completeness declined with increasing degradation, interpretable profiles were obtained across all conditions, confirming the forensic applicability of this low-cost method. Degradation-specific trends were observed, including UV-induced photochemical damage, heat-related fragmentation and protein denaturation, and partial cellular protection against hypochlorite in chemically treated samples [17, 24]. In addition, spectrophotometric measurements were poor predictors of STR success, supporting previous findings that NanoDrop metrics do not reliably reflect functional DNA integrity [21, 25].

The protocol's simplicity, safety, and accessibility makes it particularly suitable for resource-limited laboratories. However, residual salt contamination and reliance on spectrophotometric quantification, limited analytical robustness [20, 24]. Future studies should focus on degradation-specific optimization, integration of qPCR-based quality assessment, and validation using ammonium acetate extraction with targeted clean-up or bead-based enrichment strategies and casework samples. With further refinement, ammonium acetate extraction has strong potential for routine forensic analysis of degraded biological evidence.

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**Ethical Clearance/Statement of Ethics** *(Instruction to authors: Explicitly state the name of the ethics committee clearing the study, along with the date and number)*

Ethics reference UoL2025_12418

Committee UG/PGT University of Lincoln

Date of Ethical Opinion 4 April 2025

**Declaration of conflicts of interest statement if applicable:** Not applicable

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Original Article

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# Retrospective Study of Sudden Death in Medico-Legal Autopsies

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**Abstract**

**Background:** Death is considered sudden or unexpected when a person not known to be suffering from any dangerous disease, injury, or poisoning is found dead or dies within 24 hours after the onset of terminal illness, as per the WHO definition. In such cases, an autopsy plays a crucial role in determining the exact cause of death and helps the bereaved relatives rule out any suspicion of foul play. The present study aims to analyse the age, sex and system-wise distribution of causes of sudden death.

**Methodology:** This retrospective study was carried out at a tertiary care hospital, from 1st January 2023 to 31st December 2023. We reviewed autopsy reports and inquest papers and documented demographic details and cause of death. The data was analyzed statistically.

**Observation:** During the study period, a total of 556(24.7%) sudden death cases were recorded out of 2246 medico-legal autopsies. Most of the sudden deaths (82.7%) were observed in the 31- 60 years of age group with a male-to-female ratio of 3:2. The most common system involved in sudden death was the cardiovascular system (64%) followed by the respiratory system (23%), gastrointestinal system (10%) and CNS (3%). In the cardiovascular system, coronary artery disease (79.5%) was the leading cause of death.

**Keywords:** Medicolegal Autopsy, Sudden death and Natural death.

## Introduction

Sudden death remains a significant public health concern due to its unexpected occurrence, often without prior symptoms or known chronic conditions. These events frequently take place outside clinical settings, with the premortem symptom and conditions surrounding death remaining unknown[1].

Medico-legal autopsies play a crucial role in identifying the underlying causes of sudden death, providing clarity for bereaved families and ruling out unnatural causes. These examinations yield valuable data on the physiological systems involved, contributing to a broader understanding of sudden mortality patterns[2]. Such insights are essential for developing effective public health strategies aimed at prevention and early intervention.

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Although several studies have explored the causes of sudden natural deaths, limited region-specific data are available regarding the demographic distribution and system-wise causes of sudden death in medico-legal autopsy cases, particularly in tertiary care hospital settings. Variations in lifestyle, environmental factors, and healthcare accessibility may influence the patterns of sudden mortality across different populations. However, comprehensive analyses focusing on age, sex, and the physiological systems involved remain insufficiently documented in many regions.

Therefore, there is a need for systematic evaluation of medico-legal autopsy records to identify the patterns and determinants of sudden natural deaths. Understanding these patterns can help in recognizing vulnerable groups and the most commonly affected physiological systems, thereby aiding in early diagnosis, risk assessment, and preventive healthcare planning.

This study focuses on analysing the distribution of sudden natural deaths by age, sex and affected physiological systems, based on medico-legal autopsy records from a tertiary care hospital. By examining these patterns, the research aims to deepen the understanding of sudden death aetiologies and inform targeted preventive measures. The findings seek to enhance awareness of risk factors and support efforts to reduce the incidence of sudden mortality.

## Material and Method

This retrospective study was conducted at a tertiary care hospital from January 1, 2023, to December 31, 2023, reviewing autopsy reports and inquest papers to document demographic details and causes of death, with data analysed statistically. The cases were selected as per WHO definition of sudden death which state that Death is considered sudden or unexpected when a person not known to be suffering from any dangerous disease, injury, or poisoning is found dead or dies within 24 hours after the onset of terminal illness[1]. The study included individuals of all age groups who were either brought dead (e.g., those who died at home) or were hospitalized patients who died within 24 hours of admission, excluding cases with a history of injury, poisoning, or terminal/chronic illness. As the study relied on existing medico-legal autopsy records, approval from the Institutional Ethics Committee was not obtained.

## Observation

During the study period, a total of 556 (24%) sudden death cases were recorded out of 2246 medico-legal autopsies. In the study group, maximum cases of sudden death (34%) were observed in 31-40 years of age group followed by 41-50 years of age (28.6%). Over all 82.7% of participants were aged 31–60 years, showing majority of middle-aged individuals with fewer younger adults. The sample showed a higher predominance of males (male 71%and female 29%).

**Table 1: Age and gender wise distribution of cases**

| Age Group (Years) | Male Cases | Male % | Female Cases | Female % | Total Cases | Percentage (%) |
|---|---|---|---|---|---|---|
| 00–10 | 00 | 0.0% | 00 | 0.0% | 00 | 0.0% |
| 11–20 | 00 | 0.0% | 00 | 0.0% | 00 | 0.0% |
| 21–30 | 53 | 9.5% | 21 | 3.7% | 74 | 13.3% |
| 31–40 | 134 | 24.1% | 55 | 9.8% | 189 | 34.0% |
| 41–50 | 113 | 20.3% | 46 | 8.2% | 159 | 28.6% |
| 51–60 | 45 | 8.0% | 22 | 3.9% | 67 | 11.9% |
| 61–70 | 38 | 6.8% | 15 | 2.6% | 53 | 9.4% |
| 71 & above | 11 | 1.8% | 03 | 0.5% | 14 | 3% |
| Total | 394 | 70.5% | 162 | 29.5% | 556 | 100.0% |

Majority of deaths (64.0%) in this study was caused by involvement of the cardiovascular system. Additionally, 23.0% of deaths were caused by respiratory diseases, 10.0% by gastrointestinal diseases and 3.0% by central nervous system diseases.

**Table 2: Distribution of Causes of Sudden Death**

| Sr. No. | Cause of Death | No. of Cases | Percentage (%) |
|---|---|---|---|
| 1 | Cardiovascular System | 356 | 64.0% |
| 2 | Respiratory System | 128 | 23.0% |
| 3 | Gastrointestinal System | 56 | 10.0% |
| 4 | Central Nervous System | 16 | 3.0% |
| Total | | 556 | 100% |

Among cardiovascular system involvement, most frequently observed condition was coronary artery disease, present in 79.5% of the cases. This was followed by cardiomyopathy (9.8%), myocarditis (9.3%), and cardiac tamponade was noted in only five cases (1.4%).

**Table 3: Cardiovascular System Findings**

| Sr no. | Condition | Count | Percentage |
|---|---|---|---|
| 1 | Coronary Artery Disease | 283 | 79.5% |
| 2 | Cardiomyopathy | 35 | 9.8% |
| 3 | Myocarditis | 33 | 9.3% |
| 4 | Cardiac Tamponade | 5 | 1.4% |
| Total | | 356 | 100.0% |

Among respiratory system, pneumonia was the most prevalent condition, detected in 72.66% of the cases, indicating a high frequency. Pulmonary Koch's accounted for 18.8% of the cases, reflecting a notable burden of pulmonary Koch's. Additionally, interstitial pneumonitis was identified in 8.5% of the cases, contributing to the spectrum of respiratory diseases. These findings emphasize the importance of comprehensive respiratory evaluation in populations at risk for multiple pulmonary conditions.

**Table 4: Respiratory System Findings**

| Sr. No. | Cause of Death | No. of Cases | Percentage |
|---|---|---|---|
| 1 | Pneumonia | 93 | 72.66% |
| 2 | Pulmonary Koch's | 24 | 18.8% |
| 3 | Interstitial Pneumonitis | 11 | 8.5% |
| Total | | 128 | 100.0% |

Findings of table no. 5 indicated that cirrhosis emerged as the most common cirrhosis condition, identified in 36 cases (64.3%). Hepatitis was documented in 10 cases (17.9%), highlighting its significant occurrence. Acute haemorrhagic pancreatitis was observed in 6 cases (10.7%), and intestinal perforation was reported in 4 cases, accounting for 7.1% of the total. These findings illustrate the diverse range of gastrointestinal and hepatobiliary disorders encountered

**Table 5: Gastrointestinal System Findings**

| Sr. No. | Cause of Death | No. of Cases | Percentage (%) |
|---|---|---|---|
| 1 | Cirrhosis | 36 | 64.3% |
| 2 | Hepatitis | 10 | 17.9% |
| 3 | Acute Haemorrhagic Pancreatitis | 6 | 10.7% |
| 4 | Intestinal Perforation | 4 | 7.1% |
| Total | | 56 | 100% |

Among the 16 cases observed due to CNS diseases, intracerebral haemorrhage was the leading cause of death, accounting for more than half of the cases (56.3%). Subarachnoid haemorrhage contributed to one-fourth of the cases (25%), while meningitis was the least common cause, observed in 18.8% of cases. This indicates a clear predominance of cerebrovascular causes (intracerebral and subarachnoid haemorrhage together making up over 80% of cases) compared to infective causes like meningitis.

**Table No.6 Central Nervous System**

| Sr no. | Cause of Death | No. of Cases | Percentage (%) |
|---|---|---|---|
| 1 | Intracerebral Haemorrhage | 9 | 56.3% |
| 2 | Subarachnoid Haemorrhage | 4 | 25.0% |
| 3 | Meningitis | 3 | 18.8% |
| total | | 16 | 100% |

## Discussion

In the present study, majority of sudden natural deaths was observed in the 31–40 years age group (34.0%) fallowed by 41-50 year of age group (28.6%).

The similar findings were observed in study reported by Zanjad N.P. and Nanadkar S.D.[3] (28.56). However, other studies had documented lower incidences, such as Sapate A. et al[4] (23%), Ugiagbe E.E. and Ugiagbe R.A.[5] (16.8%), Azmak A.D.[6] (15.47%), and Bhoi S.B. and Tumram N.K.[7] (14.37%). The variation may be due to differences in demographic patterns and lifestyle risk factors. Although there is a difference, most studies highlight that the middle-aged group remain vulnerable**.** This predominance can be explained by the fact that conditions such as hypertension, ischemic heart disease and diabetes commonly manifest in this age group, making them highly susceptible to fatal cardiovascular events.

In terms of sex distribution, the present study showed male predominance (71%). Similar findings were observed by Zanjad NP and Nanadkar SD[3] (84.8%), Rathva VK and Bhoot RR [8] (80%), Azmak A.D. [6](76%), Kumar T. et al[9] (75.45%) and Bhoi SB and Tumram NK[7] (67.5%). These consistent results across different regions suggest that sudden natural deaths occur more commonly in males because of the higher prevalence of modifiable cardiovascular risk factors such as smoking, alcohol consumption, stress and occupational hazards.

In the present study, majority of sudden death cases was observed related to cardiovascular system (64%). This finding is consistent with the observations made by Sapate A. et al[4] (55%), Kumar T. et al[9] (72%) and Azmak A.D.[6] (55%), who also reported cardiovascular causes as the leading contributors to sudden natural deaths. The finding of the death due to respiratory causes (23%) in the present study was comparable to those reported by Zanjad N.P. and Nanadkar S.D.[3] (27.23%), Sapate A. et al[4] (26%), Kumar T. et al[9] (22%), Azmak A.D.[6] (19.1%) and Bhoi S.B. and Tumram N.K.[7] (27.20%) but, was not consistent with study finding by Ugiagbe E.E. and Ugiagbe R.A.[5](12.5%) which showed lower incidence. The present study and finding of other studies indicated that death due to cardiovascular causes had maximum fatality in young age due to change in lifestyle and it's a worrisome issue.

Coronary artery disease (CAD) emerged as the most common cause of sudden cardiac death, accounting for 79.5% of cardiovascular cases, followed by cardiomyopathy (9.8%), myocarditis (9.3%). This result aligns with global trends, as highlighted in the WHO reports on cardiovascular disease (2023) as a major global health burden.[10] Similar patterns were reported by Zanjad NP and Nanadkar SD[3] (86.47%), Bhoi SB and Tumram NK[7] (72.5%),Chaudhari S.H. et al[11] (71.83), who also reported CAD as the leading cardiovascular cause. However, Sapate A. et al[3] documented a significantly lower proportion of CAD-related deaths (41%), which is in contrast with the present study. The predominance of CAD can be attributed to the high prevalence of lifestyle-related risk factors (hypertension, diabetes, smoking, obesity, stress), making it the major contributor to sudden cardiac deaths across regions.

The present study observed pneumonia in 73% of respiratory system causes. A comparable finding was reported by Azmak A.D.[6](69.8%), showing similarity with our results. However, studies conducted by Zanjad N.P. and Nanadkar S.D.[3] (27.3%), Bhoi S.B. and Tumram N.K.[7] (27.2%), Choudhary S.H. et al[11] (25.7%) and Sapate A. et al[4] (23%) documented lower incidence of death due to pneumonia. The higher proportion of pneumonia cases in the current study may be attributed to an increased prevalence of infectious diseases such as community-acquired pneumonia and tuberculosis in the region, coupled with delayed diagnosis and treatment.

In the present study, cirrhosis was observed in 64.3% of the gastrointestinal causes of sudden death, whereas study conducted by Zanjad N.P. and Nanadkar S.D.[3] observed cases of cirrhosis in 44.4% of cases. However other studies observed different causes like hepatitis, Acute Pancreatitis. This difference may be due to variations in alcohol consumption, occurrence of liver disease, food habits, and availability of medical care which affect how often cirrhosis and pancreatitis occur in different regions.

Central nervous system (CNS) causes accounted for 3% of sudden deaths, with intracerebral haemorrhage being the leading cause (56.3%). These findings are similar to those of Azmak A.D.[6] (45%), who also reported intracerebral haemorrhage as a major CNS cause of sudden death. This may be attributed to long-standing hypertension, which predisposes to rupture of cerebral vessels, resulting in intracerebral bleeding and sudden death.

## Conclusion

In conclusion, this study highlights the critical importance of implementing preventive measures to manage cardiovascular diseases, respiratory conditions, gastrointestinal diseases and neurological health effectively. By prioritizing public health initiatives such as regular screenings, lifestyle modifications and timely medical interventions, the incidence of sudden deaths can be significantly reduced. Furthermore, advancing research and raising awareness about the early signs and risk factors associated with these conditions are essential steps toward enhancing patient outcomes and preventing sudden mortality.

The findings of this study also have important implications for forensic professionals, as medico-legal autopsies remain a vital tool in determining the precise cause of sudden and unexpected deaths. A systematic evaluation of sudden death cases can assist forensic experts in identifying common pathological patterns, improving the accuracy of cause-of-death certification, and contributing valuable data for epidemiological surveillance and public health planning.

However, certain limitations must be acknowledged. The study is based on medico-legal autopsy records from a single tertiary care hospital, which may limit the generalizability of the findings to the broader population. Additionally, the retrospective nature of the study and reliance on available autopsy records may restrict the availability of complete clinical histories and risk factor information.

Future research should focus on multicentre studies with larger sample sizes to better understand regional and population-based variations in sudden natural deaths. Prospective studies integrating clinical history, toxicological findings, and advanced diagnostic techniques such as molecular autopsy may further enhance the understanding of the underlying mechanisms of sudden death and aid in developing more effective preventive strategies.

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Original Article

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# Medico-Legal Aspects of Road Traffic Accidents in the Intensive Care Unit of Heinrich Lübke Hospital, Diourbel (Senegal)

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## Abstract

**Introduction:** Road traffic accidents (RTAs) are a major global public health concern, particularly in low- and middle-income countries. Their human, economic, and medico-legal consequences remain considerable. This study aimed to analyze the medico-legal aspects of deaths resulting from RTAs in the Intensive Care Unit (ICU) of Heinrich Lübke Hospital in Diourbel, Senegal.

**Objective:** To assess the epidemiological, injury-related, and medico-legal characteristics of RTA-related deaths in a regional ICU.

**Methods:** We conducted a retrospective, descriptive, and analytical study of 52 deaths resulting from RTAs recorded in the ICU of Heinrich Lübke Hospital, Diourbel, between January 2020 and December 2024. Data were collected from medical records and analyzed according to sociodemographic variables, accident circumstances, types of injuries, and medico-legal management.

**Results:** Most victims were male (82.7%, n=43), with a sex ratio of 4.8. The median age was 36.2 years (range: 6–72), and 34.6% were under 20 years old. The majority resided in urban areas (73%) and had an occupation (62%). Collisions between vehicles were the leading mechanism (42.3%), with motorcycles involved in 48.1% of cases. Passengers accounted for 51.9% of victims, followed by pedestrians (30.8%). Polytrauma was predominant (57.7%), with cranioencephalic injuries in 82.7% of cases. Death occurred most often within the first week of hospitalization (34.6%). Only 21.2% of victims underwent external examination or autopsy.

**Conclusion:** Road traffic accidents remain a leading cause of mortality among young adults in Senegal, dominated by fatal head injuries. The limited number of medico-legal autopsies restricts the understanding of the real causes of death. Strengthening road safety prevention, improving pre-hospital care, and systematically enforcing medico-legal procedures are essential to reduce mortality and improve forensic rigor in Senegal.

**Keywords:** Road traffic accidents – Forensic medicine – Intensive care – Head trauma – Autopsy – Senegal

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## Introduction

Road traffic accidents (RTAs) represent one of the leading causes of morbidity and mortality worldwide. According to the World Health Organization (WHO), they account for approximately 1.3 million deaths annually and nearly 50 million injuries, with an estimated economic cost of 3% of the Gross Domestic Product (GDP) for most countries[1]. Almost 90% of RTA-related deaths occur in low- and middle-income countries (LMICs), where road infrastructure, traffic regulations, and emergency care systems remain inadequate[2].

Young people aged 5 to 29 years are the most affected group, making road traffic injuries the first cause of death within this age range. "Vulnerable road users" — pedestrians, cyclists, and motorcyclists — account for more than half of all fatalities[1]. In Africa, the road traffic mortality rate is estimated at 27.2 deaths per 100,000 inhabitants, the highest in the world[3].

In Senegal, the situation is equally alarming. In 2014, 12,547 accidents were officially recorded, resulting in 22,441 victims, including 433 deaths[4]. These figures are likely underestimated due to weak reporting systems and the absence of a comprehensive national injury registry. Peripheral regions such as Diourbel are particularly affected, owing to deteriorated road networks, the increasing number of motorcycles, and frequent non-compliance with traffic regulations.

From a forensic perspective, RTAs present major challenges regarding the identification of the exact causes of death, the documentation of clinical findings, and the implementation of judicial procedures. In most regional hospitals, post-mortem examinations or medico-legal autopsies are rarely performed, limiting both the probative value of death certificates and the epidemiological understanding of fatal injuries.

Hospital intensive care units play a strategic role in the observation of severe RTA cases — polytrauma, head injuries, and delayed deaths — providing crucial data for understanding the injury mechanisms, victim profiles, and the adequacy of medico-legal management.

Although road traffic accidents are well documented globally, there is a clear lack of studies focusing specifically on fatalities occurring in intensive care units in Senegal. Most national studies address immediate deaths at the scene or cases handled in emergency departments, but the characteristics of victims who reach the ICU and subsequently die remain largely undocumented. This represents an important research gap, especially in regions like Diourbel, where road safety challenges and limited medico-legal resources coexist.

Furthermore, the scarcity of medico-legal autopsies in Senegal strongly limits the forensic accuracy of death certification. The absence of systematic forensic procedures reduces our understanding of fatal injury mechanisms and weakens the legal value of medical documentation. Therefore, this study responds to a crucial need to document the epidemiological patterns, injury characteristics, and medico-legal management of RTA-related deaths in a regional ICU.

## Materials and Methods

### Study Setting

The study was conducted in the Intensive Care Unit (ICU) of Heinrich Lübke Hospital in Diourbel, a level-2 public healthcare facility located in central Senegal. The hospital, rehabilitated in 2004 through Sino-German cooperation, has a total capacity of 188 beds, including 6 ICU beds equipped for managing life-threatening conditions. The unit is overseen by an anesthesiologist-intensivist and staffed by a team of state-registered nurses and nursing assistants.

Diourbel is situated approximately 145 km from Dakar, at the crossroads of several major intercity roads with high traffic density. The district serves a population estimated at around 378,000 inhabitants, 45% of whom live in urban areas and 55% in rural zones. The main economic activities—trade and agriculture—generate high mobility and thus significant exposure to road traffic risks.

### Study Design and Period

This was a retrospective, descriptive, and analytical study conducted over a five-year period, from January 1, 2020, to December 31, 2024.

**Study Population**

The study population included all patients who died in the ICU of Heinrich Lübke Hospital following a road traffic accident (RTA) during the study period.

**Inclusion Criteria**

- Victims of RTAs admitted to the ICU of Heinrich Lübke Hospital;
- Death occurring during ICU hospitalization;
- Availability of a complete and usable medical file, including clinical, paraclinical, and medico-legal data.

**Exclusion Criteria**

- Incomplete or illegible medical records lacking essential information for statistical analysis;
- Deaths not related to traumatic causes or unrelated to road traffic accidents.

Out of 68 identified deaths related to RTAs, 52 had complete and usable medical files, yielding a data exploitation rate of 76.5%. Files were excluded when essential clinical or medico-legal variables were missing. This approach follows standard methodological principles for retrospective studies, which recommend excluding incomplete records to minimize information bias.

**Study Variables**

The following variables were analyzed:

- **Sociodemographic data:** age, sex, occupation, area of residence, level of education;
- **Circumstances of the accident:** location, time of occurrence, mechanism, type of vehicle, type of road user;
- **Clinical and injury characteristics:** type of trauma, location of injuries, imaging and laboratory findings, presence of nosocomial infections;
- **Medico-legal parameters:** time to death after admission, type of post-mortem medico-legal procedure (simple certification, external examination, autopsy).

**Data Collection and Analysis**

Data were extracted from ICU registers, individual medical records, and death certificates. All information was entered and analyzed using Microsoft Excel® 2021.

A descriptive analysis was performed. Quantitative variables were expressed as means, medians, and ranges, while qualitative variables were presented as frequencies and percentages.

**Ethical Considerations**

This study was conducted in strict compliance with ethical principles, particularly the confidentiality and anonymity of all victims. Prior authorization was obtained from the Management of Heinrich Lübke Hospital before data collection.

## Results

**1. Sociodemographic Characteristics**

A total of 52 victims of road traffic accidents (RTAs) who died in the Intensive Care Unit (ICU) of Heinrich Lübke Hospital, Diourbel, were included in the study.

**Sex and Age**

A clear male predominance was observed, with 43 men (82.7%) and 9 women (17.3%), yielding a sex ratio of 4.8. The mean age of victims was 36.2 ± 15.8 years (range: 6–72 years). Individuals under 20 years old accounted for 34.6% of cases, followed by those aged 21–30 years (25%) and 31–40 years (17.3%).

**Place of Residence and Occupation**

Most victims lived in urban areas (n=38; 73%). Regarding occupation, 32 victims (62%) had identifiable employment. Traders were the most represented (n=12; 23.1%), followed by students (n=7; 13.5%) and motorcycle drivers ("djakartamen") (n=5; 9.6%).

**2. Circumstances of the Accidents**

**Location and Time of Occurrence**

Most crashes occurred on intercity roads (n=30; 58%), compared to urban roads (42%). Accidents occurred more frequently at night (n=23; 44.2%), with a peak around 9 p.m.

**Mechanisms and Types of Road Users**

The most frequent mechanism was collision between vehicles (n=22; 42.3%), followed by vehicle–pedestrian impacts (37.2%) and rollovers (7%). Motorcycles were involved in 48.1% of cases (n=25).

By road user category, passengers were the majority (n=27; 51.9%), followed by pedestrians (n=16; 30.8%) and drivers (n=9; 17.3%).

**3. Hospital Admission and Initial Condition**

All victims were transported by the National Fire Brigade to Heinrich Lübke Hospital. The Emergency Department (ED) served as the primary entry point before ICU transfer. A loss of consciousness was reported in 33 victims (63%).

At admission, 25 patients (48%) were unconscious, and 14 (27%) presented with psychomotor agitation. The Glasgow Coma Scale (GCS) score ranged between 8 and 9 in 48.1% of cases. The delay between ED admission and ICU transfer was less than 24 hours in 61.5% of cases.

**4. Clinical, Paraclinical, and Injury Findings**

**Types of Trauma**

Polytrauma was predominant (n=30; 57.7%), followed by isolated craniofacial or cranial trauma (n=16; 30.8%). Thoracic and spinal injuries were less common (3.8% and 1.9%, respectively).

**Sites of Fatal Injuries**

Cranioencephalic lesions were the most frequent (n=43; 82.7%), followed by thoracic and shoulder-girdle injuries (42.3%). Maxillofacial injuries were reported in 13.5% of cases, whereas pelvic or abdominal injuries were rare (1.9%).

**Imaging and Laboratory Findings**

Imaging investigations revealed central nervous system damage (cerebral hematomas, meningeal hemorrhages, or spinal cord lesions) in 78.8% of cases. Multiple bone fractures were reported in half of the medical records (50%). Laboratory findings showed anemia in 32.7% of cases, and nosocomial infections in 8 victims (15%), most frequently involving *Staphylococcus aureus*.

**5. Outcome and Medico-Legal Aspects**

**Time to Death**

Most deaths occurred within the first week of hospitalization (n=18; 34.6%). Seven victims (13.5%) died within the first 24 hours, and four (7.7%) died within one hour of admission.

**Medico-Legal Management**

Post-mortem medico-legal procedures varied considerably. Only 11 victims (21.2%) underwent an external examination or autopsy. In 78.8% of cases, only a simple medical death certificate was issued, without a medico-legal hold.

Autopsies were mainly performed in polytrauma cases (54.5%) and severe head injuries (45.5%).

**6. Analytical Findings:**

The inferential analyses showed:

- Association between road user type and fatal injury location:

Pedestrians and motorcyclists showed significantly higher rates of cranioencephalic injuries ($\chi^2$ test, $p < 0.05$).

- Association between accident mechanism and polytrauma:

Vehicle-to-vehicle collisions were significantly associated with a higher proportion of polytrauma cases compared to pedestrian impacts ($p < 0.05$).

- Association between time-to-death and initial Glasgow score:

Victims with GCS ≤ 8 tended to die earlier (<48h), although the association did not reach statistical significance ($p = 0.07$).

## Discussion

This study presents several limitations:

- Its retrospective nature exposes it to documentation bias.
- 23.5% of files were excluded due to missing key data.
- Lack of standardized recording of pre-hospital delays and initial vital signs.
- Very low autopsy rate (21.2%), reducing medico-legal accuracy.
- Small sample size limiting generalizability.

This five-year retrospective study, conducted in the Intensive Care Unit (ICU) of Heinrich Lübke Hospital in Diourbel, highlights the high lethality associated with road traffic accidents (RTAs) and underscores the predominance of cranial injuries in

post-traumatic mortality. It also confirms the male predominance, the vulnerability of young adults, and the limited medico-legal coverage in Senegal

**1. Epidemiological Profile of Victims**

The strong male predominance (82.7%) aligns with global and regional findings showing that men are disproportionately affected by road trauma. Similar proportions have been reported in Iran (79%), Jordan (81%), Turkey (77%), Lebanon (77%), Egypt (80%), and the United Arab Emirates (89%)[5–8]. This gender disparity is largely attributed to greater male exposure to traffic—particularly in professional driving and motorcycle use—as well as to risk-taking behaviors such as speeding, alcohol consumption, and failure to wear helmets.

The mean age of 36.2 years reflects the predominance of young, economically active adults, who represent the most vulnerable group in road traffic trauma. Comparable mean ages have been reported in Morocco, Senegal, and Cameroon, ranging between 32 and 40 years[9–11]. This observation carries significant socioeconomic implications, as fatalities often occur among the country's most productive population segment.

The predominance of victims from urban areas (73%) likely results from high traffic density and the widespread use of motorcycles in regional centers such as Diourbel, where helmet use and road discipline remain limited.

**2. Circumstances and Mechanisms of Accidents**

Intercity roads accounted for the majority of crashes (58%), consistent with findings by Mansouri et al. in Morocco (53%) and Ndiaye et al. in Dakar (60%) [9,10]. Poor road maintenance, inadequate lighting, and lack of signage on secondary roads increase the risk of head-on collisions and loss of vehicle control.

The most frequent mechanism was vehicle-to-vehicle collision (42.3%), corroborating findings from other African studies[9–12]. Motorcycles were involved in nearly half of all crashes (48.1%), reflecting the rapid expansion of motorcycle transportation across West Africa. These two-wheeled vehicles, with minimal structural protection, expose users—especially young males—to a significantly higher risk of fatal injury. Studies from Burkina Faso and Benin reported that over 80% of motorcyclists did not wear helmets at the time of the crash[13,14].

Most accidents occurred at night (44.2%), which can be explained by driver fatigue, reduced visibility, excessive speed, and, in some cases, alcohol consumption. Similar temporal trends have been observed in France and Morocco, where the majority of fatal accidents occur between 6 p.m. and midnight[15,9].

**3. Types of Trauma and Fatal Lesions**

Polytrauma dominated our series (57.7%), followed by isolated cranioencephalic trauma (30.8%). These results are consistent with those of Mansouri et al. (Morocco: 46.8% polytrauma) and Ndiaye et al. (Senegal: 52% severe head trauma)[9,10].

Cranial injuries, present in 82.7% of cases, played a decisive role in mortality. The head is particularly vulnerable in motorcycle crashes, especially in the absence of protective helmets. According to the WHO, correct helmet use reduces the risk of death by 40% and head injury by 70%[1].

Thoracic (42.3%) and maxillofacial injuries (13.5%) were frequent, reflecting the high kinetic energy involved in most collisions. Multiple fractures and pulmonary contusions identified through imaging support the notion of high-impact mechanisms.

The predominance of intracranial hemorrhages and polytrauma as causes of death has been consistently reported in several African studies[10,11,13], emphasizing the importance of early, multidisciplinary resuscitation.

**4. Time and Circumstances of Death**

The median time to death (approximately 40 hours) indicates that most victims succumbed within the first few days of hospitalization, before stabilization could be achieved. This finding is consistent with Mansouri et al. (median: 72 hours) and Pérez et al. in Spain (92% of deaths within 30 days)[9,16].

The prognosis is influenced by several factors: the severity of initial injuries, the delay in evacuation, the availability of ICU resources, and the quality of neurological monitoring. In our setting, delayed transport, absence of medicalized pre-hospital

care, and limited technical capacity remain major aggravating factors.

**5. Medico-Legal Aspects**

One of the most striking findings is the limited implementation of medico-legal procedures: only 21.2% of deaths were subjected to external examination or autopsy. This proportion is far below international standards, revealing a persistent lack of medico-legal culture in Senegalese hospitals.

According to Senegal's Code of Criminal Procedure and WHO recommendations, all suspicious, accidental, or traumatic deaths must be subject to a medico-legal hold ("obstacle médico-légal"), prohibiting burial before forensic examination by the prosecutor or a qualified pathologist[17].

The low autopsy rate (21%) confirms the medico-legal deficit previously highlighted by Amundsonet al.[18] and Mbassi Awa et al.[19]. Several barriers contribute to this situation: shortage of forensic physicians, reluctance of families, and insufficient awareness of the judicial importance of autopsy.

Our results emphasize the urgent need to strengthen road safety prevention, improve medico-legal training, and systematically enforce medico-legal procedures for all trauma-related deaths[20–24].

**6. Implications and Perspectives**

The findings of this study call for several key actions:

- Reinforce road safety measures, including stricter enforcement of helmet and seatbelt laws;
- Train motorcycle drivers and regulate informal urban transport;
- Improve pre-hospital emergency care by equipping fire and rescue services with advanced medical transport systems;
- Develop regional medico-legal services, ensuring the systematic implementation of medico-legal holds for all accidental deaths.

## Conclusion

Road traffic accidents remain a critical cause of mortality in Senegal, particularly among young adults. This study highlights the predominance of severe head injuries and the systemic constraints affecting medico-legal documentation. Strengthening road safety measures, improving pre-hospital care, and ensuring systematic medico-legal procedures for traumatic deaths are essential.

This study highlights several systemic shortcomings: insufficient medicalization of pre-hospital transport, delayed evacuation, limited intensive care equipment, and the near absence of medico-legal autopsies. These deficiencies not only compromise the quality of clinical management but also undermine the forensic reliability of death certification and legal documentation.

There is an urgent need to strengthen road safety measures—through public awareness campaigns, strict enforcement of helmet and seatbelt use, and speed regulation—while also improving trauma and intensive care capacity at the regional level. Furthermore, it is essential to mandate medico-legal procedures ("legal holds") for all traumatic deaths and to develop a national road trauma registry to facilitate epidemiological monitoring and forensic accountability.

Finally, training hospital physicians in medico-legal documentation and death certification will enhance forensic accuracy, justice delivery, and public health surveillance, ultimately contributing to a sustainable reduction in RTA-related mortality in Senegal.

Future studies should be multicentric, prospective, and incorporate systematic autopsies where possible. Developing a national trauma registry would significantly improve surveillance and forensic reliability.

Conflict of Interest and Funding Disclosure

**Conflict of Interest:** The author…, certifies that this manuscript is an original work and has not been submitted to or published in any other scientific journal. The study was conducted independently, without any external sponsorship or institutional support. The manuscript is submitted to the *Indian Journal of Forensic Medicine & Toxicology* in full compliance with the journal's authorship and publication policies.

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Original Article

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# Evaluate 11 Plex SNPs of the MC1R Gene for Eye Color Prediction using the SNaPshot Technique in an Iranian Population

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**Abstract**

The most ambitious DNA phenotyping goal is forecasting a full face from a DNA sample. Over the last decade, GWAS and successive predictive analyses have discovered a multitude of EVC-predictive SNPs and predictive models, most particularly for human pigmentation features. IrisPlex, the first forensic eye color prediction system, was designed mainly to differentiate between blue and brown eyes, and its evolved version, the HirisPlex-S DNA testing method, predicts eye, hair, and skin color based on DNA traces. 59 DNA samples were examined using the Multiplex SnaPshot kit (Applied Biosystems) for the simultaneous detection of 11 SNPs (rs1805005, rs885479, rs11547464, rs185008, rs1805006, rs1805007, rs1805009, rs2228479, rs1110400) taken from a large-scale GWAS research. This study adopted the genotype identification of 11 Hirisplex system markers as a prediction model of eye color and hair color. Some of these variants (rs11547464, rs885479, rs1805005, rs2228479) were found to be relevant for anticipating eye and hair color, whereas others (rs1805008, rs1805006, rs1805007, rs1805009, rs1110400, Y1520CH, N29insA) were observed to be unacceptable because of low variation. The statistical data illustrated a substantial level of agreement between the statistical model and the actual eye color of the participants.

Keywords: Hair color, Eye color, MC1R, SNaPshot, Iranian Population

## Introduction

Forensic DNA phenotyping (FDP) uses basic genetic knowledge to understand more about an unknown crime scene donor. FDP is being introduced into the field of forensic genetics using large-scale sequencing methods for two reasons. First, it overcomes the limitations of conventional

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human identification techniques, and second, it is evidently advantageous as a police investigative tool to narrow the group of suspects[1]. DNA phenotype allows the projection of phenotypic traits based on genetic information. In cases where there is no proof to identify a person, this reduces the number of likely suspects. The forensic community's interest in phenotypic trait prediction has grown as more genes associated with various physical traits have been discovered[1,2]. Most human traits are considered complex because they are influenced by multiple genes as well as environmental factors, and their variability is continuous (Serrano, 2020). Eye, hair, and skin pigmentation are among the most studied human phenotypic traits, as well as facial features, since they provide the most data in criminal cases when searching for and identifying suspects. Because the former phenotypic traits are less complicated, studies on the genetics of human pigmentation are more sophisticated than studies on facial traits. This is because they have semi-Mendelian heredity, meaning that a small number of genes provide most of the phenotypic information [1,3]. Pleiotropic effects, in which a single SNP affects more than one phenotypic trait, accelerate the genetic difficulty of color[4]. The same is true for the occurrence of epistasis, which takes place when many SNPs interact in the creation of a single trait[1]. SNPs are single nucleotide polymorphism markers that regulate the phenotype of an individual. They have now been demonstrated to be very suitable alternative markers for STRs in criminological studies[5]. SNPs have several benefits over STRs in forensic studies, including a lower mutation rate, which is critical in kinship testing. Another benefit of SNPs is that they can be examined quickly, comprehensively, and automatically[6]. Today, attempts are being made to study more than 50 SNPs simultaneously in a short period of time. Although several research efforts have been conducted to evaluate the viability of using SNPs in different contexts, no unified approach for using SNPs in criminal investigations has been offered[7].

The most ambitious aim of DNA phenotyping is to predict an entire face based on a DNA sample[8]. Several attempts have been made to develop a predictive face model [8–11]. GWAS and subsequent predictive analyses have found several EVC-predictive SNPs and predictive models over the past decade, notably for human pigmentation traits[9]. The first forensic eye color prediction system, IrisPlex [12] was explicitly developed to distinguish blue and brown eyes[13]. The HIrisPlex-S prediction system is the latest test that can predict eye, hair, and skin color simultaneously [1,13]. HIrisPlex-S DNA testing technology enables simultaneous projection of eye, hair, and skin color based on DNA traces. The FDP system consists of two SNaPshot-based multiplex assays targeting a total of 41 SNPs, including 24 SNPs for eye and hair color prediction and 17 SNPs for skin color prediction [9]. Walsh et al. conducted a study on 6168 Dutch populations to confirm the HIrisPlex system. The outcomes of this study showed that some variations of the melanocortin-1 receptor gene (MC1R) have high penetrance [12]. In the study conducted by Spichenok et al. on 544 individuals, they concluded that the iris plex system has a low error rate in determining eye color in different races in the United States. In fact, this study confirmed the accuracy and correctness of this marker collection [14]. Allwood et al. and Kastelic et al. also confirmed the capability of the iris plex system to predict eye color [15(p2013)]. (In order to provide forensic intelligence SNP data from latent DNA, Young et al. report the first use of direct PCR combined with MPS by examining the HIrisPlex System [16]. Breslin et al. provide massively parallel sequencing (MPS) possibilities for the HIrisPlex-S (HPS) system using the two MPS technologies frequently used in forensics, Ion Torrent and MiSeq, to cover all 41 DNA variants in a single test. They also exhibit the forensic developmental validation of the two HPS-MPS tests[17].

In this field, many similar experiments have been conducted worldwide, but in Iran, such a study based on the allele frequency of SNPs of the HIrisPlex system has not been conducted yet. The objective of this investigation was to investigate the frequency of some SNP markers (1805005, rs885479, rs11547464, rs185008, rs1805006, rs1805007, rs1805009, rs2228479, rs1110400) related to hair and eye color in a sample of Iranians.

## Materials and Methods

Blood samples were randomly collected from 59 subjects, including 22 men and 37 women of different Iranian ethnicities, among unrelated individuals; DNA was then extracted from all samples using a

standard salting-out protocol. Multiplex design and genotyping methodology A multiplex single-base extension reaction test was utilized to examine 59 DNA samples. All participants were tested for 11 SNPs simultaneously. We developed a multiplex single-base extension approach for the simultaneous identification of every 11 SNPs (rs1805005, rs885479, rs11547464, rs185008, rs1805006, rs1805007, rs1805009, rs2228479, rs1110400) taken from a large-scale GWAS research (Liu et al., 2010), employing the Multiplex SNaPshot kit (Applied Biosystems) for the reaction, followed by capillary electrophoresis on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems). The PCR primers were designed with OLIGO Primer Analysis Software Version 7 and Gene Runner Version 6.5.52x64 Beta to generate PCR fragments ranging from 106 to 158 bp. To eliminate primer-primer interactions, all primer sequences were analyzed using the Auto Dimer software application [47] before laboratory analysis. Table 1 contains all of the primer sequences. All 11 SNPs were amplified using PCR primers (5 pmol concentration), 1 µl genomic DNA (50 ng concentrations), and attempts to use master mix Red2x (AMPLIQON) in a single multiplex PCR assay in a total volume of 25 µl.

**Table 1: The quantity of AUCs estimated for the hair and eye color SNPs in the research**

| | Hair color | | | Eye color | |
|---|---|---|---|---|---|
| **SNP** | **Black Hair** | **Brown Hair** | **Red Hair** | **Intermediate eye** | **Blue eye** |
| rs11547464 | 0.532 | 0.658 | 0.636 | 0.531 | 0.531 |
| rs885479 | 0.524 | 0.505 | 0.532 | 0.542 | 0.542 |
| rs1805005 | 0.526 | 0.608 | 0.638 | 0.510 | 0.510 |
| rs2228479 | 0.559 | 0.540 | 0.503 | 0.522 | 0.522 |

All amplifications were performed on a Minicamp™ Plus Thermal Cycler (Thermo Fisher scientific) with the following conditions: 95°C for 10 minutes, 35 cycles of 94°C for 1 minute, 64.4°C for 1 minute, and 72°C for 1 minute. The last extension was 10 minutes at 72 degrees Celsius. The amplified PCR products were purified using EXO I (exonuclease I) and SAP (shrimp alkaline phosphatase) and incubated for 60 minutes at 37°C and 15 minutes at 75°C. In the following single-base extension technique, the SBE primers annealed around the single-nucleotide polymorphism (SNP) region. Poly (dt) tails of varying lengths were added to single-base extension primers (Table 2).

**Table 2: Sequences of Single-Base Extension Reaction Primers and PCR Primers**

| SNP | primer | Sequence (5′-3′) |
|---|---|---|
| rs12913832 | PCR For | TAATTCAAAATGCCCCCAAG |
| | PCR Rev | TGATAGCGTGCAGAACTTGACA |
| | SNaPshot | TGATGATAGCGTGCAGAACTTGACA |
| rs1800407 | PCR For | TGAAAGGCTGCCTCTGTTCT |
| | PCR Rev | TATGGTCACAGGCGTGAAGA |
| | SNaPshot | GACTGACTGACTGACTGACTGACTGACTGACGTGGCCAGGCATACC GGCTCTCCC |
| rs1393350 | PCR For | GATGCGTGCATATCCACCAACT |
| | PCR Rev | GGTGAATGATAACACGAACAGATT |
| | SNaPshot | GACTGACTGACTGACTGACTGACTGACTGAGTAAAAGACCACACAGATTT |
| rs16891982 | PCR For | GGAAAACACGGAGTTGATGC |
| | PCR Rev | CCAGAGGTGGAGAAGCAGAG |
| | SNaPshot | GACTGACTGACTGACTAGAGGAGTCGAGGTTGGATGTTGGGGCTT |

Cont....

| | | |
|---|---|---|
| rs12896399 | PCR For | CTGGCGATCCAATTCTTTGT |
| | PCR Rev | GACCCTGTGTGAGACCCAGT |
| | SNaPshot | ttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttTCTTTAGGTCAGTATATTTTGGG |
| rs12203592 | PCR For | AGGGCAGCTGATCTCTTCAG |
| | PCR Rev | GCTTCGTCATATGGCTAAACCT |
| | SNaPshot | ttttttttttttttttttttttttttttttttttttttttttttttttTCCACTTTGGTGGGTAAAAGAAGG |

The SNaPshot reaction contained 1 µL of primer mix, including all 11 single-base extension primers (final concentration of 1 pmol each), 1.5 µL of purified PCR products, and 2.5 µL of SNaPshot Multiplex kit (Applied Biosystems by Thermo Fisher Scientific) in a large volume of 5 µL. The PCR condition consisted of 25 cycles of 10 seconds at 95°C, 5 seconds at 50°C, and 30 seconds at 60°C. After the reaction, the 5′-phosphoryl groups of unincorporated dideoxy nucleotide triphosphates were removed by adding 1.0 U of CIP (Calf Intestine Phosphatase) and incubating the enzyme at 37°C for 45 minutes and 75°C for 15 minutes. Capillary electrophoresis was carried out using an ABI PRISM 3130 Genetic Analyzer, and for allele calling and data analysis, Gene Mapper ID, Ver. 3.7 (Thermo Fisher Scientific) was used. A number of samples were submitted for sequencing to confirm the observations of the SNaPshot approach from all 11 SNPs. Iris Photography Tocompares and assess, the iris of all participants was photographed in two ways. Due to the sensitivity of the study and inclusion criteria, the expert physician was supported in identifying the samples that did not fulfill the inclusion condition in the first technique. The second method was to examine HD-quality photographs of the iris of study participants taken using a digital camera and the same photographic conditions. The aim of iris photography was to discover a reasonable and effective link between each individual's iris color and their IrisPlex profile in the study population. Topcon camera photography (by an ophthalmologist) After validating the inclusion criteria, all participants were evaluated by a specialist who photographed their iris. It should be mentioned that the ophthalmologist investigated the natural color of the iris, illnesses, injuries, and the application of effective medications in the color of all the volunteers' eyes. We used a Topcon IM 900 camera (Fig. 1) and EyeSuite software to process the photographs. To analyze the iris with Eyesite software, we needed standard and high-quality photographs taken in standard conditions of the volunteer's eyes. The Canon EOS 70D professional camera with the Canon EF 100mm Macro F2.8 L IS USM lens was used for all photography in similar conditions in terms of distance and amount of light. We also developed software to identify eye color using IMI and RGB indices in partnership with the Department of Biomedical Engineering at Islamic Azad University.


**Fig 1: The spectrum of different decreasing colors of the iris from 1 to 12, photographed with a Canon PowerShot digital camera and a macro lens by a professional photographer under the identical environmental conditions for all participants**

**inquiry into hair color**

The red hair color was assessed using people's self-reports, family history, and the completion of a questionnaire. The photograph was shot with a Topcon camera at a distance of 7 cm from the back of the head to examine the color of the hair.

In this study, we employed a multi-nominal regression model and the HIrisPlex system's resultant profile to reliably predict blue and intermediate eye color, as well as black, brown, and red hair color.

## Results

The multiplex SNaPshot approach was confirmed by assessing 10 DNA samples genotyped earlier by DNA sequencing for each examined SNP. To ensure the performance of SNaPshot (Fig. 2) from each series of samples associated with an SNP detected by the SNaPshot diagnostic system, we sequenced several samples, and the sequencing findings validated the SNaPshot results (Fig. 3).

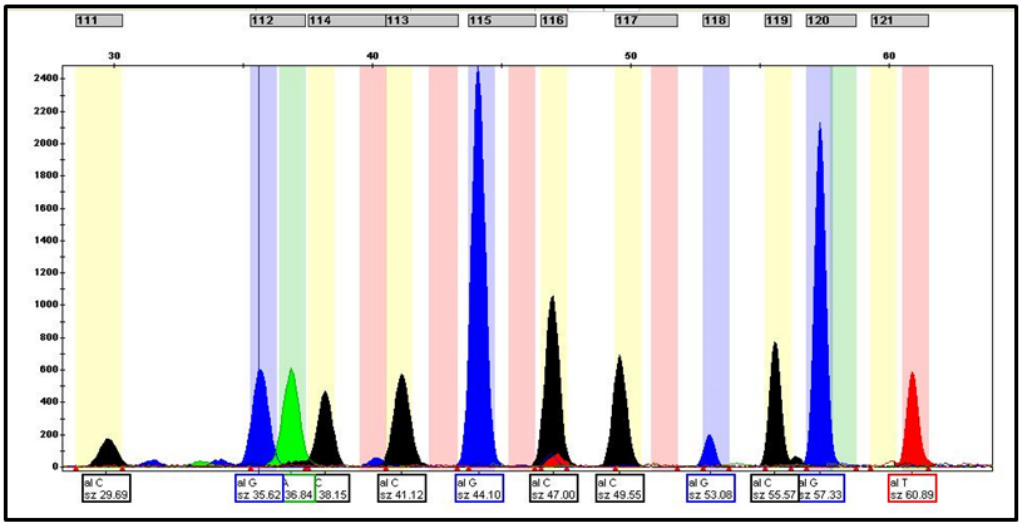


**Fig 2: The SNaPshot Profile of a person with red hair. Each peak corresponds to a single SNP: 111(N29insA), 112 (rs11547464), 113(rs885479), 114(rs1805008), 115(rs1805005), 116(rs1805006), 117(rs1805007), 118(rs1805009), 119(Y152OCH), 120(rs2228479), and 121(rs1110400).**

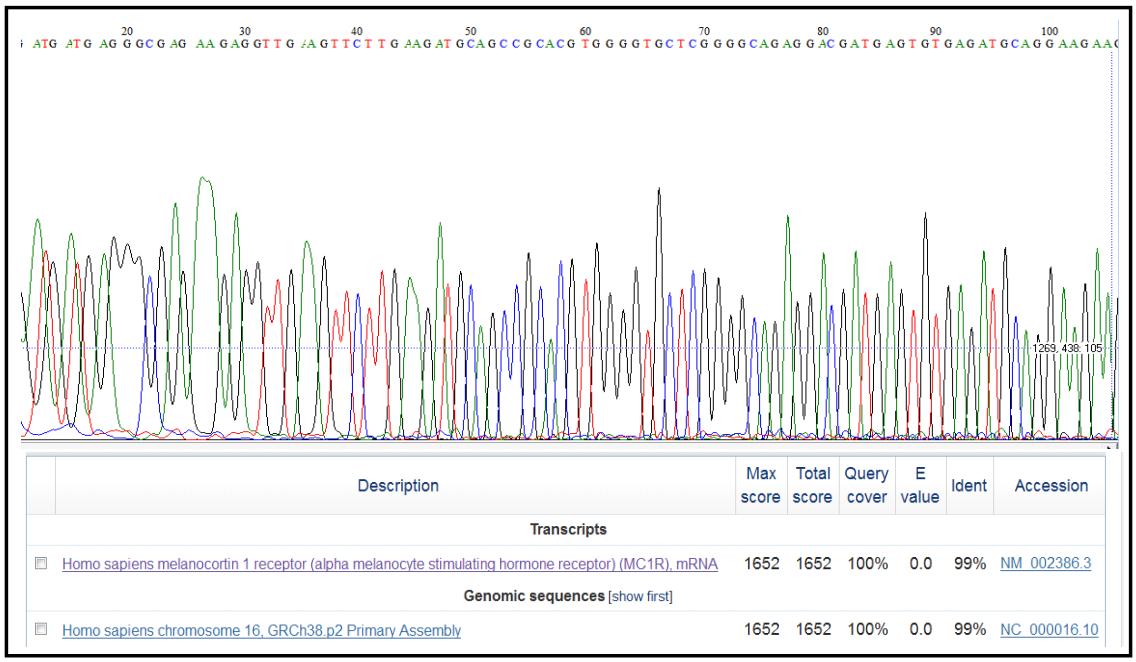


**Fig 3: The match is significant since the E-value is zero.**

In this study, genotype identification of 11 HIrisPlex system markers [12]. was employed as a prediction model of eye color and hair color for each of the 59 samples. Statistical research revealed that certain variations (rs11547464, rs885479, rs1805005, rs2228479) were acceptable for predicting eye and hair color, but others (rs1805008, rs1805006, rs1805007, rs1805009, rs1110400, Y1520CH, N29insA) were unacceptable owing to low variation. The probability of having any of the eye colors (blue and intermediate)

and any of the hair colors (black, brown, and red) has been employed in prior research through authorized and published multinomial logistic regression models [3,18]. The ROC curve was utilized to enhance the performance of predictive models, including the area under the ROC curve (AUC), which shows the value of some sensitivity and specificity of the eye and hair color prediction model (Fig. 4). The determined AUC for our markers is shown in Table 1. The statistical findings revealed a significant degree of agreement between the statistical model and the participant's real eye color.

## Discussion

Human hair color is one of the most noticeable characteristics. Twin studies estimate that heritable elements may explain up to 97% of the difference in hair color, and genome-wide association studies (GWAS) have found various chromosomal regions associated with hair color and other pigmentation traits [9]. Eye color is a highly polymorphic trait that has been proven to be multigenic in the European population by genome-wide association studies [18]. The accuracy of the HIrisPlex systems SNP markers in the diagnosis and projection of eye and hair color in the European population are now well established [12,17,19]due to the impending ability to predict externally visible characteristics (EVCs. The outcomes of the HIrisPlex system assessment in European populations with large sample sizes demonstrate that the alleles associated with blue and brown eye color correspond to the parameters of the logistic regression model [12]due to the impending ability to predict externally visible characteristics (EVCs. The logistic regression model has an adequate value for predicting blue and brown eye color; however, it was less valid for intermediate eye colors [20]. The HIrisPlex system is intended to study tiny quantities of DNA material, including damaged DNA. It is employed in forensic genetics for this reason, and for populations of European origin, its prediction has proven reliable. A thorough evaluation of its accuracy for people from other nations has not been made [7]as well as in studies of ancient human populations. However, the accuracy of this tool has been verified on the West and Central Europeans only, while populations from border regions between Europe and Asia (like Caucasus and Ural. We assessed the HIrisPlex system in a target population of Iranians for the first time in this study, which was verified and analyzed to reliably detect eye color and hair color owing to the presence of numerous ethnicities and races in the Iranian community. These findings are consistent with studies conducted in previous years. Hair color prediction and categorization are more prone to mistakes because elements such as darkness and intensity, as well as environmental factors, particularly lifespan, influence hair color variations. The most popular age-related hair color changes are from bright blonde in childhood to dark blonde and bright or dark brown in maturity. This might be described by hormonal changes throughout puberty. However, its chemical basis is unknown at this time. The HIrisPlex system is unable to differentiate between these variations in individuals and the stability of hair color from childhood to adulthood. Some variants on the MCR1 gene consist of N29insA, rs11547464, rs1805005, rs1805006, rs1805007, rs1805009, and Y152OCH, and rs1805009 are very important variations in Walsh's study, while the variants rs885479, rs1805005, and rs2228479 indicate a varied distribution in the European population and its nearby areas. These polymorphisms of the MC1R gene were explored in research done in a chosen population of Tehran, and the results were verified in persons with red hair. We employed a statistical logistic regression model in this investigation to properly predict eye color. We predicted the precise color of blue, brown, and intermediate colors using the HIrisPlex model. In this model, the correct prediction rate (AUC) for blue eyes was 0.66, for the intermediate color 0.66, for black hair 0.68, for brown hair 0.68, and 0.82 for red hair. The sensitivity level for intermediate eye color is 100%, whereas the sensitivity level for blue eye color is 0%, indicating that these indicators are ineffective for predicting blue eye color, and it cannot tell the difference between blue and intermediate eye colors. And the specificity for blue eyes is 100%, whereas the specificity for intermediate eyes is 0%. In addition, the sensitivity of this model is 91% for black hair, 94.3% for brown hair, and 63.6% for red hair. Furthermore, the application of these SNP markers facilitates the categorization of eye color; nevertheless, little research has been undertaken with this scheme in the classification of eye color so far [19]. The optimum concentration of DNA in the SNaPshot multiplex

reaction for eleven HIrisPlex system markers was between 0.3 and 0.5 ng, but for the AmpF/STR Minifiler identification test for eight STRs, we require 125 pg. In reality, the HIrisPlex system has a far higher sensitivity than the AmpF/STR Minifiler [21]. Because the sensitivity of SNP testing is expected to be higher than that of STR tests, the combined system of the SNP/STR multiplex is proposed in forensic investigations owing to the significant significance of the subject [12]due to the impending ability to predict externally visible characteristics (EVCs. In conclusion, to gain an improved understanding of the high-scale research of SNP markers and their capacity to predict EVCs in individuals with mixed genetic backgrounds, we need to examine a variety of populations with different genetic backgrounds and increase the sample size. Furthermore, additional SNP markers are required to discriminate eye colors, notably intermediate colors, with confidence.

## Conclusion

Predicting an entire face from a DNA sample is the most demanding DNA phenotyping challenge[11]. Several EVC-predictive SNPs and predictive models have been found during the past ten years through GWAS and subsequent predictive analysis, most notably for human pigmentation traits [20(p2018)]. The first forensic eye color prediction system, IrisPlex [17], was created primarily to distinguish between blue and brown eyes. Its upgraded version, the HirisPlex-S DNA testing technique, uses DNA traces to predict eye, hair, and skin color [1]. In this investigation, the Multiplex SNaPshot kit was utilized to simultaneously identify 11 SNPs that were obtained from a large-scale GWAS study for 59 DNA samples belonging to an Iranian population. A model for predicting eye color and hair color was developed using the genotype identification of 11 markers from the HIrisPlex system. Only some of these variations, according to the results, are suitable for predicting eye and hair color, while others are unsuitable owing to limited variation. The statistical data showed that the statistical model and the individuals' real eye colors agreed to a significant extent. Due to the diversity of nationalities and races within Iranian society, we evaluated the HIrisPlex system in this study's target group of Iranians for the first time. The system was confirmed and examined to precisely determine eye color and hair color. These results are by research from earlier years. In closing, we need to explore a range of populations with varied genetic origins and expand the sample size to better understand the high-scale research of SNP markers and their ability to predict EVCs in individuals with mixed genetic backgrounds. To confidently distinguish between eye colors, especially intermediate colors, additional SNP markers are needed.

There are no conflicts of interest to declare.

The study was conducted in accordance with ethical standards, and informed consent was obtained from all participants.

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Original Article

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# Prospective Study of Sudden Natural Deaths in Youngand Middle Age Group at Tertiary care Hospital, Surat, Gujarat

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**Abstract**

In the post–COVID-19 pandemic period, an increasing number of sudden deaths have been observed, particularly among young and middle-aged individuals in India. This trend has created a major challenge for the healthcare system in understanding the underlying pathological mechanisms responsible for such unexpected fatalities. Sudden deaths frequently generate public concern, medicolegal scrutiny, and allegations, making accurate determination of the cause of death essential. A prospective study on sudden natural deaths was carried out at a tertiary carehospital in Surat from 1st January 2024 to 31st December 2024. The study demonstrated that most sudden deaths occurred in adults, with a higher incidence among individuals above 30 years of age. A clear male predominance was noted, with a male-to-female ratio of 6:1. cardiovascular diseases were identified as the leading cause of sudden natural deaths, accounting for 42.72% of cases. Respiratory system diseases were the second most common cause, contributing to 36.89% of deaths. These findings emphasize the significant contribution of cardiovascular pathology to sudden deaths in the adult population. The study also highlighted the importance of histopathological examination in establishing the definitive cause of death. Histopathology played a crucial role in correlating clinical history and autopsy findings, thereby improving diagnostic accuracy and strengthening medicolegal conclusions. Hence, comprehensive autopsy evaluation supported by histopathological analysis is vital in cases of sudden natural death.

**Key Words**: Sudden death, Natural Death, Unexpected Death, coronary artery disease.

## Introduction

Natural deaths constitute a substantial proportion of cases subjected to medicolegal autopsy for the purpose of death investigation.[3] A forensic autopsy is a systematic scientific medical procedure carried out to determine the cause and manner of death, particularly in cases that are sudden, unexpected, or unexplained.[4] According to the World Health Organization, a death is considered sudden or unexpected when an individual who was not known to be suffering from any life-threatening disease, injury, or poisoning is found dead or dies within 24 hours of the onset of terminal illness.[1,2]

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The medicolegal importance of sudden death lies in the potential for suspicion, as such deaths frequently raise questions regarding the possibility of foul play.[5] Sudden natural deaths, especially those occurring in apparently healthy individuals, have a profound impact on society and often result in social concern, emotional distress among relatives, and legal scrutiny. In many instances, individuals with no known significant medical history are found dead at home or at the workplace, circumstances that naturally necessitate a thorough medicolegal investigation.[5] In such cases, a comprehensive forensic post-mortem examination, supported by detailed histopathological analysis, plays a crucial role in establishing the exact cause of death and excluding unnatural causes.

Although sudden natural deaths are encountered routinely in forensic practice, the underlying causes and their distribution show considerable variation based on demographic factors, lifestyle patterns, healthcare access, and geographical location. Most available data are derived from Western populations, and there is a relative paucity of region-specific studies from developing countries, particularly from urban and semi-urban areas of India. Moreover, limited literature is available that systematically analyzes sudden natural deaths using autopsy findings in the context of the local population of South Gujarat.

There is a lack of comprehensive autopsy-based studies evaluating the causes and system-wise distribution of sudden natural deaths in and around Surat. The absence of localized data hampers accurate assessment of population vulnerability and limits the usefulness of mortality statistics for public health planning and medicolegal decision-making.

A detailed evaluation of sudden natural deaths through forensic autopsy is essential to improve understanding of their underlying causes, enhance the accuracy of cause-of-death certification, assist legal authorities, and provide clarity to the relatives of the deceased. Additionally, identifying vulnerable population groups can contribute to better public health planning and preventive strategies. A prospective study was conducted at a tertiary care hospital in Surat, Gujarat, over a one-year period from 1st January 2024 to 31st December 2024.

**Objectives:**

1. To assess the effectiveness of post-mortem examination in determining the cause of death and its correlation with histopathological findings.
2. To identify the pattern and distribution of causes of sudden natural deaths.

## Material and Methodology

A prospective study was conducted at a tertiary care hospital in Surat, Gujarat, over a one-year period from 1st January 2024 to 31st December 2024. During the study period, a total of 103 cases of sudden natural death were included. The study material comprised cases in which the deceased had died suddenly and/or unexpectedly and were subjected to medicolegal autopsy. In each case, a thorough post-mortem examination was performed following standard forensic protocols. All major organs were preserved and submitted for histopathological examination. The collected specimens were fixed in 10% formalin, after which gross examination was carried out and representative tissue sections were selected. Tissue processing was performed according to standard laboratory procedures. Routine Haematoxylin and Eosin staining was applied to all sections, and special stains were employed whenever indicated. Final opinions regarding the cause of death were formulated after receipt and correlation of histopathological findings with autopsy observations. All findings were systematically documented and subjected to statistical analysis.

A prospective study design was adopted to ensure systematic and uniform data collection with real-time documentation of autopsy and histopathological findings, thereby minimizing bias. Inclusion of histopathological examination was essential to accurately establish the cause of sudden natural deaths, especially in cases with inconclusive gross findings. Conducting the study at a tertiary care centre over one year ensured adequate case representation and seasonal variation.

**Inclusion Criteria:**

- Both male and female individuals were included in the study.
- Individuals belonging to young and middle-aged groups were considered.
- All cases of sudden natural death subjected to medicolegal autopsy were included.

**Exclusion criteria:**

Cases involving road traffic accidents, drug-related deaths, poisoning, blast injuries, deaths due to asphyxia (including suicides and homicides), and decomposed bodies were excluded from the study.

## Results and Observations

**Table 1: Distribution of cases according to Age and Sex**

| Age Range (Years) | MALE (%) | FEMALE (%) | Total |
|---|---|---|---|
| 17-20 | 5(4.85%) | 0 (0.00%) | 5(4.85%) |
| 21-25 | 8(7.77%) | 3 (2.91%) | 11(10.68%) |
| 26-30 | 16(15.53%) | 4(3.88%) | 20(19.42%) |
| 31-35 | 16(15.53%) | 2(1.94%) | 18(17.48%) |
| 36-40 | 22(21.36%) | 2(1.94%) | 24(23.30%) |
| 41-45 | 21(20.39%) | 4(3.88%) | 25(24.27%) |
| Total | 88(85.44%) | 15(14.56%) | 103(100%) |

A total of 103 cases of sudden natural death were analysed, comprising 88 males (85.44%) and 15 females (14.56%). The highest incidence was observed in the 41–45-year age group, which accounted for 25 cases (24.27%), followed closely by the 36–40-year age group with 24 cases (23.30%). The lowest number of cases was recorded in the 17–20-year age group, with only 5 cases (4.85%), all of whom were male. Male predominance was evident across all age groups. The gender disparity was most pronounced in the 36–40-year and 41–45-year age groups. Female cases were most frequently observed in the 26–30-year and 41–45-year age groups, with each group contributing 4 cases (3.88%). These findings are comparable with observations reported by Zanjad et al[3]., Gupta et al[4]., and Angam et al [8]., who also documented a higher prevalence of sudden natural deaths among males.

**Table 2: Distribution of cases according to Habits and Co-morbidity**

| Age (Years) | Alcohol | Smokers | Tobacco chewing | Hypertension | Diabetes |
|---|---|---|---|---|---|
| 17-20 | 0 | 0 | 0 | 0 | 0 |
| 21-25 | 2 | 0 | 5 | 0 | 0 |
| 26-30 | 13 | 6 | 15 | 1 | 1 |
| 31-35 | 11 | 2 | 16 | 1 | 0 |
| 36-40 | 18 | 6 | 20 | 5 | 0 |
| 41-45 | 17 | 7 | 21 | 5 | 2 |
| Total | 61 (59.22%) | 21 (20.38%) | 77 (74.75%) | 12 (11.65%) | 3 (2.91%) |

Tobacco chewing was the most frequently reported habit, observed in 77 cases (74.75%), followed by alcohol consumption in 61 cases (59.22%) and smoking in 21 cases (20.38%). The highest prevalence of tobacco uses and alcohol intake was noted in the 36–40-year and 41–45-year age groups. Hypertension was documented in 12 cases (11.65%), predominantly among individuals aged between 36 and 45 years. Diabetes mellitus was identified in 3 cases (2.91%), all occurring in individuals aged 26 years and above, with the highest frequency in the 41–45-year age group. Overall, a progressive increase in lifestyle-related habits and associated comorbidities was observed with advancing age, particularly after the age of 30 years.

**Table 3: Distribution of Cases according to the body system involved**

| Involved System | Male | Female | Total |
|---|---|---|---|
| Cardiovascular | 38 (36.89%) | 6 (5.83%) | 44 (42.72%) |
| Respiratory | 32 (31.07%) | 6 (5.83%) | 38 (36.89%) |
| Gastrointestinal | 10 (9.71%) | 0 (0%) | 10 (9.71%) |
| Gastrourinary | 5 (4.85%) | 2 (1.94%) | 7 (6.80%) |
| Other | 3 (2.91%) | 1(0.97%) | 4 (3.88%) |
| Grand Total | **88 (85.44%)** | **15 (14.56%)** | **103 (100%)** |

In the present study, the cardiovascular system was the most commonly involved system, accounting for 44 cases (42.72%) of sudden natural death. This was followed by respiratory system involvement in 38 cases (36.89%). Gastrointestinal system involvement was observed exclusively among male subjects, with 10 cases (9.71%) and no cases reported in females. Genitourinary system involvement was comparatively less frequent, with 7 cases (6.80%), showing a male-to-female ratio of approximately 2.5:1. Male predominance was observed across all organ systems, reflecting the overall sex distribution of the study population. Together, cardiovascular and respiratory system pathologies constituted more than 79% of all sudden natural deaths, indicating a disproportionate burden of cardiopulmonary disorders in the studied population. This pattern highlights the significant contribution of these systems to sudden mortality. Despite the wide range of conditions known to cause sudden death, cardiovascular diseases emerged as the leading cause in the present study. Of the 103 cases analysed, 44 cases (42.72%) were attributed to cardiovascular causes, of which 38 cases (36.89%) occurred in males and 6 cases (5.83%) in females. These findings are consistent with standard forensic literature, as described by K. S. Narayan Reddy and P. C. Ignatius, who reported that cardiovascular diseases account for approximately 45–50% of sudden deaths. Comparable results have also been documented in previous studies, including those by Zanjad et al. (49.55%)[3], Gupta et al. (58.73%)[4], Angam et al. (44.8%)[8], Dayananda R et al. (63%)[11], and BK et al. (39.18%)[12]. The consistency of these findings across multiple studies reinforces the dominant role of cardiovascular pathology in sudden natural deaths.

**Table 4: Distribution of Cases according to the Cardiovascular System.**

| Cardiac Disease | Male | Female | Total |
|---|---|---|---|
| Atherosclerosis/Chronic coronary artery disease | 16 | 2 | 18 |
| Cardiac Tamponade | 1 | 0 | 1 |
| Myocardial Infarction/ Ischemic heart disease | 18 | 4 | 22 |
| Myocarditis | 3 | 0 | 3 |
| **TOTAL** | 38 (36.89%) | 6 (5.83%) | 44 (100%) |

In the present study, out of 103 cases of sudden natural death, cardiovascular system involvement was identified in 44 cases (42.72%), making it the most frequently affected body system. Among these cardiovascular cases, myocardial infarction/ ischemic heart disease was the predominant pathology, observed in 22 cases (50%), followed by atherosclerosis/chronic coronary artery disease in 18 cases (40.91%). Myocarditis was noted in 3 cases (6.82%), all of which occurred in males, while cardiac tamponade was identified in 1 male case (2.27%). Overall, males constituted 86.36% of cardiovascular deaths, whereas females accounted for 13.64%. The cardiovascular system contributed to the highest proportion of sudden natural deaths in this study, with 44 cases (42.72%). This observation is consistent with findings reported in multiple studies conducted across different geographical regions, where cardiovascular pathology has been identified as the leading cause of sudden death. Comparable results have been reported by G. Angam et al. (44.8%)[8], Zanjad N. P. et al. (49.55%)[3], Gupta S. et al. (58.73%)[4], Dayananda R. et al. (63%)[11], and Gunthethi B. K. et al. (42.55%)[12]. Theconsistent predominance of cardiovascular causes across these studies underscores the critical role of cardiac pathology in sudden natural deaths and highlights the need for early identification an management of cardiovascular risk factors in the adult population.

**Table 5: Distribution of Cardiac cases of sudden death according to Atherosclerosis change**

| Cardiovascular disease | Classification of Coronary Atherosclerosis | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Grade 1 | Grade 2 | Grade 3 | Grade 4 | Grade 5 | Grade 6 | Grade 7 | Total |
| Chronic Coronary artery Disease | - | - | 3 | 7 | 5 | 2 | 1 | 18 |
| Ischemic heart disease | - | 1 | 1 | 2 | 5 | 2 | - | 11 |
| Myocardial Infarction | - | - | - | 2 | 4 | 1 | 4 | 11 |
| Myocarditis | - | - | - | - | 3 | - | - | 3 |
| Cardiac Tamponade | - | - | - | - | 1 | - | - | 1 |
| Total | 0 | 1 | 4 | 11 | 18 | 5 | 5 | 44 |

In the present study, the pattern of sudden cardiac deaths was analyzed in relation to the severity of coronary atherosclerosis. A total of 44 cardiovascular cases were evaluated and graded from Grade 1 to Grade 7 based on the extent of atherosclerotic changes. A progressive increase in the number of sudden death cases was observed with increasing grades of atherosclerosis, with a notable rise beginning from Grade 3 onwards. The highest number of cases was recorded in Grade 5 (18 cases), followed by Grade 4 (11 cases), indicating a strong association between moderate to severe coronary atherosclerosis and sudden cardiac death. Deaths due to chronic coronary artery disease and ischemic heart disease were predominantly seen in the higher grades of atherosclerosis. Myocardial infarction was mainly observed in Grades 4 to 7, suggesting its close relationship with advanced atherosclerotic disease. In contrast, myocarditis and cardiac tamponade were infrequently encountered and showed minimal correlation with the severity of coronary atherosclerosis. The marked increase in cases from Grade 3 onwards highlights a significant association between progressive atherosclerotic severity and the occurrence of fatal cardiac events.

**Table 6: Distribution of sudden death cases according to System**

| System | Cause of death | Male | Female | Total |
|---|---|---|---|---|
| CVS | Chronic Coronary artery Disease | 16 | 2 | 18 |
| | Ischemic heart disease | 8 | 3 | 11 |
| | Myocardial Infarction | 10 | 1 | 11 |
| | Myocarditis | 3 | 0 | 3 |
| | Cardiac Tamponade | 1 | 0 | 1 |
| | Grand Total | 38 (36.89%) | 6 (5.83%) | 44(42.71%) |
| RS | Chronic lung disease | 2 | 0 | 2 |
| | Intra-alveolar haemorrhage | 3 | 2 | 5 |
| | Pneumonia | 13 | 4 | 17 |
| | Pulmonary oedema | 13 | 0 | 13 |
| | Pulmonary Tuberculosis | 1 | 0 | 1 |
| | Grand Total | 32 (31.07%) | 6 (5.83%) | 38(36.89%) |
| GIS | Chronic liver disease | 4 | 0 | 4 |
| | Cirrhosis | 4 | 0 | 4 |
| | Hepatitis | 2 | 0 | 2 |
| | Grand Total | 10(9.71%) | 0 (0) | 10(9.71%) |
| GUS | Acute Tubular Necrosis | 2 | 1 | 3 |
| | Chronic kidney disease | 2 | 0 | 2 |
| | Chronic pyelonephritis | 1 | 1 | 2 |
| | Grand Total | 5(4.85 %) | 2(1.94%) | 7(6.80%) |
| Other | Septicaemia | 3 | 1 | 4 |
| | Grand Total | 3(2.91%) | 1(0.97%) | 4(3.88%) |
| TOTAL | | 88(85.44 %) | 15(14.56%) | 103(100%) |

In the present study, the cardiovascular system was identified as the leading cause of sudden deaths, accounting for 44 cases (42.71%). Among cardiovascular causes, chronic coronary artery disease emerged as the most common individual pathology, observed in 18 cases, followed by myocardial infarction and ischemic heart disease, with 11 cases each. The respiratory system was the second most commonly involved system, contributing to 38 deaths (36.89%). Pneumonia was the predominant respiratory cause (17 cases), followed by pulmonary oedema (13 cases). Gastrointestinal system involvement was noted in 10 cases (9.71%), all of which occurred in male individuals, with chronic liver diseases being the principal cause. Genitourinary system disorders accounted for 7 cases (6.80%), involving both acute and chronic renal conditions. Septicaemia, categorized under miscellaneous causes, was responsible for 4 deaths (3.88%). Overall, males constituted 85.44% of the total cases, demonstrating a marked male predominance across all organ systems. The most commonly affected system was the cardiovascular system (42.71%), the most frequent individual cause was chronic coronary artery disease, and the leading non-cardiac cause was pneumonia. These findings highlight the dominant contribution of cardiovascular and respiratory diseases to sudden deaths, along with a significant gender disparity favouring male involvement.

## Discussion

India has witnessed a noticeable increase in the incidence of sudden deaths in recent years, largely attributable to a rising burden of coronary artery disease (CAD). Sudden death continues to represent a major medicolegal and public health concern, particularly among young and middle-aged adults. The present study analyzed 103 cases of sudden natural death with emphasis on age and sex distribution, lifestyle habits, comorbid conditions, system-wise involvement, and cardiovascular pathology. A clear male predominance was observed, with males accounting for 85.44% of cases and a male-to-female ratio of approximately 6:1. This finding is consistent with earlier studies by Hajra K. Mehdi et al[5]. and Modi R. A. et al[10], who reported male predominance with ratios of 10:1 and 4:1 respectively. Similar observations across multiple Indian studies suggest that males are more frequently exposed to cardiovascular risk factors such as tobacco use, alcohol consumption, occupational stress, and sedentary lifestyles. The majority of cases in the present study belonged to the 36–45-year age group, with the highest incidence noted in individuals aged 41–45 years (24.27%), followed by 36–40 years (23.30%). This distribution indicates that sudden death predominantly affects individuals in their most productive years of life. Comparable age patterns have been reported by Zanjad and Nanadker[3], Gupta et al[4]., Mehdi et al[5]., Pandian et al[9]., and Modi et al[10]., where most victims were between 30 and 50 years of age. Standard forensic textbooks by K. S. Narayan Reddy, O. P. Murty[1], and P. C. Ignatius[2] also emphasize that sudden natural deaths commonly occur in the fourth and fifth decades, largely due to premature cardiovascular pathology. However, studies by Angam et al [8]. and Dayanandra et al[11]. have reported a slightly higher incidence in older age groups, which may reflect regional, dietary, and lifestyle variations.

Analysis of lifestyle habits revealed tobacco chewing as the most prevalent habit (74.75%), followed by alcohol consumption (59.22%) and smoking (20.38%). The high prevalence of these habits, particularly in the 31–45-year age group, suggests a strong association between lifestyle factors and sudden death. Similar associations have been reported by Gupta et al[4]., Bansal et al [6]., Bhagora et al[7]., and Angam et al[8]. While Western studies emphasize cigarette smoking as a major risk factor, Indian studies—including those by Mehdi et al[5]. and Guntheti & Mohsin[12]—highlight the role of smokeless tobacco, supporting the findings of the present study. Among documented comorbidities, hypertension (11.65%) was more frequently observed than diabetes mellitus (2.91%). This relatively lower recorded prevalence may be explained by the fact that many individuals remain undiagnosed or inadequately treated. Textbook references by Reddy & Murty[1] and Ignatius[2] emphasize that undetected or poorly controlled hypertension is a common contributor to sudden death, particularly through its role in accelerating atherosclerosis and precipitating fatal cardiac events.

System-wise analysis revealed that the cardiovascular system was the most commonly

involved, accounting for 42.72% of sudden deaths, followed by the respiratory system (36.89%). Gastrointestinal (9.71%), genitourinary (6.80%), and other causes (3.88%) constituted smaller proportions. These findings align with multiple Indian studies that have consistently identified cardiovascular pathology as the leading cause of sudden natural death, with reported rates ranging from 40% to 65%. Among cardiovascular causes, ischemic heart disease and myocardial infarction together constituted the majority of cases, followed by chronic coronary artery disease. Myocarditis and cardiac tamponade were relatively rare. These observations are consistent with studies by Gupta et al[4]., Mehdi et al[5]., Pandian et al[9]., Modi et al[10]., and Guntheti & Mohsin[12], all of whom identified ischemic heart disease as the most frequent cause of sudden cardiac death. According to Reddy & Murty and Ignatius, ischemic heart disease remains the single most common cause of sudden natural death in adults, strongly supporting the present findings. Grading of coronary atherosclerosis demonstrated that Grade 4 and Grade 5 lesions were the most frequently encountered, indicating that advanced coronary artery disease plays a pivotal role in sudden cardiac death. The presence of severe atherosclerosis even in younger individuals highlights the silent and progressive nature of coronary artery disease. Similar findings have been reported by Mehdi et al[5]., Modi et al[10]., and Pandian et al[9]. Textbook literature further emphasizes that severe atherosclerosis, even in the absence of acute thrombosis, can precipitate fatal arrhythmias, explaining sudden death in apparently stable individuals. Respiratory causes of sudden death were mainly due to pneumonia and pulmonary oedema, particularly among males. Gastrointestinal causes were largely related to chronic liver disease and cirrhosis, often associated with prolonged alcohol consumption. Genitourinary causes included acute tubular necrosis and chronic kidney disease, while septicaemia accounted for deaths categorized under miscellaneous causes. From a medicolegal perspective, sudden deaths in young and middle-aged individuals frequently raise suspicion of foul play. A meticulous autopsy examination, including detailed cardiac evaluation and coronary artery assessment supported by histopathological analysis, is essential for establishing the natural cause of death. Accurate identification of underlying disease not only aids in excluding unnatural causes but also assists legal authorities and provides reassurance to the bereaved family.

**Limitations of the Study:**

The present study was conducted at a single tertiary care centre with a relatively limited sample size, which may restrict the generalizability of the findings to the wider population. Information regarding premorbid conditions and lifestyle factors was limited in some cases due to reliance on available records and relatives' history. Additionally, advanced investigations such as molecular autopsy and genetic analysis were not performed.

## Conclusion

Meticulous autopsy examination supported by comprehensive histopathological analysis plays a pivotal role in accurately determining the cause of sudden death. The present study demonstrated that a substantial proportion of sudden deaths occurred among adults, particularly those above 30 years of age, highlighting a growing public health concern in contemporary society. Cardiovascular causes accounted for the majority of cases, with coronary artery disease—primarily due to atherosclerosis—emerging as the leading underlying pathology. These findings underscore the urgent need to strengthen preventive strategies targeting non-communicable diseases. Effective implementation of the National Programme for Prevention and Control of Cancer, Diabetes, Cardiovascular Diseases and Stroke (NP-NCD), early screening for cardiovascular risk factors, promotion of healthy lifestyle practices, and increased public awareness regarding the hazards of tobacco and alcohol consumption are essential. Regular health check-ups for young and middle-aged adults, along with sustained community-level awareness programs, may significantly contribute to reducing the incidence of sudden deaths due to cardiovascular causes. Future multicentric studies with larger sample sizes are recommended to better understand regional and national patterns of sudden natural deaths. Incorporation of advanced diagnostic modalities, including molecular and genetic studies, may further aid in identifying occult causes of sudden death, particularly in young individuals. Long-term epidemiological studies integrating clinical,

pathological, and lifestyle data would be valuable for developing targeted preventive strategies.

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Original Article

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# A Comprehensive Autopsy-Based Study of Profile of Poisoning Cases Brought for Postmortem at Tertiary Hospital

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## Abstract

**Introduction**: Poisoning is a leading cause of preventable mortality worldwide, with trends shaped by changing societal, environmental, and toxicological factors. This study examines poisoning-related fatalities in Patiala, Punjab, using autopsy data to identify patterns, demographic correlations, and toxicological profiles, aiming to inform preventive and intervention strategies.

**Aims and Hypothesis**: The study hypothesizes that poisoning trends are shifting due to the increased availability of hazardous substances and changing socio-economic pressures. It aims to analyze these trends and explore innovative approaches to mitigate poisoning-related mortality.

**Materials and Methods**: A descriptive prospective study was conducted over 1.5 years on 360 autopsy cases with alleged poisoning. Data was analyzed on parameters including demographic profiles, type of poison, route and timing of exposure, and the socio-environmental context of incidents. Statistical analysis, including Fisher's Exact Test, identified significant associations, with $p \leq 0.05$ considered significant.

**Results**: Among 360 cases, 301 (83.6%) were males, and 59 (16.4%) were females, with the highest incidence in the 21–30 years age group (30.56%). Aluminum phosphide was the most commonly detected poison (21.39%), followed by alcohol (11.11%). Suicide was the predominant manner of death (37.22%), strongly associated with aluminum phosphide, while alcohol was a key factor in accidental and overdose cases.

Innovative findings revealed that 29.44% of cases had pending toxicology reports, underscoring systemic delays that hinder timely legal and preventive interventions. Furthermore, most incidents (77.78%) involved oral ingestion, with 59.17% occurring in domestic settings, highlighting the need for stricter regulation and storage of household toxins. Seasonal analysis identified a significant spike in poisoning cases during the rainy season (43.61%), suggesting the role of agricultural practices and pesticide use during this period.

**Conclusion**: This study emphasizes the critical need for gender- and age-specific interventions, enhanced mental health services, and stricter regulations on toxic substances, particularly aluminum phosphide. Innovative measures, such as real-time toxicological diagnostics, community-based poison control programs, and educational campaigns targeting rural and urban populations, are essential to reduce the burden of poisoning-related mortality.

**Keywords**: Autopsy, Post-Mortem, Poisoning, Suicide, Toxicology

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## Introduction

The term "poison" originates from the Latin word "potion," initially meaning a healthful drink, while "toxicology" derives from the Greek word "toxican," referring to poisonous substances used on arrowheads. Poisons, whether liquid, solid, or gas, are defined as substances that cause injury or death when introduced to a living body in small amounts. Poisoning has been a known cause of death since ancient times, affecting all age groups and both sexes.[1] Poisoning remains a significant public health issue worldwide, with an estimated 193,460 deaths occurring annually due to unintentional poisoning, as reported by the World Health Organization (WHO).[2] In India, poisoning contributes to 20–30% of medico-legal autopsies, with substances such as pesticides, household chemicals, and pharmaceuticals being the most common agents.[3] Regional variations exist, with rural areas reporting higher cases due to increased agricultural pesticide use, while urban areas see more poisoning from pharmaceuticals and household chemicals. [4]

Modern poisoning trends have shifted due to the widespread use of insecticides, pesticides, cleaning acids, and hair dyes. Historically, substances like arsenic and copper sulfate were prevalent, but newer agents such as aluminum phosphide, alcohol, and carbamates are now common. Autopsies in suspected poisoning cases are conducted under strict medico-legal guidelines to determine cause of death and legal culpability. [5, 6]

Poisoning can be categorized as suicidal, homicidal, or accidental, each requiring unique investigative approaches. Understanding regional poisoning trends is essential for improving management and reducing mortality. [3, 7] This study focuses on poisoning-related fatalities in Patiala, Punjab, utilizing autopsy data to analyze patterns, demographic distributions, and causes, offering critical insights for better prevention and treatment strategies.

## Materials & methods

This prospective study was conducted in the mortuary of the Department of Forensic Medicine & Toxicology over a period of one and a half year. Total 360 cases were brought for autopsy with alleged history of poisoning. After receiving police inquest papers, they were examined to ascertain their inclusion in the study based on the following criteria:

**Inclusion Criteria:**

- Cases of deaths due to alleged poisoning.
- Cases with clinical / postmortem findings of poisoning were included.

**Exclusion Criteria:**

- Cases in which there was no alleged history of poisoning
- Cases with no clinical / postmortem findings suggestive of poisoning.

Subsequently the details of the cases were entered into a predetermined proforma and the data was collected. The data was subjected to descriptive statistical analysis and was tabulated as results and observations in tabular form, graphs, and charts. Fisher's Exact Test was used to gauge if there was a statistically significant difference between the proportions of the categories in two group variables. A p-value less than or equal to a predetermined significance level (0.05) indicated a statistically significant result, meaning the observed data provide strong evidence against the null hypothesis.

## Results and Discussion

In our study, out of a total of 360 cases, 301 were males (83.6%) and 59 were females (16.4%). The highest incidence of poisoning was observed in the 21-30 years age group, comprising 30.56% of the total cases. The most common poison detected was aluminum phosphide, accounting for 21.39% of cases, followed by alcohol at 11.11%. A significant portion of cases (29.44%) remained pending for poison detection due to delayed receiving of chemical examination reports. Suicide was the leading manner of death, predominantly associated with aluminum phosphide poisoning. Drug overdose cases were largely linked to alcohol.

**Table 1: Distribution of poisoning cases in relation to type of poison, age group and gender ($X^2$=71.501, p=0.022)**

| Age Groups (years) | Alcohol | | Aluminum Phosphide | | No Poison Detected | | Oleandrin | | OPC | | Opium | | Paraquet | | Phenyl | | Snake Bite | | Pending | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | M | F | M | F | M | F | M | F | M | F | M | F | M | F | M | F |
| 1-10 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 11-20 | 7 | 0 | 1 | 2 | 1 | 0 | 0 | 0 | 1 | 1 | 2 | 1 | 0 | 1 | 1 | 1 | 5 | 0 | 4 | 5 |
| 21-30 | 5 | 1 | 17 | 4 | 11 | 2 | 0 | 0 | 7 | 1 | 8 | 0 | 4 | 1 | 0 | 2 | 9 | 0 | 27 | 11 |
| 31-40 | 12 | 0 | 23 | 3 | 7 | 3 | 0 | 1 | 3 | 1 | 2 | 0 | 1 | 0 | 1 | 1 | 4 | 2 | 27 | 3 |
| 41-50 | 6 | 0 | 13 | 1 | 7 | 2 | 0 | 0 | 1 | 0 | 5 | 0 | 1 | 0 | 3 | 0 | 6 | 1 | 18 | 2 |
| 51-60 | 5 | 0 | 6 | 1 | 7 | 0 | 0 | 0 | 4 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 2 | 0 | 4 | 1 |
| 61-70 | 3 | 0 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 2 | 3 | 1 |
| 71-80 | 1 | 0 | 1 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Total | 39 | 1 | 65 | 12 | 37 | 7 | 0 | 1 | 16 | 3 | 18 | 1 | 8 | 2 | 6 | 4 | 29 | 5 | 83 | 23 |

**Table 2: Shows various parameters studied in the present study**

| Variables | | Total (N=360) | |
|---|---|---|---|
| | | n | % |
| Route of exposure | Bite | 37 | 10.28% |
| | Inhalation | 6 | 1.67% |
| | Intravenous | 13 | 3.61% |
| | Oral | 280 | 77.78% |
| | Unknown | 24 | 6.67% |
| Gender | Female | 59 | 16.39% |
| | Male | 301 | 83.61% |
| Time of intake | Day Time | 173 | 48.06% |
| | Evening Time | 26 | 7.22% |
| | Night Time | 161 | 44.72% |
| Marital status | Divorced | 1 | 0.28% |
| | Married | 248 | 68.89% |
| | Separated | 2 | 0.56% |
| | Unmarried | 106 | 29.44% |
| | Widower | 1 | 0.28% |
| | Unknown | 2 | 0.56% |
| Manner of death | Accidental | 46 | 12.78% |
| | Drug Overdose | 75 | 20.83% |
| | Homicide | 10 | 2.78% |
| | Insect Bite | 4 | 1.11% |
| | Snake Bite | 33 | 9.17% |
| | Suicide | 134 | 37.22% |
| | Not Know | 58 | 16.11% |
| Duration of survival | 0 Days | 134 | 37.22% |
| | 1-5 Days | 213 | 59.17% |
| | 6-10 Days | 10 | 2.78% |
| | >10 Days | 3 | 0.83% |

Cont.....

| | | | |
|---|---|---|---|
| Site of Incident | Home | 213 | 59.17% |
| | Field | 72 | 20% |
| | Outside | 66 | 18.33% |
| | Jail | 7 | 1.94% |
| | Hostel | 1 | 0.28% |
| | Shop | 1 | 0.28% |

Suicide emerged as the most prevalent manner of death, accounting for 37.22% of cases. The study showed a significant gender association with young males being more affected. Seasonally, most cases occurred during the rainy season (43.61%), followed by summer (20.28%). The predominant route of exposure was oral ingestion, accounting for 77.78% of cases. Daytime ingestion accounted for the majority of cases at 48.06%, followed by night- time incidents at 44.72%, and evening incidents at 7.22%. Most of deaths occurred within 1-5 days of ingestion (59.17%). Site of incident is mostly at home (59.17%).

| **Study Location** | **Year** | **Common poison** | **Age group (years)** | **Gender** | **Marital status** | **Route** | **Suicidal** |
|---|---|---|---|---|---|---|---|
| Present study | 2022-23 | Aluminum phosphide 21.39% | 21-30 | Male 83.6% | Married 68.9% | Oral 77.78% | 37.22% |
| Chandigarh [8] | 1999 | Aluminum phosphide 65% | 14-30 | Male | Married 57.5% | Oral 69.21% | 46.29 |
| Rohtak,Haryana[9] | 1995 | Aluminum phosphide 67.8% | 11-20 | Male | Married | Oral 87.91% | 91.4% |
| Amritsar,Punjab[10] | 2001 | Organophosphorus comp 52.7% | 21-30 | Male | Married | Oral | 61.2% |
| Yavatmal,Maharashtra [11] | 2003 | Aluminum phosphide 55.4% | 21-30 | Male | Married 63% | Oral 72% | 63.4% |
| Ludhiana,Punjab [12] | 2015 | Organophosphorus comp 34% | 21-40 | Male | --- | Oral 95% | 94% |
| Kota,Rajasthan [13] | 2016 | Organophosphorus comp 17.39% | 21-30 | Male 61.7% | Married 55.27% | Oral 42% | 79.82% |
| Udaipur,Rajasthan[14] | 2020 | Aluminum phosphide 37.47% | 21-30 | Males 54.6% | --- | Oral 38% | --- |
| Lucknow,Uttar Pradesh [15] | 2023 | Snake bite 26.1% | 21-30 | Males 63.2% | Married 57.5% | Oral | 78% |
| Chandigarh [3] | 2002 | Aluminum phosphide 18.9% | 21-30 | Males 69.9% | Married 60.40% | Oral | 78.1% |
| Bengaluru,Karnataka [16] | 2018 | Medications 51.6% | 21-30 | Females 52.6% | Married 49.6% | Oral 81.2% | 69.1% |
| Linkoping,Sweden [17] | 2004 | Propoxyphene | 40-50 | Females 51% | | | 44% |

The table presents a comparative analysis of poisoning cases across various regions and years, focusing on common poisons, demographics, route and manner of death in poisoning cases. Aluminum phosphide and organophosphorus compounds were the most frequently reported poisons in India, while medications and propoxyphene were significant in urban and international studies. The 21–30 age group emerged as the most affected in most studies, with males being predominantly impacted, except in Bengaluru and Linkoping, where females' cases were more prevalent. A considerable proportion of cases involved married individuals, and oral ingestion was the predominant route of poisoning in various

studies. The proportion of suicidal cases varied significantly. These findings underscore regional differences and patterns in poisoning incidents, emphasizing the need for tailored prevention and intervention strategies.

## Conclusion

This comprehensive study on poisoning cases autopsy reveals critical insights into the patterns and disparities associated with such incidents, underscoring the necessity for targeted interventions and a multifaceted approach to mitigate risks and reduce mortality. The findings indicate a higher incidence of poisoning cases among males and young adults, suggesting the need for gender-specific strategies and focused educational campaigns to raise awareness about the dangers of poisoning and preventive measures. The study also reveals a significant number of cases being suicides, underscoring the urgent need for improved mental health services. Strengthening mental health infrastructure, providing counselling and support services, and implementing stricter regulations on sale of hazardous substances can help prevent such incidents.

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Original Article

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# Near-Hanging in a Tertiary Care Hospital attached to a Medical College: A Ten-Year Retrospective Analysis of Demography, Prognostic Indicators, and Immediate Outcomes (2012–2021)

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## Abstract

**Background:** Near-hanging is frequently reported in trauma and emergency, compelling immediate treatment and forensic intervention. Early predictors of outcome help guide clinical and medico-legal decisions. This study provides decade-long data highlighting prognostic significance in a developing country setting.

**Aim:** To study demographic attributes and prognostic implications of Glasgow Coma Scale (GCS) at admission in near-hanging cases over ten years.

**Materials and Methods:** A retrospective observational study of 71 consecutive near-hanging patients admitted alive between January 2012 and December 2021 was conducted. Variables included age, gender, admission GCS, and outcome (survived/expired).

**Results:** The mean age was 32.1 years. Young adults (21–30 years) constituted 36.6% of cases. Males accounted for 54.9%. Overall survival was 54.9% and mortality 45.1%. Patients with GCS ≤8 on admission had significantly higher mortality ($\chi^2 = 12.84$; $p < 0.01$). The study highlights admission GCS as a simple yet robust predictor of outcome in resource-limited settings.

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**Conclusion:** Near-hanging predominantly affects young adults. Admission GCS is a statistically significant predictor of mortality. Early neurological assessment and aggressive emergency care are crucial.

**Keywords:** Glasgow Coma Scale; Mortality; Near-hanging; Suicide attempt; Prognostic indicators.

## Introduction

Hanging remains a method of choice for suicide globally and contributes significantly to preventable mortality.[1,2] On constriction of the neck following hanging, there is compression of the neck structure, resulting in unconsciousness, making it nearly impossible to prevent the death. Hence, literature reports a high mortality between 70% to 95% among hanging victims before reaching the hospital.[1,3,4] However, in a few instances (10-30%) where timely intervention and the institution of life-saving measures are undertaken, a few victims are brought alive to the medical care facility. This is referred to as 'Near hanging'.[5] Among these patients, 18% survival was reported in victims developing cardiac arrest complications, and 90% survival in patients without neurological compromise.[1,6,7] The Glasgow Coma Scale (GCS) is extensively recognised for evaluating neurological status in emergency settings as a predictor of outcome.[8,9] However, recent multicentric study emphasised the utility of additional prognostic indicators, including injury severity and duration of hypoxia.[10]

In India and other developing countries, there is limited longitudinal data spanning a decade, especially comparing evaluating admission neurological scores and the outcome in victims of hanging brought alive for medical intervention. This study fills this gap by analysing near-hanging cases over ten years.

## Materials and Methods

This retrospective observational study was undertaken at a tertiary care teaching hospital between January 2012 and December 2021. Seventy-one patients reported alive after attempted hanging were included. Demographics (age, gender), admission Glasgow Coma Scale (GCS) scores, and hospital outcomes (survived or expired) were documented for analysis. Descriptive statistics were used to summarise the data, and the association between GCS category and mortality was analysed using the Chi-square test, with $p < 0.05$ considered statistically significant. Although no interventions were performed, Institutional Ethical Committee approval was obtained before the study.

**Ethical clearance was obtained from the Institutional Ethics Committee (Ethical Approval No.:** AJEC/REV/287/2021 dated 22.11.2021.

## Results

Out of the 71 patients reported alive after a failed attempt of hanging, most of the victims belonged to the age group of 21–30 years (36.6%), followed by 31–40 years (25.4%) as illustrated in Table 1. Individuals aged less than 20 years accounted for 11.3% of cases, and 16.9% were in the age group between 41years and 50 years. The mean age of the patients was 32.1 ± 9.8 years, indicating that most cases occurred among younger individuals.

**Table 1: Age Distribution (n=71)**

| Age Group (Years) | Number | Percentage (%) |
|---|---|---|
| ≤20 | 8 | 11.3 |
| 21–30 | 26 | 36.6 |
| 31–40 | 18 | 25.4 |
| 41–50 | 12 | 16.9 |
| >50 | 7 | 9.8 |
| Total | 71 | 100 |

Table 2 depicts the gender distribution of the study population. Of the 71 near-hanging patients, 39 were males (54.9%), while 32 were females (45.1%). The attempted hanging was slightly common among males in this cohort.

**Table 2: Gender Distribution**

| Gender | Number | Percentage (%) |
|---|---|---|
| Male | 39 | 54.9 |
| Female | 32 | 45.1 |
| Total | 71 | 100 |

The correlation between Glasgow Coma Scale (GCS) score at admission and hospital outcome is explained in Table 3. Amongst32 near-hanging patients with severe neurological impairment (GCS 3–8), 22 expired and10 survived, emphasising a

high mortality in this group. In comparison, among 21 patients with moderate GCS scores (9–12), 15 survived, and 6 expired. Among 18 patients with mild impairment (GCS 13–15), 14 survived, and only 4 died, indicating the best outcome. Statistical analysis using the Chi-square test showed a significant association between GCS at admission and fatality ($\chi^2 = 12.84$; $p < 0.01$), indicating that lower GCS scores at admission were significantly associated with higher mortality.

**Table 3: GCS Category and Outcome**

| GCS Category | Survived | Expired | Total |
|---|---|---|---|
| 3–8 | 10 | 22 | 32 |
| 9–12 | 15 | 6 | 21 |
| 13–15 | 14 | 4 | 18 |
| Total | 39 | 32 | 71 |

## Discussion

Hanging remains a common method of suicide globally and contributes significantly to preventable mortality. Epidemiological data from across the globe indicate that hanging accounts for a substantial proportion of suicides in both developed and developing nations. This is attributed to the fact that any material can be used as a ligature easily and the perceived notion of certain lethality. Most of the research highlights that 70–95% of hanging victims succumb at the scene before institution of medical intervention, reflecting the rapid lethality associated with the compression of neck structure and ensuing cerebral hypoxia.[1,2,4] However, a marginal number of victims survive long enough to reach the hospital and are termed as near-hanging cases, representing approximately 10–30% of attempted hanging incidents.[5]

The survival of near-hanging victims largely depends on the extent and period of cerebral hypoxia resulting from vascular occlusion and airway obstruction. Impeding of the blood flow through the carotid arteries can rapidly lead to cerebral ischemia and loss of consciousness, while venous occlusion may result in cerebral congestion and raised intracranial pressure. In certain circumstances, although in miniscule in number, in instances of partial/incomplete suspension, early release of neck constriction, rapid initiation of resuscitative measures, or incomplete vascular occlusion, victims may be rescued well before irreversible brain injury occurs and thus reach the hospital alive.[3]Early intervention, including airway stabilisation, oxygenation, and intensive monitoring, plays a critical role in improving survival in such cases.

The present study demonstrates that near-hanging patients are predominantly young adults, the majority of them in the age group between 21 and 30 years (36.6%), followed by those in the age group of 31–40 years (25.4%). This observation is in concurrence with studies indicating that suicidal tendencies and attempted hanging are more common among young and economically productive age groups, often linked to psychosocial stressors and mental health disorders.[2]In the current cohort, an insignificant male predominance (54.9%) was observed, in sync with global suicide trends showing higher rates of completed suicide among males, although gender differences may vary across regions. A significant finding of this study is the association between GCS at admission and outcome. Patients presenting with GCS scores between 3 and 8 had highest mortality, whereas those with GCS scores of 13 to15 had the most favourable outcomes. The statistical analysis showed a significant relationship between lower GCS scores and increased mortality ($\chi^2 = 12.84$; $p < 0.01$). These findings are in concurrence with the previous studies that highlighted the prognostic value of early neurological assessment in near-hanging victims. Early and prompt identification of patients with severe neurological impairment can facilitate swift intensive care management and may aid clinicians in anticipating potential complications and outcomes.[6,7]

The present study adds to existing literature by providing a decade-long retrospective dataset from a tertiary care centre, emphasizing the practical utility of GCS as an early prognostic tool in near-hanging cases. This is particularly relevant in resource-constrained settings where rapid clinical decision-making is essential.

The present research provides longitudinal data spanning over a decade from a tertiary care setting, contributing crucial understandings into the demographic profile and prognostic indicators of near-hanging cases in a context of developing country. In many developing countries, long-term analyses of near-hanging cases are limited. The findings highlight the significance of rapid rescue,

early neurological evaluation, and aggressive emergency management in aiding positive outcomes among these patients. Furthermore, from a forensic and public health perspective, these findings may aid in planning preventive strategies and prompt emergency response systems.

Nonetheless, some limitations should be acknowledged, which may be overcome in future studies. Being a retrospective study, the study relied on hospital records, and some related variables, such as duration of suspension, ligature type, pre-hospital resuscitation, and psychiatric history, could not be evaluated. Future prospective research incorporating these variables may provide a more inclusive understanding of prognostic determinants in near-hanging victims.

## Conclusion

Near-hanging poses a considerable clinical and medico-legal concern due to the near-total lethality attributed to hanging. Although significant victims die before reaching medical care, a small proportion survive to receive medical care. The present study shows that near-hanging predominantly affects young adults and shows a slight male predominance. Importantly, the Glasgow Coma Scale (GCS) score elicited on admission is a significant predictor of hospital outcome, with lower GCS scores being greatly associated with enhanced mortality. Early neurological evaluation and prompt emergency management are therefore vital in improving survival outcomes. Long-term analyses such as this provide vital epidemiological intuitions and emphasize the need for enhanced preventive strategies, timely rescue, and efficient emergency care systems to reduce mortality associated with hanging attempts.

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Review Article

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# Fingerprint Evidence and National Security: A Global Review

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**Abstract**

Fingerprint evidence has long been regarded as one of the most reliable forms of personal identification, and it continues to serve as a cornerstone in both forensic science and national security. In an era marked by terrorism, organized crime, cyber threats, and large-scale migration, fingerprint and biometrics have become indispensable tools for law enforcement, border management, and civil identification. This review examines the scientific foundations of fingerprint individuality and permanence, evaluates operational workflows and examiner's reliability, and explores applications across criminal justice, counter-terrorism, immigration control, and digital identity systems. Particular attention is given to emerging threats such as spoofing, fingerprint alteration, and vulnerabilities in contactless capture, alongside countermeasures including liveness detection and multimodal biometric fusion. Ethical and governance concerns ranging from surveillance creep and privacy erosion to the challenges of proportionality in systems like Eurodac and Aadhaar are analyzed to highlight the political as well as technical dimensions of biometric infrastructures. A comparative analysis of U.S., EU, Indian, and INTERPOL systems underscores both the enduring value of fingerprint evidence and the evolving challenge of balancing security imperatives with civil liberties. This review contributes by integrating technical, legal, and ethical perspectives to provide a holistic understanding of fingerprint evidence in contemporary security regimes.

**Keywords:** Fingerprint evidence, Forensic Science, Biometric identification, Security and Privacy, Ethical governance

## Introduction

National security today spans physical borders, digital spaces, and public safety. Biometric technologies play a pivotal role in ensuring security, with fingerprints being the most widely used due to their universality, permanence, and uniqueness.[1] While DNA and facial recognition have expanded forensic and security capabilities, fingerprints remain the gold standard because they are less affected by environmental changes and intentional disguise.[2]

Globally, countries have built large-scale databases: the FBI's Next Generation Identification (NGI) in the U.S.[3], Eurodac and Entry/Exit System (EES) in the EU[4], NAFIS and Aadhaar in India[5], and

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INTERPOL AFIS.[6] This review critically examines the foundations, applications, threats, and future of fingerprint evidence in national security contexts.

Despite the existence of available research regarding fingerprint uniqueness, examiner accuracy, or biometric system performance, there has not been sufficient coverage in terms of their application in regional national security decision-making. Current studies exist in varying levels of forensic science or regional security, but not in comprehensive terms regarding scientific, operational, governance, technical, legal, or ethical applicability. There has also been a lack of assessments concerning international discrepancies in terms of system applicability, governance structures, privacy rights, or scalability. In this regard, this current study aims to provide a synthesis in terms of scientific, operational, or governance applicability of major international biometric systems in enhancing regional national security within appropriate civil liberty requirements.

**Scientific Foundations of Fingerprint Evidence**

**Uniqueness and Permanence**

Fingerprint uniqueness has been statistically validated: the likelihood of two individuals sharing identical ridge detail is negligible.[1] Longitudinal studies confirm stability over decades, making fingerprints highly reliable for lifetime identification.[2]

**Reliability and Error Rates**

NIST's Fingerprint Vendor Technology Evaluation (FpVTE 2012) showed false match rates below 0.01% with high-quality images.[7] However, forensic latent fingerprint analysis introduces examiner subjectivity. Black-box studies showed examiners are highly accurate but not infallible, with non-zero false positive rates, reinforcing the need for quality control.[8]

The intrinsic value and scientific foundation of fingerprint evidence are well-recognised in forensic science and security. According to the INTERPOL Factsheet, fingerprints are unique—even for identical twins and are permanent except in cases of deep injury. This means fingerprints are reliable for identifying people throughout their lives, mainly because matching focuses on small, distinct features in the patterns.[9]

Even though fingerprints are now considered stable and reliable, there are still challenges in using them. Marasco and Ross things like spoofing and deliberate damage to fingerprints can create problems, especially at the border controls and security checkpoints. For example, some people have tried to use fake fingers made from materials like gelatin or Play-Doh, or have altered their fingerprints on purpose. To respond to these challenges, they suggested using advanced liveness detection techniques so that the system can tell a real finger from a fake one.[10]

In addition to this, explained that fingerprint recognition now benefits from deep learning with new network designs such as convolutional neural networks (CNNs), which improve the way fine details are found and matched even when the prints are unclear or incomplete.[11] These modern methods works better than older ways that depended on manually selected features.

Researchers have looked at how fingerprint science fits into systems that use more than one biometric technique. Fingerprints have been used for a long time with great success; they noted that poor print quality can still affect results.[12] To overcome these issues, they came up with combining fingerprint identification with other biometric systems, such as iris and face recognition, which can lead to more accurate and dependable identification overall.

Taken together, these studies show why fingerprints continue to play a central role in forensic and security work today. Their biological uniqueness and permanence make them vital, but researchers also point out the importance of addressing the security risk and on the use of new technologies to overcome these weakness.

**Standards, Quality, and Interoperability**

Biometric interoperability across nations requires standardization. ISO/IEC 19794 specifies minutiae and image formats,[13] while NIST's NFIQ 2.0 provides a benchmark for assessing fingerprint image quality.[14] Recent work emphasizes the need for certification of contactless capture devices, as new technologies (e.g., mobile scanners, smartphones) enter law enforcement and border workflows.[15] Aadhaar API 2.0 & 2.5 stress data security, registered devices, encryption, and

best-finger detection for quality assurance.[16] The same is being systematized for National Automated Fingerprint Identification System (NAFIS). The inclusion of Virtual IDs (VIDs) in 2.5 reflects a move towards privacy-preserving standards. INTERPOL requires adherence to NIST standards for fingerprint exchange, ensuring member countries can share prints across borders. Eurodac highlights interoperability within EU systems: Eurodac now links with SIS, VIS, ETIAS, and ECRIS-TCN, expanding beyond asylum into law enforcement and migration management.[17]

**National Security Applications**

Fingerprint-based systems have become integral to national security infrastructures worldwide, underpinning law enforcement, migration management, welfare distribution, and counter-terrorism. From large-scale domestic databases such as the FBI's *Next Generation Identification (NGI)* in the U.S., *Aadhaar* in India, and *Eurodac* in the EU, to international platforms like INTERPOL's *AFIS*, these systems illustrate the breadth of fingerprint applications while also reflecting the political and historical legacies embedded in biometric governance. The following subsections examine these applications in greater detail.

**Criminal Justice and Law Enforcement**

Fingerprints underpin criminal record systems worldwide. In the U.S., the Rap Back service within NGI enables continuous monitoring of individuals in sensitive positions.[18] Latent print matches remain critical in cold case investigations.[19]

**Border Security and Immigration Control**

The EU's Eurodac stores asylum applicants' prints, while the EES will register fingerprints and faces of all non-EU travelers.[4] The U.S. IDENT/HART databases support immigration and traveler vetting.[20] India's Aadhaar program demonstrates large-scale civilian integration, with fingerprints linked to national ID for both governance and security.[5]

**Counter-Terrorism and Intelligence**

Fingerprints collected from conflict zones, crime scenes, and international travel points are checked against global watchlists.[21] Rapid mobile fingerprint systems (e.g., RISC) enable instant field verification by law enforcement.[3]

**Cybersecurity and Digital Identity**

Biometric authentication is central to digital banking, e-governance, and online security. Fingerprint sensors in consumer devices form part of the national cybersecurity strategy, reducing risks of identity theft.[22]

**Forensic Workflows and Investigative Value**

Forensic workflows for fingerprint evidence integrate collection, enhancement, and AFIS search, forming a cornerstone of modern identification practices. Latent fingerprint recovery remains one of the most common and probative forms of physical evidence,[19] with INTERPOL outlining operational protocols in which recovered prints are searched in Automated Fingerprint Identification Systems (AFIS). These searches may run in 'lights-out' mode for automated identifications or require manual examiner review for confirmation. To do so with assurance, examiner writings emphasize blind proficiency testing and standardized quality controls particularly.[8] Similarly, the centralization of forensic and civil AFIS systems augmented investigative reach, but, simultaneously spawned controversies surrounding proportionality, privacy, and ethical control.

In spite of these developments, operational and security issues continue to prevail. Researchers highlight that spoofing attacks and intentional alteration of fingerprints have the potential to compromise the integrity of workflows, particularly in high-throughput operations like border control checkpoints.[10] To address these issues, researchers have set forth the use of multimodal biometric systems that combine fingerprint recognition with other modalities such as iris and face recognition.[12] Such integrated approaches not just reduce the incidences of false matches but enhance robustness against adversarial attack and modality imposed limitations of single-modality systems. Therefore, they enhance the evidentiary and legal value of fingerprint data, subsequently guarantying its continued relevance within both forensic and security contexts.

**Threats and Countermeasures**

Fingerprint-based systems in national security face a spectrum of adversarial threats that challenge their reliability and raise governance concerns. One of the most prominent vulnerabilities is presentation attacks, where artificial fingerprints made from materials such as silicone or gelatin deceive recognition systems.[10] Countermeasures have evolved from early aliveness detection to deep learning classifiers, yet even advanced CNN-based models remain susceptible to adversarial manipulation.[11] Beyond spoofing, fingerprint alteration through surgical modification, burning, or ridge obliteration continues to pose risks, though algorithms have become more effective in detecting such manipulations.[2] Contactless capture technologies, while hygienic and convenient, introduce new weaknesses related to lower image quality and spoofing potential, leading NIST to propose evaluation and certification standards. This highlights that threats to fingerprint systems are not only technical but also socio-political.[15]

The researcher have classified these threats into spoofing (e.g., gelatin fingers), obfuscation (ridge mutilation), and impersonation, while proposing aliveness detection as a critical countermeasure.[10] National initiatives such as India's Aadhaar system have emphasized cryptographic protections, including PID block encryption, secure registered devices, and audit trails, to safeguard biometric transactions.[23] However, the security debate extends beyond technical dimensions. Mordini and Massari identify ethical threats such as surveillance creep, privacy erosion, and the reduction of personal identity to data points.[27] Similarly, critics of the Eurodac system highlight the risks of storing children's fingerprints from as early as age six, arguing that such practices expand surveillance to vulnerable populations and raise profound human rights questions.[17] In addition to these technical and ethical deliberations, they highlight that, like the models of national security, the sustainability of fingerprint data relies on innovation in technology and good governance.

**Privacy, Governance, and Ethical Concerns**

The increasing use of fingerprint databases has raised concerns over how these databases are managed, especially concerning how secure the information is, whether people truly agree to their use, and the potential for constant watching. Biometric technologies can lead to widespread surveillance, taking away the unique qualities of individual identities and turning them into simple data points. These significant concerns are present in rules and laws both within countries and internationally.[24,25]

The fast-growing use of fingerprint databases has today raised various sets of questions about the management and ethical considerations, especially in data security, genuine consent, and continuous surveillance. Researchers like Lyon and Breckenridge have focused on how these biometric technologies can lead to extensive monitoring and reduce individual identities to simple data points. These important issues are addressed in laws and regulations nationally and internationally.[24,25]

This ongoing tension between protecting human rights and managing migration is evident in the expansion of the Eurodac database, which now requires fingerprinting, children as young as six years old.[26] In a similar way, India's Aadhaar card program is one of the world's largest biometric identification initiatives, has also faced legal challenges over privacy concerns, potential misuse of data, and questions about the adequacy of consent procedures.[16] Issues of fairness and surveillance have also arisen with the NGI/HART system in the United States.[28]

These examples show the importance of ethical risk and its importance in biometric systems. Poor data protection, weak systems for holding people accountable, and not having proper ways to get user consent all make it more likely that privacy will be violated and fingerprint information will be used improperly. Furthermore, putting all biometric information in one central place also raises questions about fairness: while it might be justified by national security needs, the expanding reach of fingerprint surveillance often targets vulnerable groups like migrants, asylum seekers, and children. This, in turn, worsens problems related to fairness and accountability.

**Emerging Trends and Future Directions in Fingerprint Biometrics**

Biometric technologies are continuously changing in how fingerprints are used to identify

people. Deep learning methods, for example, have greatly improved fingerprint recognition, especially in handling unclear, partial, or poor-quality prints that have previously been difficult for automatic systems to process.[11] At the same time, contactless fingerprint scanning has become part of digital environments. This offers clean and user-friendly alternatives to traditional scanners and also makes mobile and remote identification easier. However, this shift also brings new technical demands related to how different systems work together and how to ensure authentication security. Beyond systems that use only one type of biometric, multimodal biometric systems-which combine fingerprints with other identifiers like iris scans and face recognition- have been shown to significantly increase accuracy, reduce mistakes in matching, and provide greater reliability in various operating conditions.[12]

Future research is increasingly focusing on biometric systems that protect privacy and on making these systems secure. The goal is to find a good balance between their use for forensic purposes and protecting individual freedom. Approaches based on homomorphic encryption now make it possible to compare fingerprints directly on encrypted templates without revealing the confidential data during processing.[29] Blockchain technology are now being explored to create unchangeable records of activity and to allow decentralised control of biometric documents. This will help us to lower the chance of misuse or unauthorised access.[30]

There are systems that are developed to bring various institutions to collectively train for powerful recognition models, while making sure that sensitive fingerprint data to not stored in on central location.[31] These ongoing efforts show a shift towards more reliable biometric systems, where the guiding principle is towards a reliable biometric system where privacy and accountability are priorities over previous systems that only focused on being accurate.

These developments suggest that the future of fingerprint biometrics will be closely connected to areas like the ethics of artificial intelligence, cybersecurity, and the evolving rules surrounding digital rights.

**Comparative Global Perspectives**

The Biometric systems are now widely used nationally and internationally for security purposes by various organizations. It has now grown exponentially in integrative design and governance, enhancing and utilization for security and access.

In the United States, the FBI Next Generation Identification (NGI) system and the Department of Homeland Security's, Homeland Advanced Recognition Technology (HART) are examples of data collection of mass criminal, border, and civil biometric data. The NGI improves traditional fingerprint collections by adding multimodal capabilities, while the HART seeks to centralise fingerprints, facial recognition, iris patterns, and voice identification within a common framework-raising questions of proportionality, oversight, and the duration of retention of the stored information.[28,32]

In the European Union, migration and asylum management are provided for by Eurodac, and the future Entry/Exit System (EES) will digitally collect fingerprints and facial images of every non-EU visitor. The systems support border management and migration policy but evokes criticism for enhancing surveillance, specifically the collecting and storing of children's fingerprints and the possibilities of rerouting use of the data beyond its initial purpose.[4,26]

In India, Aadhaar is one of the biggest biometric networks worldwide, connecting fingerprints not only for identity but also for delivery of welfare benefits, financial inclusion, and national security. Though Aadhaar demonstrates the value of centralised government biometrics, it has been contested many times before the court on privacy, consent, and security of data counts.[5]

On the global stage, INTERPOL's AFIS offers a worldwide platform for the cross-border matching of fingerprints, facilitating collaboration against organized crime and terrorist acts. The system highlights the international strategic importance of fingerprint forensic evidence in global policing but also signals challenges for technical standards harmonization, accuracy across heterogeneous national databases, and the promotion of human rights in cross-border information sharing.[6] In combination,

the cases recognize how various jurisdictions strike a balance among operational effectiveness and rights preservation in varied manners.

Compared to the frameworks supported by the U.S. and the EU, the Indian biometric systems give great importance to the management of national identity and the provision of these services on a massive scale. As far as the aspect of the protection of privacy, the management of accountability, and regulation by the law of the land is considered, the Indian biometric systems still have less-developed frameworks, in contrast to the European Union. At the same time, in comparison to the U.S. biometric frameworks, the Indian biometric databases have less linkage to the international criminal intelligence networks, which is a constraint on the global level of investigations. It should be noted that the operational conditions of the Indian biometric systems related to demographic diversity, climate, and rural capture conditions affect the quality of fingerprint matching performance, which has less severity in technologically non-diverse conditions.

## Conclusion

Fingerprint evidence has become an indispensable component of national security frameworks, primarily owing to its inherent reliability and persistence. The pervasive application of this technology is evident across a multitude of sectors, including criminal justice, border control, counter-terrorism initiatives, and digital identity verification. Notwithstanding persistent challenges such as spoofing vulnerabilities, the inherent subjectivity of examiner-based evaluations, pervasive privacy concerns, and limitations in interoperability, the future trajectory of fingerprint evidence is intrinsically linked to the strategic integration of AI-supplemented analytics, the advancement of multimodal biometric technologies, the incorporation of privacy-enhancing technical solutions, and the implementation of more robust governance frameworks. A global comparative perspective offers invaluable insights into these dynamics. As fingerprint systems are poised to retain their central role in identification and security, their ongoing justification will increasingly hinge upon the capacity to effectively balance their investigative utility with the protection of civil liberties—a critical equilibrium that will ultimately determine both the legitimacy and public trust vested in contemporary biometric infrastructures.

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**Purpose & Scope:** Indian Journal of Forensic Medicine & Toxicology is a peer reviewed six monthly Journal. It deals with Forensic Medicine, Forensic Science, Toxicology, DNA fingerprinting, sexual medicine and environmental medicine. It has been assigned International standard serial No. p-0973-9122 and e-0973-9130 **website: www.ijfmt.com**. This journal is also indexed with Index Copernicus (Poland).

The journal encourages research from theoretical perspectives, research reports of evidence based practice as well as praxis research work that focuses on the interface between theory and practice and how each can support the other. In addition, the journal strongly encourages reports of research carried out within or involving countries in the Asia- Pacific region.

**Invitation to submit papers**:
A general invitation is extended to authors to submit journal papers for publication in IJFMT.

**The following guidelines should be noted:**

1. The article must be send by E-mail in word only as attachment. Hard copy need not be send.
2. The article should be accompanied by a declaration from all authors that it is an original work and has not been sent to any other journal for publication.
3. References should be in Vancouver style.
4. As a policy matter, journal encourages articles regarding new concepts and new information.

**Please submit paper in following format as far as applicable**

1. Title
2. Names of authors
3. Your Affiliation (designations with college address), email id
4. Corresponding author- name , designations, address,email id
5. Abstract with key words
6. Introduction or back ground
7. Material and Methods
8. Findings
9. Discussion / Conclusion
10. Conflict of Interest
11. Source of Support
12. Ethical Clearance
13. References in Vancouver style.
14. Word limit 2500-3000 words, MSWORD Format, single file
15. Please. quote references in text by superscripting

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# Indian Journal of Forensic Medicine & Toxicology

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