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Elevated Skull Fracture in a 15-Year-Old boy Following fall from bicycle: A Rare Autopsy Case Report

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Abstract

Background: Elevated skull fractures are an extremely rare type of cranial injury, especially in the pediatric age group. They occur due to tangential blunt force trauma and differ markedly from more commonly encountered depressed or linear skull fractures.

Case Description: We present a rare case of a 15-year-old male who sustained fatal head and thoracic injuries following a fall from a bicycle during rainy weather. He was conscious initially but later deteriorated and succumbed despite medical management. Autopsy revealed an unusual outward displacement of a segment of the parietal bone — an elevated skull fracture — with associated extradural and subdural hemorrhages, comminuted cranial fractures, and a penetrating chest injury involving the right lung.

Conclusion: This case emphasizes the importance of recognizing elevated skull fractures as a distinct traumatic pattern. Proper documentation during autopsy can significantly aid in understanding injury dynamics, especially in pediatric accidental deaths.

Keywords: Elevated skull fracture, pediatric head injury, bicycle accident

Introduction

Cranial fractures represent one of the most frequent consequences of head trauma, with patterns varying according to the **direction, magnitude, and nature of force** applied. The most common types are linear and depressed fractures, while rarer forms include diastatic and elevated fractures. Elevated

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skull fractures are particularly uncommon and were first described by Adeloye (1971)[1]as a distinct pathological entity. Elevated skull fracture are mostly reported in the age group of 5 to 15 years. Elevated skull fracture are not always fatal. Chances of survival depends on the severity of the intracranial injury and prompt neurosurgical care. If the dura is intact and there is no major intracranial bleed, chances of survival can be good.Unlike depressed fractures, which occur following perpendicular blows that drive bone fragments inward, elevated fractures result from **tangential blunt force trauma** that produces shearing forces strong enough to displace a bone fragment outward (Reddy, 2017)[5]. Recent studies continue to highlight their rarity and unique biomechanics in children and adults (Borkar et al., 2015)[7]; Srinivasan et al., 2016[6]; Zoghi et al., 2024[8].

The recognition of this pattern carries both **clinical and forensic importance**. Clinically, elevated fractures may not always cause direct brain laceration but are often accompanied by **extradural and subdural hemorrhages** that can prove fatal if untreated (Ersahin&Mutluer, 1993)[3]. From a forensic standpoint, documenting this type of fracture provides vital information about the **mechanics and direction of impact**, which is particularly useful when distinguishing accidental falls and road accidents from inflicted trauma (Sharma et al., 2005)[2].

Mahapatra&Tandon (1987)[4] observed that the nature of cranial fractures in different age groups is influenced by skull elasticity and developmental stage, explaining why linear and depressed fractures are more frequently encountered. Within this broader context, elevated fractures remain exceedingly rare, and their occurrence provides important insights into the dynamics of impact.

The present case describes an **unusual elevated skull fracture following a fall from a bicycle**, associated with multiple cranial hemorrhages and thoracic trauma. By presenting this case, we aim to expand on the **biomechanics, pathological features, and forensic implications** of elevated skull fractures, while emphasizing the value of thorough autopsy documentation in clarifying the mechanism and manner of injury.According to our experience we have rarely seen such a case in our institute and documented such a case.

Case Report

History

Taking in account of the guardians and police inquest a 15-year-old male adolescent sustained a head injury after falling from a bicycle on a wet road. He was initially conscious and was referred to a tertiary care hospital for treatment. However, his condition deteriorated, and he was declared dead the same day. A medico-legal autopsy was performed to determine the cause and manner of death.

External Examination

- Moderately nourished male, age ~15 years.
- Rigor mortis present in all limbs.
- Postmortem hypostasis fixed over the back.

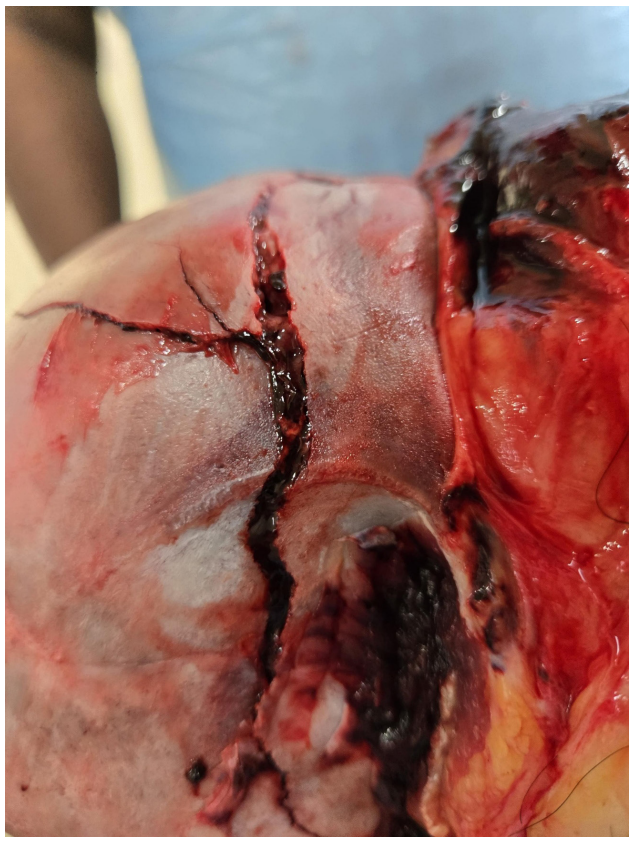


Figure 1: Close-up of linear fracture lines extending near base of skull.



Figure 2: Radiating fracture lines from the impact site extending toward frontal, occipital, and skull base.

Antemortem Injuries Noted

- **Chest Injuries:**
  - Bruise on right lateral chest wall measuring 6 × 1 cm from nipple 5 inches laterally.
  - Bruises just below the clavicles (right: 2 × 1 cm; left: 3 × 1 cm).
  - Incised penetrating wound on the right lateral chest wall, located 6 inches lateral to the midline and 3 inches below the axilla. It pierced through the 5th intercostal space into the thoracic cavity.
- **Abdominal Injury:** Bruise on the right lumbar region measuring 4 cm × 2 cm. at L2-L4, 2 inches lateral to the midline.
- **Scalp Laceration:** 8 cm × 1 cm × Bone deep over the right parietal region running obliquely, with irregular margins.

Internal Examination

Scalp and Skull

- Hematomas diffusely present over bilateral frontal, right parietal, and temporal regions.
- On farther examination it was found that the outer table of the triangular region got detached leaving the inner table intact without any elevation as shown in figure 4.
- Triangular elevated fracture of the outer table of the mid-frontal bone.
- Comminuted fissured fractures radiating from both frontal bones to the left temporal and mastoid regions.
- Corresponding laceration over the right parietal region.
- Dura mater intact

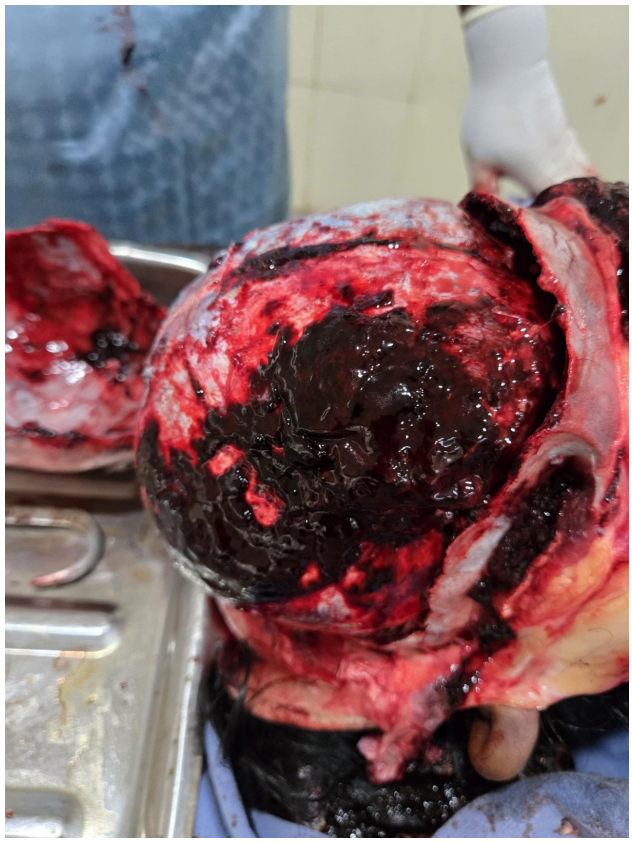


Figure 3: Extensive extradural over right cerebral hemisphere with cerebral edema.



Figure 4: Showingthe inner table of the skull which is not elevated after outer table detachment.

Brain and Meninges

- Extradural hemorrhage over the right frontal and parietal lobes.
- Diffuse Subdural hemorrhages over bilateral frontal and parietal lobes.
- Mild cerebral edema present.
- No laceration of brain tissue.

Thoracic and Abdominal Organs

- **Lungs:** Congested; hemorrhage on the lower lobe of the right lung; left lung collapsed.
- **Heart, liver, spleen:** Appeared normal.
- **Kidneys:** Congested.
- **Stomach:** Contained approximately 100 ml of semi-digested rice.
- **Bladder:** Contained ~100 ml clear urine.
- **Intestines:** Contained fecal matter and gases.
- No internal injury to abdominal viscera.

Musculoskeletal System

- No long bone fractures or dislocations.
- Vertebrae intact.

Cause of Death

Death was due to the effects of head injury. The elevated skull fracture, along with associated extradural and subdural hemorrhages, was sufficient to cause death in the ordinary course of nature. All injuries were antemortem in nature.

Discussion

Elevated skull fractures are rare lesions first described by Adeloye (1971)[1], who emphasized their distinction from depressed and linear fractures. They occur when **tangential blunt force** is applied, producing outward displacement of bone fragments rather than the inward displacement seen in depressed fractures (Reddy, 2017)[5]. In contrast to the more pliable pediatric skull, which generally withstands displacement (Mahapatra&Tandon, 1987) [4], the occurrence of such an injury in an adolescent makes this case exceptional.

The mechanism in our case — a **bicycle fall on a wet road** — is consistent with the glancing or shearing impact described in earlier literature (Sharma et al., 2005)[2]. The outward detachment of the outer table, with the inner table remaining intact, further supports a tangential force dynamic rather than perpendicular compression. Notably, despite the elevated fracture, the dura was intact and no direct brain laceration was present, highlighting the potential for significant hemorrhage without parenchymal disruption (Ersahin&Mutluer, 1993)[3]. This observation is consistent with more recent institutional experiences and meta-analyses underscoring both the clinical and forensic relevance of this injury pattern (Borkar et al., 2015)[7]; Srinivasan et al., 2016[6]; Zoghi et al., 2024[8].

From a **forensic perspective**, careful recognition of this fracture pattern is important in reconstructing

injury mechanics. Unlike depressed fractures that may raise suspicion of assault, elevated fractures point toward accidental mechanisms such as falls or road accidents (Sharma et al., 2005)[2]. In the present case, the absence of defense wounds or multiple impact sites further supported an accidental manner of death.

Clinically, reports suggest that early diagnosis of elevated fractures by imaging can guide management, though surgical intervention may not always be necessary if neurological compromise is absent (Ersahin&Mutluer, 1993)[3]. Unfortunately, our patient rapidly deteriorated due to associated intracranial hemorrhages and thoracic injuries, underscoring the potential lethality of combined trauma.

Comparison of Reported Elevated Skull Fractures

Author / Year	Age Group	Mechanism of Injury	Location of Fracture	Outcome	Key Observation
Adeloye (1971)[1]	Adult	Assault (blunt force)	Parietal	Survived	First description of elevated fracture
Ersahin&Mutluer (1993)[3]	Children	Accidental falls	Parietal/ Temporal	Survived (surgery)	Pediatric cases, intact dura reported
Sharma et al. (2005)[2]	Adults	Road traffic accidents	Frontal	Survived	Forensic significance highlighted
Mahapatra&Tandon (1987)[4]	Children	Various cranial injuries	Mostly linear/ depressed	Variable	Elevated fractures extremely rare in pediatric skulls
Present Case (2025)	15 years	Bicycle fall (accidental)	Frontal/ Parietal	Fatal	Outer table elevated, inner table intact, associated thoracic injury

This case adds to existing literature by demonstrating:

- The **rarity of elevated skull fractures in children and adolescents** (Adeloye, 1971[1]; Mahapatra&Tandon, 1987[4]).
- **Distinct biomechanics** of tangential impact (Reddy, 2017)[5].
- **Medico-legal importance** in differentiating accidental falls from intentional trauma (Sharma et al., 2005)[2].
- A unique autopsy finding of **outer table detachment with intact inner table**, expanding understanding of fracture morphology.

Conclusion

This case highlights a rare elevated skull fracture in a pediatric patient following a bicycle fall, associated with fatal intracranial hemorrhage and thoracic trauma. Recognition of this uncommon injury pattern is not only clinically relevant for guiding management but also **forensically significant** in reconstructing the mechanism of trauma and excluding foul play. Detailed autopsy documentation, as emphasized in earlier reports, remains essential for clarifying the biomechanics of injury and contributing to the limited body of literature on pediatric elevated skull fractures.

- Conflict of Interest: None declared by any of the authors.
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Case Study

Human Sacrifice: A Forensic Approach – A Review with a Case Study of Narabali

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The Deadly Amoeba: A Rare Case Report of Primary Amoebic Meningoencephalitis by Naegleria Fowleri

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Abstract

Introduction: Naegleria fowleri (N.fowleri), a thermophilic, free-living amoeba found in warm freshwater, causes primary amoebic meningoencephalitis (PAM), a rare, highly fatal brain infection with over 97% mortality. PAM mimics bacterial meningitis, presenting vague symptoms like fever and headache, progressing to severe neurological signs.

Case Report: Post mortem viscera of a 46 year old male was received with the alleged history of altered sensorium. The gross examination of brain showed opaque, granular leptomeninges with multiple necrotic greybrown areas of 1.5–2.5 cmwere noted in the frontal, parietal and temporal lobesand both cerebellum. Microscopy revealed neutrophilic exudate, necrosis, hemorrhage,dense inflammatory infiltrates and round to oval PASpositive, amoebic trophozoites, consistent with N.

Conclusion: This case marks only the second reported case of N. fowleri meningoencephalitis in Haryana.Increased awareness, public education and water monitoring are crucial for prevention. Early detection through improved diagnostic protocols can significantly reduce the associated high mortality rates.

Keywords: Amoeba, fatal, meningoencephalitis, Naegleria fowleri.

Introduction

Primary amoebic meningoencephalitis (PAM) is a rapidly progressing and often fatal infection of the central nervous system, caused by the amoeba Naegleria fowleri.[1] It has been reported in various parts of the world, including the Americas, Australia, Thailand, Hong Kongand Taiwan. The first documented case was reported by Fowler and Carter in 1965 in Australia.[2] Fewer than two dozen cases have been reported in India till now according to articles published in various newspapers.From existing literature search, the first case of PAMcaused by Naegleria fowleri in India was reported in 1971.[3]

N.fowleri is a thermophilic, free-living amoeba typically found in warm freshwater environments

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such as lakes, ponds, rivers, and hot springs, particularly those contaminated with soil.[1] This amoeba primarily infects individuals by entering the body through the nasal cavity when they come into contact with contaminated water. Once inside, the amoeba penetrates the nasal mucosa, crosses the cribriform plate, and travels along the olfactory nerves to reach the brain.[4] It has a life cycle consisting of three stages: cyst, trophozoite and flagellate. Under specific conditions, it can undergo transition between these forms, but infection in humans occurs through trophozoite stage.[5]

N.fowleri is often referred to as the "brain-eating amoeba" due to its ability to cause severe acute encephalitis in the brain, with mortality rate exceeding 97% with only a few documented survivors. The disease progresses rapidly, with an incubation period ranging from 2 to 15 daysand death usually occurs within 3 to 7 days after symptoms begin.[1] Its early symptoms are typically vague, with common complaints like fever and headache mimicing symptoms of bacterial meningitis making its timely diagnosis a challenging task.[6] As the disease progresses and more brain tissue is affected, neurological symptoms begin to emerge.A detailed patient history, especially recent exposure to freshwater can help in suspecting the etiology with analysis of cerebrospinal fluid (CSF) for confirmation of the diagnosis. Neuroimaging techniques can also be useful for visualising the affected areas of the brain, particularly in the later stages of the disease.[7]

The rarity of PAM is evident and its severity and swift progression underscores the importance of prompt recognition and intervention, especially in areas where such cases are almost unheard of. To best of our knowledge, this case being only second reported case of N.fowleri meningoencephalitis from the state of Haryana.

Case Report

We received post mortem viscera of a 46 year old male in the department of pathology with alleged history ofaltered sensorium.No other significant details regarding past history or investigations were available in the post mortem papers except that he was a known alcoholic. On gross examination, whole brain was received weighing1200 gms and measuring 15x12x10 cm. The leptomeninges appeared opaque, slightly granular with thin layer of purulent exudation. On cut section, cerebral hemispheres showed multiple grey brown poorly defined necrotic areas in the frontal, parietal and temporal lobes measuring 1.5-2.5 cms in diameter. Both sides of cerebellum also revealed multiple focal areas of necrosis and congestion (Figure 1).



Figure 1: Gross examination showed multiple grey brown poorly defined areas of necrosis cerebral cortex (black arrow) and cerebellar cortex (red arrow).

Microscopic examination from meninges revealed presence of neutrophilic exudate, prominent dilated vessels and edema. Sections from cerebral cortex shows multiple large areas of necrosis,haemorrhage, oedema, dense mixed acute and chronic inflammatory cell infiltrate comprising of neutrophils, macrophages and few eosinophils. The section also revealed singly scattered and small groups of round to ovalorganisms, 15 - 25 µm in diameter with vacuolated cytoplasm, single vesicular nucleiconspicuous karyosomes surrounded by clear haloand prominent nucleoli with chromatin lining the nuclear membrane. There were foci of

necrotizing vasculitis, thrombosis and fibrinoid necrosis. These trophozoites were periodic acid shiff (PAS) positive, morphologically consistent with amoebic trophozoites. The diagnosis on the basis of histomorphological features was given as acute amoebic meningoencephalitis by N. fowleri. (Figure 2)

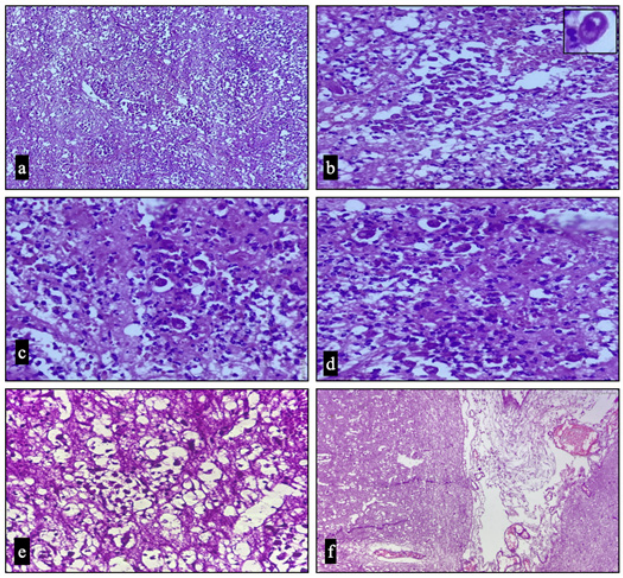


Figure 2: Microphotograph shows (a) multiple large areas of necrosis and dense mixed inflammatory infiltrate in cerebral cortex (H&E X40), (b)foci of necrosis, neutrophils, macrophages and a few eosinophils along with round to oval organisms in cerebral cortex (H&E X100), inset showing round to oval organisms with vacuolated cytoplasm, single vesicular nuclei surrounded by clear halo and prominent nucleoli(H&E X oil immersion), (c, d) multiple round to oval organisms with prominent nucleoli (red arrow) (H&E X400), (e) multiple magenta coloured round to oval organisms (PAS X400). (f)presence of edema, congested blood vessels, neutrophils and macrophages in the meninges (H&E X100).

Specimen of heart weighed 230 gm and measured 10x7x5 cm. On cutting open, the right ventricular wall, left ventricular wall and interventricular septum thickness were 0.3 cm, 1.3 cm and 1.1 cm respectively. A piece of lung weighing 60 gm & measuring 9x5x4.5 cm. Piece of liver weighing 160 grams and measuring 11×8×4 centimetres, piece of spleen weighing 140 grams and measuring 7.5x5x2 centimetres and both pieces of kidneys weighing 100 grams each and measuring 10x6x1 centimetres and 10x7x1 centimetres respectively were also received. No abnormality was identified in thevarious viscera pieces on gross and microscopic examination.

Discussion

N. fowleri is a freeliving amoeba, is responsible for PAM which is rare and highly lethal infection mimicing bacterial meningitis clinically.In 1965, Australian pathologist Malcolm Fowler identified N. fowleri while investigating cases of fatal PAM and it was later named in his honor for his contributions to its discovery and characterisation. It rapidly progresses through the central nervous system, causing extensive brain tissue destruction and severe cerebral edema.While there are over 40 genera of freeliving amoebae, only four are known to cause diseases in humans: Acanthamoeba sp, Balamuthia mandrillaris, N. fowleri and Sappinia dialoidea, of which first two are associated with granulomatous amoebic encephalitis (GAE).Also,of 47 identified species of Naegleria, only N. fowleri is responsible for PAM.[8]

N. Fowleri is waterbornefree living amoeba thriving in freshwater especially that contaminated with soil. Its a thermophilic organism which survives in temperatures between 30°C to 45°C and occurs in 3 forms - inactive cyst form, transitory flagellate form and trophozoite form. The cyst form is 7 - 12 µm spherical that can survive in low temperatures of winter. Transitory flagellate form is 10 - 16 µm pear shaped with 2 flagella which is neither reproductively active nor pathogenic. Trophozoite form isnearly 22 µm long and 7 µm wide oval to pear shaped reproductively active and pathogenic. It can be cultured on nonnutrient or lownutrient agar plates or grown in Nelson's growth medium. Diagnostic tests include direct visualisation or polymerase chain reaction (PCR). Direct visualisation of cerebrospinal fluid can be done to detect motile trophozoites under light microscopy. Reverse transcriptase PCR is useful for accurately identifying the genus and species.Diagnostic challenges further

hinder timely identification as direct microscopy relies on skilled operators, PCR tests may not be accessible in rural areas and culturing the amoeba requires specialised media and time. Increased awareness and better diagnostic resources are essential to prevent fatalities.[9,10]

In 2009, Gupta et al were first to report a case of PAM by N. fowleri in Haryana of a 20yearold male with a history of acute lymphoblastic leukemia (ALL) in remission. He was admitted with complaints of fever, headache, vision and hearing loss with neck stiffness and hypertonic limbs. Laboratory results showed leukocytosiswith neutrophilia. CSF was turbid with neutrophil predominance and showed N. fowleri trophozoites on wet mount preparation. Amoebae also grew in culture from the CSF, confirming the diagnosis.Even after treatment with ampotericin B, patient succumbed to death.[11]

In a study by Regu et al conducted in Kerala, the first laboratory confirmed case of PAM of that region was reported in Alappuzha in March 2016. Since then, eight confirmed cases were documented out of which, 87.5% were male, with ages ranging from 10 to 62 years. All patients had a clear history of swimming or diving in contaminated water. In 87.5% of the cases, Giemsastained CSF wet mounts revealed trophozoites resembling N. fowleri. Water samples from five exposure sites were tested, with two (40%) showing amoebic growth. It was further suggested that initial nonspecific symptoms and later progression resembling bacterial meningitis can hinder accurate diagnosis by clinicians. They concluded that the number of undiagnosed cases may be even higher in countries lacking a surveillance system for PAM or adequate diagnostic testing facilities for N. fowleri.[12]

In another study from Kerala by Susheela et al, a 62 year old male presented with suddenonset generalized tonicclonic seizures, fever, headache, vomiting and photophobia. On examination, he was stuporous, had fever with neck stiffness. A diagnosis of acute meningoencephalitis with seizures was made. Lumbar puncture revealed cloudy CSF with elevated proteins, low sugar and leukocytosis and wet mount microscopyrevealed motile amoebic trophozoites, identifying N.fowleri. Despite prompt treatment the patient deteriorated and died on day nine.[13]

In a systematic review of twenty one studies by Zamzuri et al in 2023, recent case reports on N. fowleri infections were consolidated to inform healthcare professionals about its epidemiology and clinical features. A thorough literature search of databases was done, including PubMed and Scopus, for identificationof these 22case reports for analysis from 2013 onwards of which 4 were from India. The cases spanned globally, with a 72.7% fatality rate. Patients ranged from 11 days to 75 years old, with freshwater exposure being a common risk factor. The study suggested a probable case definition in accordance with the findings which includes the acute onset of fever, headache, and vomiting with meningeal symptoms following exposure to freshwater within the previous 14 days which will ensure an early index of suspicion during medical consultation for diagnosis and treatment. The study concluded thatimproved public awareness is essential for prevention and to seek immediate treatment.[8]

In a study by Panda et al, water samples were collected from 107 reservoirs, including ponds and lakes in Rohtak and Jhajjar districts of Haryana, every 14 days during summer and autumn of 2012 and 2013. Five samples from each site were combined, filtered, and processed for culture on non-nutrient agar with Escherichia coli. Cultures were observed microscopically, and suspected free-living amoeba (FLA) colonies were cloned. DNA was extracted, and PCR was used to identify Naegleria species. Of the 107 samples, 43 (40%) were FLA-positive, with 37 confirmed as Naegleria by PCR, and one identified as Naegleria fowleri. This study marks the first PCR-based detection of Naegleria species in northern India, emphasising the health risks associated with these water bodies and the need for public awareness and prevention.[14]

The limited availability of NGSfacilities in rural or district hospitals poses a challenge. In the present study, no history or investigations were available in the postmortem records. The gross examination

of the brain revealed multiple necrotic areasand microscopic analysis showed the presence of amoebic trophozoites. Diagnosis of amoebic encephalitis can be difficult, only on clinical symptoms and high degree of clinical suspicion and advancements in laboratory techniques allow for timely and accurate identification. Review of literature revealed similar cases in which histopathological examination of autopsy brain revealed amoebic trophozoites (Table 1).

Table 1. Review of literature of cases of PAM by N.fowleri diagnosed on autopsy.

Author	**Sugita[15] et al**	**Cogo[16] et al**	**Chomba[17] et al**
Year	1999	2004	2017
Country	Japan	Italy	Zambia
Age/sex	25/F	9/M	24/M
Symptoms	High Fever, headache, neck stiffness and deterioration of consciousness.	Fever, persistent headache	Seizures and fever for 1 day. Stiff neck and decorticate posturing.
History of swimming/ exposure to water bodies	-	10 days before onset of symptoms	-
GCS	-	-	6
Lumbar puncture	Day 3 - 4616 cells/ cm^3, all of which were neutrophils or large monocytic cells. CSF glucose - 10 mg/100 mL and the total protein - 431 mg/100 mL. Clusters of amoeba showing a conspicuous karyosome that was surrounded by a clear nuclear halo were detected on microscopy.	Cloudy CSF with 2.5 mmol/L glucose, 4.54 g/L protein, CSF TLC - 6120/mm3	Day 1, day 3 - Normal Day 8 - Direct wet mount microscopic examination showed numerous highly motile amoebic trophozoites and cysts.
TLC	-	Day 1 - 6,800/$mm^3$with 90% neutrophils. Day 3 - 19600/$mm^3$with 91% neutrophils	-
Gram-stain CSF	-	No bacteria	-
Blood and CSF culture	Grown in culture and the pear-shaped biflagellates were identified as N.fowleri.	Viruses, bacteria, and fungi were negative	-
CT scan	-	Lesion in the right frontal lobe and diffuse cerebral edema	No abnormality seen.

Continue....

Gross findings	The orbitofrontal cortex and the olfactory bulbs and tracts showed foci of recent hemorrhage with softening of brain stem. Massive infiltration of the frontal, temporal base and basal meninges was seen on the external surface of the brain.	Swollen and edematous brain with opaque and congested leptomeninges. A cerebellar tonsillar herniation and soft, easily breakable frontal lobes were found. Coronal sections of cerebral hemispheres showed diffuse and multiple foci of hemorrhagic necrosis in both gray and white matter.	The leptomeninges were thin and transparent with vascular congestion, fibrinopurulent exudates. There was no displacement of the cingulate gyrus, medial temporal lobe, or cerebellar tonsils.
Histopathology findings	Massive infiltration of the frontal, temporal base and basal meninges was seen on the external surface of the brain. Inflammatory cells infiltrated the leptomeningeal space extensively. There were numerous amebic trophozoites mingled with macrophages in the perivascular spaces. There were no amoebic cysts. Clusters of amoeba showing a conspicuous karyosome that was surrounded by a clear nuclear halo were detected.	Diffuse and multiple foci of hemorrhagic necrosis in both gray and white matter. Massive and diffuse inflammatory infiltrate, characterized by a high number of neutrophils, few eosinophils, macrophages and numerous large clusters of cells that morphologically resembled amoebic vegetative forms, tentatively classified as Entamoeba or Naegleria.	Leptomeninges were thin and transparent with vascular congestion, fibrinopurulent exudates. Edema, lymphocytes and neutrophils features consistent with meningitis. Multiple amoeba were present in the brain parenchyma.
Indirect immunofluorescent (IIF)	-	Reactivity of amoeba in tissue sections with anti N.fowleri serum was identified	-
DNA sequencing and PCR	-	PCR done viarRNA gene was amplified by using primers NF-ITS-F1	PCR done via DNA primers NF-ITS-F1

The cases discussed, including ours, highlight the importance of considering amoebic encephalitis as a differential diagnosis for specially in endemic areas or with history of fresh water swimming.

Conclusion

Despite the known prevalence of N.fowleri in freshwater bodies, only a few cases have been reported from India. This deadly infection is often misdiagnosed as bacterial meningitis, posing a growing global concern. The rapid disease progression and lack of awareness within the medical community pose significant diagnostic challenges, suggesting many cases may go undetected. To combat this, health education activities including increased awareness prior to engagement in freshwater activities and routine water source surveys are essential to reduce the morbidity and mortality associated with the entity.

Source of Funding: Nil

Conflict of Interest: There is no conflict of interest

Ethical Clearance: Not required as it is a case report. The identity of the deceased is masked and therefore permission from relatives was not obtained.

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A Cyclist's Catastrophe: An Unusual Case of Road Traffic Accident with A Unique body Trajectory of the Victim, and Associated Baffling Injuries, Defying Stereotypes

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Abstract

Introduction: Fatalities from road traffic accidents have resulted in loss of life and material resources. In India, 1374 accidents and 400 deaths take place every day, due to RTAs. Globally, RTA is the main cause of death among the 15–to 29-year-old population. Thus, all such cases must be meticulously studied to identify the mechanisms and causations to reduce such mishaps.

Case History: A young man, riding on a bicycle, was dashed to death by an unknown vehicle running perpendicular to his trajectory. He was declared brought dead by the EMO upon arrival at a tertiary care hospital. Accordingly, a post-mortem examination was conducted.

Autopsy Findings: On external examinations, rigor mortis was present. Multiple incised-looking lacerations were noted, including those in the scalp, and all over the face and neck, anterior chest wall, shoulders and upper extremities. On dissection, the base of the skull fractures and the combination of various intracranial haemorrhages were noted. The conspicuous finding was the presence of a very minimal number of abrasions over the body.

Discussion and Conclusion: The injuries, produced by unusual mechanics, baffled the autopsy surgeons by simulating multiple chop wounds. Reconstruction of the event with the help of information forwarded by the police and crime scene photographs solved the puzzle.

Keywords: Road Traffic Accident, Bicyclist Impact, Body Trajectory, Lacerations, Chop wound.

Introduction

"Accidents are not accidents but precise arrivals at the wrong time."

A road traffic accident (RTA) is any vehicular accident occurring on the roadway that originates on, terminates on or involves a vehicle partially on the roadway[1]. Road traffic injuries are expected to take third place in the rank order of disease burden by the year 2027.[2] Worldwide, the number of people killed in road traffic crashes each year is estimated to be around 1.2 million, while the number of injured could be as high as 50 million[3], contributing to a major non-communicable cause of death all over the world.

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The essential driving force in an RTA is a tremendous, instantaneous, blunt force.

1. Primary impact- Struck by the bumper of a car and by the front of the bonnet, which injures the upper part of the leg or pelvis.
2. Secondary impact- a person is rotated so that the upper half of the body, head, shoulders and chest are thrown against the bonnet or windscreen
3. Tertiary impact- The Victim falls off the front of the car, finally coming to rest after sliding across the road surface. The greatest abrasions, contusions and lacerations are sustained here.

Several Subcategories of the same would be

1. Flat projections/over injuries- flat-fronted vehicles, the victim is thrown forward onto the road surface in front and is exposed to subsequently being run over.
2. The wing top person is struck by the front corner of the vehicle and is carried over the wing, falling to the ground at the side or behind of vehicle.
3. Rooftop occurs either at high speeds or when a vehicle accelerates after impact. Victims slide up the windscreen and over the roof.
4. Somersault - A feature of high speed that occurs when the lower part of the body somersaults to the ground.

In the following report, we have presented a case of a road traffic accident between an unknown vehicle and a bicycle. What makes this case essentially unique or off the record is the unusual set of injuries sustained by the victim, and the complex interplay of factors that led to the genesis of the same, a few of which we have been attempting to explain henceforth.

Case History

The deceased was shifted to a Medical College and Hospital in Kolkata, with a history of being dashed by an unknown vehicle and resultant injuries on 26.05.24 at about 17:30 hrs. He was declared dead by the on-duty EMO at the Medical College and Hospital, Kolkata, on 26.05.24 at 18:25 hrs.

Autopsy Findings

External Findings

1. Average built and nourished male subject, rigor mortis present all over the body.
2. Conjunctivae congested corneas hazy.
3. Blood is coming out of both the ears and the nostrils.
4. Multiple lacerated wounds on the scalp, in the parietal and occipital regions, the largest measuring around 2"x 0.6"x muscle.
5. Multiple incised-looking lacerated wounds- along an oblique line starting from the lateral margin of the right eyebrow across the face, the anterior aspect of the neck, and the left half of the anterior chest wall, the largest measuring around 5"x2"x muscle. (Fig. 1, Fig. 2, Fig. 3)

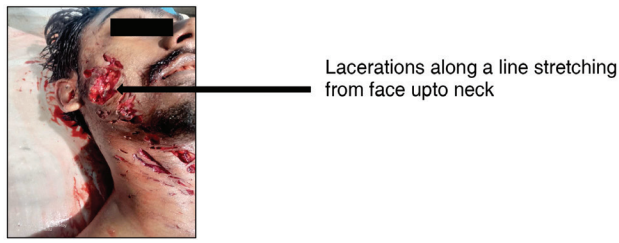


Figure 1



Figure 2

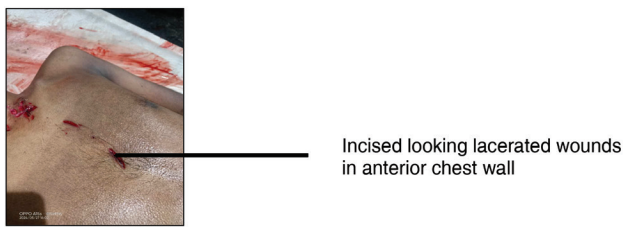


Figure 3

6. On dissection, neck wounds show diffuse extravasations of blood in and around. (Fig. 4)

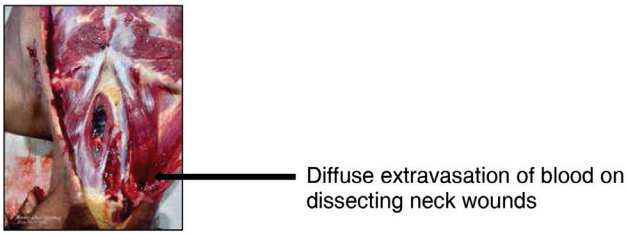


Figure 4

7. Lacerations are noted on the right clavicle, suprasternal notch, posterior aspect of left arm, forearm, and left elbow, left foot, the largest one with the dimensions 4.5"x2"x muscle. (Fig. 5)

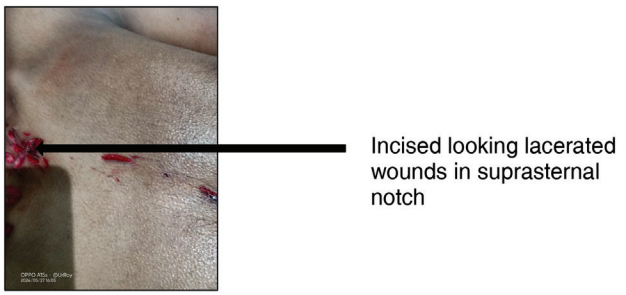


Figure 5

8. Minor abrasions are present over the right shoulder and left knee joint, showing extravasations of blood on dissection.
9. One bruise over the right knee joint

Internal Findings

1. Diffuse extravasations of blood into the layers of scalp tissue involving the right parietal and occipital regions- corresponding to the lacerations over an area of 6" x 4.5" (Fig. 6)

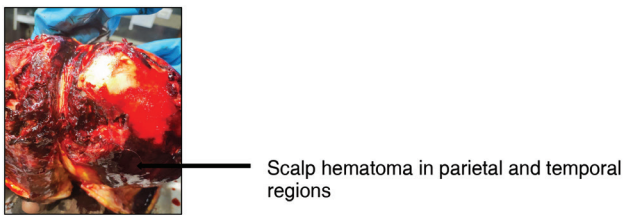


Figure 6

2. Global Diffuse Subdural haemorrhage involving the cerebral and cerebellar hemispheres of both sides. (Fig. 7)

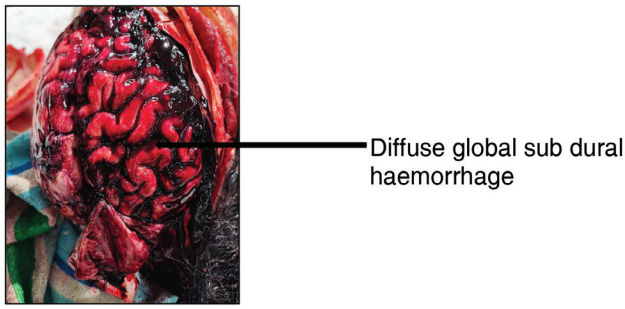


Figure 7

3. Fracture of the right supraorbital margin, right side of the base of the mandible, transverse fracture in the anterior cranial fossa. (Fig. 8)

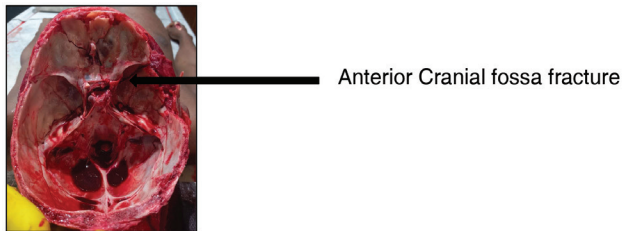


Figure 8

4. Bilateral lung contusion- right lung middle and lower lobes- left lung upper lobe. (Fig. 9)

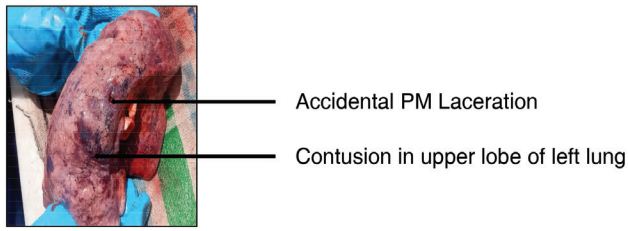
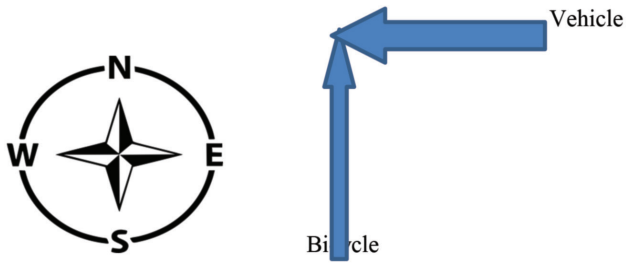


Figure 9

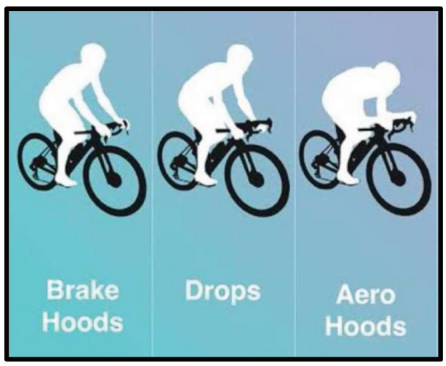
All the injuries noted above show signs of vital reaction.

Discussion

Case Scenario- The deceased was moving on a bicycle from south to North while the vehicle was moving from East to West.



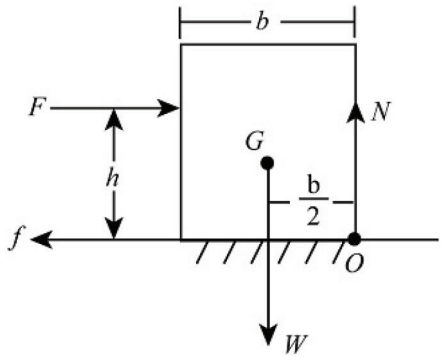
1. Primary impact- When the bicyclist sees the vehicle rushing towards him, there are two options for him: either halt and back off to steer clear or accelerate rapidly to get over the pre-estimated diametric boundary of the vehicle. The latter seems to be the case in this scenario. And for that instantaneous, rapid acceleration, the bicyclist changes the aerodynamic position to gain speed at the cost of losing control over the brakes. This changes the posture from "hoods " to "drops" or "aero hoods", a posture characterised by an almost horizontal forearm on handlebars, arched back and minimisation of frontal surface area. It is at this spot that the head protrudes ahead, assuming an almost "4-legged animal" posture.[6] A sudden blunt hit to the facial and thoracic region (to the forward-pushing half of the body) could be held accountable for the acute absence of abdominal trauma. The facial hitcaused the supraorbital and mandibular base fractures.



Also, since the deceased was on a bicycle, the autopsy did not reveal any blunt trauma to the lower limbs, a characteristic feature of pedestrian injury.

2. Effects of the primary impact-[7]

   a. Rotational component- Riding a bicycle will automatically increase the effective height centre of gravity of the rider(considering the algebraic sum of the COG of the bicycle and the COG of the rider). It is safe to assume that the vehicle had hit the rider at a spot below the centre of gravity, thus effectively decreasing the distance of application of force from the point of rotation, causing what is commonly referred to as the "Toppling effect".



The net torque produced will guide an anticlockwise rotational force, propelling the rider towards the bonnet of the vehicle, instead of a run down.

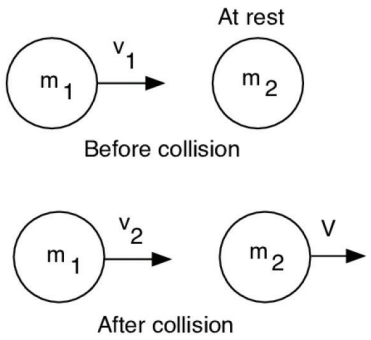
Atypia noticed the presence of bilateral lung contusion, predominantly in the posterior basal and lateral basal segmentsof the middle lobe of the left lung and the apical segment of the right lung, but without the presence of any rib fracture. This shifts our deduction to a contre coup injury rather than a primary one.

A person hits a windshield ⟹ Thoracic viscera undergoes relative motion within ribcage ⟹ opposite to the site of strike, an area of negative pressure is generated ⟹ increase in volume of dissolved gases at pulmonary and bronchial vessels(as per Boyle's Law) ⟹ vessels rupture, creating a local area of decreased perfusion match ventilation perfusion ratio, ventilation is decreased ⟹ peripheral asphyxia.

Lung contusion: Blunt chest trauma → alveolar-capillary damage → haemorrhage + oedema in alveoli & interstitium

This further explains why the heart is found intact and cushioned between the pulmonary viscera.

   b. Translational component- The given case scenario can be pictured as an inelastic head-on collision between an extremely heavy mass and a comparatively lighter mass.



Consider m1 = mass of vehicle,

m2 = mass of bicycle+rider system,

v1 initial velocity of the vehicle,

v2 final velocity of the vehicle

Final velocity of the system.

Here, the bicycle was travelling at right angles to the vehicle before the crash. Since cos 90= 0, we have no component of the velocity of the bicycle in the line of motion of the vehicle, so we have considered the bicycle+rider system to be stationary initially.

Now, following the Law of conservation of momentum for a one-dimensional head-on collision,

$$m1v1 + m2.0 = m1v2 + m2v$$

$$v = m1(v1 – v2)/m2$$

Since $v1 \geq v2$, $v \geq 0$, but this is an absolute velocity.

So when observed from a non-inertial frame of motion, the relative velocity of the rider and the vehicle comes out negative, which means that the rider acquires a force propelling it against the motion of the vehicle, responsible for the characteristic scooping up the vehicle, and ramming possibly against the windshield.

3. Secondary impact injuries- On ramming against the windshield, the consequences were as follows

    a. Extradural haemorrhage, producing diffuse bleeding over the parietal and occipital region under the scalp due to rupture of bridging scalp veins

    b. Transverse fractures (type III) in the anterior cranial fossa, responsible for the presenting feature of bleeding through the nose, are produced by general deformation of the skull. Cerebrospinal rhinorrhea, a possible option, was not inspected for.

    c. Diffuse global subdural haemorrhage, involving bleeding from the cerebral as well as cerebellar parts.

    d. Windshields are made of outer and inner layers of glass with a thicker plastic coating in between. Possibly the forceful propulsion of the right aspect of the rider caused the windscreen to crack and split, producing a long chain of lacerations, coalescing with each other, starting from the lateral aspect of the right supraorbital margin to the lower border of the base of the right jaw, continuing further downwards into the neck region and the anterior chest wall as the rider skids down the windscreen in an oblique direction from superolateral to inferomedial aspect, which then crosses midline of the body and continues on the inferolateral aspect as well.

    Further rolling of the body on the sharp surface could have produced the lacerations on the scalp, coupled with those on the extensor aspect of the left forearm.

    These wounds, at first glance, looked like chop wounds and were misleading, only to be ruled out on further meticulous examination.

5. The strangest atypia in this case is probably the acute scarcity, if not complete absence, of the abrasion marks, the most prominent features of road traffic accidents due to blunt force. This throws light on the fact that possibly the rider had not been inflicted upon the ground due to the slowing down of the vehicle, and thus had not received the tertiary impact injuries due to the rough ground surface. This could also possibly lessen the chances of the case bearing a classical “hit and run” tag, and erode the

malicious or homicidal intent of the driver of the vehicle, presenting the case more to be an "accidental" one.

Opinion after Autopsy

It was opined that death was due to the effects of antemortem injuries.

Consent

Informed consent was taken from -1. Next of kin of the deceased, 2. Investigating Police Officer 3, the autopsy surgeon of the present case, for access to the autopsy room, to capture photographs of significant findings and publish the same, keeping the identity anonymous.

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Conclusion

RTA victims, especially the ones brought dead, bring forth the immense task of a meticulous autopsy. The primary objectives during such an autopsy would be to determine the cause of death, confirm that death was caused by injuries suffered or sustained in the accident, and detect any disease or factor, for example, drugs that could have precipitated or contributed to the accident or death and document all findings for subsequent use in either criminal or civil actions [8]

This is usually coupled with several other investigations for the complete reconstruction of the scene of the RTA, such as the study of the accident scene and mechanical and engineering examination of the involved vehicles, and of each component whose failure could affect safety in motion[9]

With each factor executed to near perfection, it is possible to ascertain the disposition of appropriate judicial measures for all the individuals entangled with the incident.

Abbreviations

RTA- Road Traffic Accident

EMO-Emergency Medical Officer

COG- Centre of Gravity

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A Review on Arrhythmias due to Snakebite

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Abstract

An estimated 1.8 to 2.5 million venomous snakebites occur worldwide each year and result in at least 100,000 to 125,000 deaths. It is a public health problem particularly in tropical and subtropical regions. It is known that 4 species of snakes (Viperidae) Elapidae, Colubridae, and Lamprophiidae are determined to cause harm to humans. Envenoming by these snake species may cause myocardial damage and electrocardiographic deterioration. Common manifestations of cardiovascular toxicity are acute myocardial infarction, electrocardiogram abnormalities and arrhythmias. In this narrative review, we aimed to conduct a comprehensive analysis of cardiovascular toxicity due to snake bite.

Keywords: Snakebite, envenomation, arrhytmia

Introduction

Snake envenoming is a common medical emergency particularly in tropical countries [(1)]. Every year, approximately 2.5 million cases of snake bite occur and 81,000-138,000 deaths occur due to snake bites [(2)]. Most of the snake bites result in local symptomsincluding bleeding, edema, and ecchymosis, However, they may also cause systemic reactions such as acute kidney injury, rhabdomyolysis, stroke, coagulopathy, thrombocytopenia, neurotoxicity and cardiotoxicity. Up to now, 4 species of snakes are determined to cause harm to humans. These are Viperidae, Elapidae, Colubridae, and Lamprophiidae. Each species has potential to cause cardiovascular toxicity [(3)].

Envenoming by certain snake species may cause myocardial damage and electrocardiographic deterioration [(4)]. Common manifestations of cardiovascular toxicity are acute myocardial infarction, electrocardiogram abnormalities and arrhythmias [(5)]. Atherothrombotic disease may occur as a result of coagulation disorders which may lead to acute coronary syndromes, left ventricular function impairment, and arrythmia. The most common rhythm disturbances are atrial fibrillation, ventricular fibrillation, ventricular tachycardia, atrioventricular block, and non-specific ECG abnormalities (ventricular extrasystoles, prolonged QTc intervals, left bundle branch block, T-wave inversion, T-wave flattening, tall T-waves, and presence of U waves). Bradyarrhythmia is a less common manifestation. However, atrioventricular block or sinus node dysfunction (sinus node arrest) may also be observed [(3)].

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In snake envenomation; besides acute manifestations, subacute and late-onset complications may be determined. Thus, observation period and ECG follow-up period should be longer than typical cardiac patients [3]. Treatment modality for snake envenomation should be selected on a case-specific basis. When cardiac arrest due to ventricular arrhythmias occur, IV continous amiodarone should be administered. Also, cardiopulmonary resuscitation and intubation may be required. In case myocardial infarction emerges, standart procedure for revascularization should be initiated. If thrombocytopenia and coagulopathy is determined, the risk for bleeding increases and thus, the use of thrombolytic agents may be limited. If myocarditis occurs, anti-inflammatory medications should be initiated [3].In a study, 52 patients with snake envenomation were investigated in terms of treatment modality. Better results were obtained in the group that received both hydrocortisone and chlorpheniramine when compared to single hydrocortisone group and placebo group. Additionally in group that received both hydrocortisone and chlorpheniramine, less adverse reactions were observed[6].

In this narrative review, we aimed to perform a comprehensive analysis of snake envenomation literature and create awareness on optential cardiovascular effects emerge in patients with snake bite.

Action Mechanism of Snake Venom

Snake venom has both presynaptic and postsynaptic neurotoxins [1]. In certain species, venom is produced in specific glands and secreted to their saliva. The venom is a mixture of protein toxins and various enzymes [7].

Potential mechanisms of venom toxicity are increased capillary permeability, endothelial damage, and disruption of the coagulation system leading to hemorrhagic activity and edema. These mechanisms are executed with proteins and enzymes in the venom. However, myotoxicity, myonecrosis, and cardiotoxicity due to venom are rare [8].

There are two classes of venom toxins that may cause myotoxicity; phospholipases and L-aminoacidoxidases [8]. The main components of venoms are phospholipase A2, three-finger peptides, serine proteases and metaaloproteinases. Phospholipase is a compound found in all snake venom. Phospholipase is cardiotoxic and can cause hypotension when combined with other components such asnatriuretic peptides, bradykinin potentiating peptides, vascular endothelial growth factors, snake venom metalloproteinases, and snake venom serine-protease [3].

Even though mechanism of cardiotoxicity is in snake envenomation is not clear, there are some hypotheses that were proposed. Some of these are as follows: [1] direct cytotoxic effect, [2] toxin-induced arrhythmia, [3] acute coronary syndrome secondary to coagulopathy, [4] toxin-mediated coronaryspasm, [5] hyperkalemia secondary to acute renal failure and [6] inflammatory processes secondary to venom-induced hypersensitivity [8]. It was also reported that fatal cardiac rhythm changes ranging from bradycardia to ventricular tachycardia/fibrillation may develop in a short period following snakebite [9].

Venom components directly may cause hypotension. Also, hypotension may occur as a result of implications of envenomation (coronary ischemia or arrhythmias with hemodynamic instability) [3].

Experimental studies have shown that venom induces alteration of genes expressed in the heart, which is responsible for the mediators of immune response, apoptosis, ion transport, signal transduction, hypotension, energy metabolism and electron transport [10].

Primary action site of snake venom is known to be neuromuscular junction. Hence, it causes flaccid paralysis. Multiple toxin types in a single venom presents various actions [2]

Materials and Methods

We conducted a narrative review entering keywords “snakebite” and “arrhythmia” into the scientific database, Pubmed©. If the full text of the

article was not available and the researchers failed to retrieve it, then the article has been excluded if the abstracts were nonexplanatory. During the database search, we excluded studies, which did not relate with the objective by reading the title and abstract. Studies that were not published in English, studies without explanatory abstracts and studies that do not focus on cardiovascular effects of snake envenomation were excluded. Also, animal studies relevant to snake envenomation were excluded. It was observed that the majority of the studies in the literature consist of case reports on snake bites. Two reviewers conducted independent screening and data extraction. First, the reviewers independently screened titles and abstracts of the returned articles to decide if they met the inclusion criteria.

Results

A total of 64 articles were determined relevant to the topic. It was observed that most of the articles on snake envenomation in literature consisted of case reports. Twelve of the publications were excluded from the study because they were about dog exposure to snakebite. Two of them were horse exposure, 1 of them was swine exposure and 1 was rat exposure. Thus, when these articles were excluded, a total of 48 articles were included into the study.

Discussion

Snakebite is a common problem in rural areas of Asia[11]. Snake bites rarely cause cardiac manifestations and when present, main cause is vipers [1].

Cardiotoxicity is a rare manifestation of Russell's Viper's (Daboia russelii) bite.Russell's Viper toxicities are mainly bleeding disorder, nephrotoxicity, neuromuscular paralysis and respiratory failure[1,11]. Cardiac rhythm abnormalities include sinus bradycardia, sinus tachycardia, an atrioventricular conduction abnormality, and ventricular arrhythmia [12].

In a report, a healthy man presenting with a Russell's viper bite developed cardiac arrest from ventricular tachycardia. He was successfully treated by cardiovertion and amiodarone. Subsequently, antivenom for Russell's Viper was administered for correction of a bleeding disorder [11].In another report, a 35-year-old male patient presented with snakebite on his left ankle in 2 hours. The dead snake was detected and identified as a viper. The ECG of the patient revealed sinus node dysfunction characterized by sinus arrest with junctional escape rhythm and retrograde P waves with a rate of 40 beats per minute. It is probable that the venom alters electrophysiological features of cardiac cell membrane by effecting impulse generation and conduction [7].

A 14-year-old female patient was bitten by Russell's viper. The patient presented with mucosal bleeding, ptosis, and muscle weakness. A coagulopathy was determined in the laboratory analysis. The initial ECG of the patient revealed sinus tachycardia. Then, she developed bradycardia and her ECG revealed a delayed onset sinus node dysfunction[12].

Three young farmers died 15, 52 and 36 h after being bitten by Russell's vipers were presented byThan-Than et al. Clinical features included local swelling, spontaneous systemic bleeding, defibrination, shock, cardiac arrhythmia, hypoglycemia, coma and oliguria [13]

In another report a 60-year-old male presented to the ED following Russell's viper bite on the left ankle. His heart was irregularly irregular with a rate of 146 beats/minute. His ECG revealed atrial fibrillation with fast ventricular rate of 126 beats/minute. An echocardiography was performed and it was normal without wall motion abnormality. Russell's viper venom contains factor V and factor X activators within. These factors induce coagulopathy with their procoagulant potential. Rarely, Russell's viper venom causes myocardial infarction and atrial fibrillation [12].

In a study on patients with European adder (Vipera berus) bites were retrospectively analyzed. Of 26 patients, 14 patients presented systemic symptoms that require antivenom. The cardiac dysrhythmias

observed in 2 patients (ventricular arrhythmias) on admission and did not require treatment [14].

Vipera berus envenomation is well-known with its morbidity while its mortality rate is low. A 69-year-old male patient was bitten by V. Berus on the right thumb and presented to the Emergency Department 30 minutes after being bitten. In his medical history, it was determined that he had heart attack 10 years previously. His initial complaints were abdominal pain and vomiting. As he presented to the Emergency Department, he developed Diarrhoea. An ECG was performed and it revealed showed intermittent 2: 1 second degree heart block [15]. In another study with V. berus, 76 patients were determined in 7 years. In concordance, electrocardiographic finding of cardiac effect (T-wave inversion) was seen in 9% of the 54 patients in whom an electrocardiography had been performed [16].

A male patient at the age of 56 presented with severe coagulopathy following Vipera ammodytes ammodytes envenomation. The patient was hypotensive and a tachycardia was determined in the ECG [17].

In a study with 30 patients with snake bite, it was determined that majority of the patinets were bitten by Viperine snake and the rest by elapide snake. In 25% of cases with Viper bite, cardiotoxicity was determined. The most common cardiac manifestations were disturbance in heart rate, rhythm disturbance, tachycardia and bradycardia, respectively. Also, evidence for myocardial ischemia was detected in 10% of the patients. Another significant finding was that 10% of the patients died and all of these patients had abnormal electrocardiogram [18].

A 42-year-old healthy male presented to the Emergency Department due to hump-nosed pit viper (Hypnale hypnale) bite. The patient was admitted to the Emergency Department with cardiac arrest and following CPR the patient developed atrial fibrillation on ECG. Atrial fibrillation reverted to normal rhythm following synchronized electrical cardioversion was applied. However, the patient died due to multi organ failure in 16th day of follow-up [19].

A 49-year-old male patient with hump-nosed pit viper (Hypnale hypnale) experienced cardiac arrest 30 minutes after bite. Hump-nosed pit viper, up to then, was known only with its local effects. An ECG was performed and it revealed ST elevation in leads II, III and aVF with reciprocal changes in leads I and aVL, suggestive of an inferior wall infarction. Also, atrial fibrilllation with a heart rate of 132 per minute was determined [20].

In a report, a 14-year-old boy presented to a hospital mild envenomation symptoms after a spitting cobra (Naja sumatrana) bite. Premature multiple ventricular complexes were determined on his ECG. On the physical exam, ptozis as a sign of paralytic envenomation was determined. After antivenom administration, ptozis and ECG abnormalities have improved [21].

A 23 year-old male patient was bitten by a cobra. He had complaints of nausea and vomiting. On his examination, hypotension and tachycardia were determined. On ECG, infrequent ventricular ectopics that progressed to ventricular bigeminy was detected. Sinus rhythm was obtained following monovalent antivenom against Naja kaouthia venom was administered [22].

Agarwal et al. presented a case of a 26 -year -old male farmer was admitted to the hospital with complaints of breathlessness six hours after being bitten by a snake while working in the fields. The dead snake was brought in and identified as Bungarus caeruleus. On admission, the patient had repeated bradycardia and tachycardia episodes. Patient was also sweating despite sedation and maintenance of normoxemia. Patient developed pulmonary edema due to snakebite. This clinical finding was found to be associated with myocarditis due to venom. It was emphasized that neurotoxic snake venoms may cause cardiotoxicity. In order to prevent and complications like pulmonary edema, recognition of cardiac involvementis essential in these patients [1].

In a case, a healthy 39-year-old patient was bitten by Heloderma suspectum. The patient developed

tongue, lip swelling and stridor. When he was tranferred to Intensive Care Unit, he was hypotensive with hypokalemia. He developed a state of shock with atrial fibrillation and a electrical cardioversion was performed. Following cardioversion, patient's ECG revealed sinus tachycardia at 130 bpm, associated with diffuse ST depression in the majority of leads, particularly in aVR. In the follow-up, ECG returned to normal aside from an anteroseptal 1–2mm J point elevation. The elevation of troponin suggested non-ST elevation myocardial infarction. Even though it was not clear if myocardial infarction was strongly related to snake venom, the authors emphasized three possibilities following snake bite: angioedema, fluid loss associated with hypokalemia and metabolic acidosis,and cardiac disorders simulatingischemia[23].

In another study, 33 patients with snake bite were investigated. Sixteen of them were bitten by kratis and 14 of them manifested local swelling at the bite site and haemorrhagic manifestations. Three of the patients were bitten by cobras. However, in this study, authors do not focus on cardiovascular effects of the envenomation[24].

Khaldi et al. reported a 20-year-old male who developed atrial fibrillation following D.palaestinae snakebite. The patient was administered polyvalent anti-venom in the emergency department and relief in his pain was obtained. Then, he was transferred to the Intensive Care Unit for close monitorization. On the second day, the patient developed palpitations and shortness of breath. The patient did not mention a chest pain. His heart rate was 155 bpm, blood pressure130/65 mmHg, O2 saturation 95%, temperature 36.6. After appropriate consultations and medications, the patient was discharged with full recovery in three days[8].

In a study, 65 patients with snake bite were investigated in a 3-year period in terms of cardiovascular effects. Cardiovascular effect was defined as occurence of at least one of the followings: myocardial injury, shock, ventricular dysrhythmia, or cardiac arrest. In 9 patients, cardiovascular effects of envenomation were determined. Underlying cardiac problems were more common in these patients. None of the patients developed ventricular dysrhythmia or cardiac arrest[25].

A prospective study investigated electro-cardiographic changes in patients with snake bite inPapua New Guinea. Sixty-nine patients were involved into the study and 36 of these patients were bitten by taipan (Oxyuranus scutellatus), 2 were bitten by death adders (Acanthophis sp.) and 1 was bitten by the brown snake (Pseudonaja textilis). The most common cardiac abnormalities determined were septal T wave inversion and bradycardias, including atrioventricular block. Even though there were no patients with hemodynamic deterioration, 2 patients presented troponin elevation revealing myocardial damage[4].

23-year-old man bitten from finger was admitted to a hospital due to brown snake bite. On ECG; Intraventricular conduction delay, asymptomatic QRS and QT prolongation were determined. Following antivenom and supportive treatment, his symptoms resolved in 11 days (26).

In a report, a 73-year-old patient with co-morbidities bitten to his lower limb by Crotalinae was presented. Following hospitalized in the Intensive Care Unit, his electrocardiogram revealed new-onset atrial fibrillation. Amiodorone treatment was initiated and a sinus rhythm was obtained. A transthoracic echocardiogram revealed mild concentric left ventricular hypertrophy and an ejection fraction of 72%. The patient was discharged with baseline first-degree atrioventricular block as a sequela [5].

Of 10 patients with puff-adder (Bitis arietans) bite, 6 showed local signs and 4 showed systemic manifestations. Cardiovascular effects were hypotension, and bradycardia. Two patients died due to circulatory collapse and renal failure. Antivenom and supportive treatment helped with blood pressure in hypotensive patients[27]. The venomous snakes, their cardiac effects and treatment methods are summarized in Table 1.

Table 1. Commonly Seen Venomous Snakes Involved in the Review

Snake Species	Cardiac Involvement (Reference Number)	Treatment (Reference Number)
Russell's Viper's (Daboia russelii)	Sinus bradycardia, sinus tachycardia, an atrioventricular conduction abnormality, and ventricular arrhythmia (12), cardiac arrest from ventricular tachycardia (11), sinus node dysfunction characterized by sinus arrest with junctional escape rhythm and retrograde P waves with bradycardia (7), bradycardia and her ECG revealed a delayed onset sinus node dysfunction following tachycardia (12), cardiac arrhythmia and coma (13), atrial fibrillation with fast ventricular rate, myocardial infarction and atrial fibrillation (18).	Cardiovertion and amiodarone(11), anti-venom (7,11,12)
European adder (Vipera berus)	Ventricular arrhythmias (14), intermittent 2: 1 second degree heart block (15), T-wave inversion (16)	Did not require treatment (14), anti-venom, antibiotics when cellulitis occurs (15)antivenoms, corticosteroids (controversial) (16)
Vipera ammodytes ammodytes	Tachycardia and hypotension (17)	Intravenous fluids, antihistamines, antibiotics, glucocorticosteroids, antivenom (17)
Hump-nosed pit viper (Hypnale hypnale)	Atrial fibrillation, cardiac arrest (19),inferior wall infarction, atrial fibrillation (20)	Synchronized electrical cardioversion (19)
Spitting cobra (Naja sumatrana)	Premature multiple ventricular complexes (21), infrequent ventricular ectopics that progressed to ventricular bigeminy (22), no cardiac involvement (24)	Anti-venom (21,22)
Bungarus caeruleus	Bradycardia and tachycardia episodes, myocarditis (1)	Antivenom, mechanical ventilation when needed (1)
Heloderma suspectum	Atrial fibrillation, hypotension, non-ST elevation myocardial infarction, shock (23)	Electrical cardioversion (23)
Daboiapalestinae	Atrial fibrillation (8)	Anti-venom (8)
Taipan (Oxyuranus scutellatus)	Septal T wave inversion and bradycardias, including atrioventricular block (4)	Anti-venom (4)
Death adders (Acanthophis sp.)	Septal T wave inversion and bradycardias, including atrioventricular block (4)	Anti-venom and supportive treatment (26)
Brown snake (Pseudonaja textilis)	Septal T wave inversion and bradycardias, including atrioventricular block (4), intraventricular conduction delay, asymptomatic QRS and QT prolongation (26)	Anti-venom and supportive treatment (26)
Crotalinae	Atrial fibrillation, concentric left ventricular hypertrophy, first-degree atrioventricular block (5)	Amiodorone treatment (5)
Puff-adder (Bitis arietans)	Hypotension, bradycardia,circulatory collapse (27).	Anti-venom and supportive treatment (27)

Limitations

There are also some limitations of our article. Firstly, the fact that the majority of the articles in the literature consist of case reports makes it difficult to establish a certain standard for the treatment of snake bites.Another reason why such a standard cannot be established is that snake species vary greatly depending on geographical regions and the spread of snake speciesaround the world for commercial purposes is increasing.Additionally, another limitation of our study, as in all other review articles on snake bites, is the possibility that there are still unidentified potentially venomous snakes and their effects on the cardiovascular system are unknown.

Conclusion

In summary, cardiovascular toxicity secondary to snake bite should be treated with antivenom therapy combined with hemodynamic support and specific cardiovascular interventions. Cardiac involvement in snake bite may complicate clinical status and alter the treatment course. If abnormalities are detected in ECG, the patients should be monitored serial ECGs and cardiac enzymes. As people interact with wildlife more, their chances of encountering poisonous snakes will increase.Clinicians' awareness of snake envenomation and timely intervention will contribute significantly to the reduction of morbidity and mortality.As a future recommendation, every snakebite should be assumed to be dangerous and poisonous on cardiovascular system as well as other systems until proven otherwise, and ECG should be considered a priority modality in these patients. It should be known that the initial ECG is not sufficient and serial ECGs should be taken to monitor potential ECG changes.Long-term follow-up of patients should be provided for long-term complications.The cases collected from all over the World can be compiled in an international database and the correlation between snake species and symptoms can be made easily accessible.Artificial intelligence (AI) can also be used to create these databases. If a photograph of the snake is available, it can be uploaded to an AI application, and clinicians can be informed about po ssible symptoms depending on the snake species.Additionally, ensuring collaborative working conditions between international laboratories, a method proven successful during the COVID-19 pandemic, will be beneficial in preventing deaths due to snake bites.

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Exploring Microscopic and Spectroscopic Techniques in Forensic Hair Analysis: A Comprehensive Review

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Abstract

Hair is valuable forensic evidence due to its ability to link suspect, victims, and crime scenes. Traditional microscopic methods support morphological analysis and structural insights. This scoping review outlines key approaches used in forensic hair examination.A systematic PRISMA-based search was performed in the ScienceDirect and R-Discovery for studies published between 2014 and 2025. The search identified 2,399 records, and after removal of duplicate, 2,395 remained. Screening of titles and abstract excluded 2,189 articles, leaving 146 for full-text review. Following full text assessment, 39 studies were included for final analysis summarizing themajor microscopic and spectroscopic techniques for human hair analysis, highlighting their advantages and limitations.

Keywords: Hair, UV-VIS, Vibrational, Mass,Atomic, NMR, ESR Spectroscopy

Introduction

Trace evidence is hard-to-find evidence, which often proves significant in assisting investigators in solving a crime when other evidence fails to do so. Even the most skilled criminals overlook this evidence, leading to their apprehension. Hair is a type of trace evidence often overlooked or restricted to morphological analysis, undermining its evidentiary value[1,8]. Basic morphological parts of hair include a root adjoined to the dermis layer through follicular cells, and a shaft made of keratin proteins, lipids, and mineral elements[2]. Forensically, hair is evident in most of the cases. Hair can serve as valuable evidence in determining ancestry, human body area, and distinguishing between animal and human hair through the analysis of microanatomy and morphological differences [5,6,7]. Hair can aid in personal identification through unique marks from treatments and damage, such as straight or angled cuts, color changes from bleaching or dyeing, and conditions like pili annulati or monilethrix [6].

Nuclear DNA (Deoxyribonucleic Acid) can be found in the follicular cells of hair which can aid in personal identification[1,9]. However, for n-DNA analysis, hair must contain root or follicular cells. The presence of follicular cells can be determined by the stage of hair growth. Anagen and catagen stage is suitable for n-DNA analysis but Telogenroot

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is not viable for n-DNA analysis[3].Additionally, mitochondrial DNA obtained from the hair shaft can assist in tracing maternal lineage[4].A case report revealed that mitochondrial DNA analysis of hairs identified the victim in a child abuse case. Short Tandem Repeat (STR) analysis showed a strong match to the victim, while only a few STRs were typed from 10 naturally-shed hairs, suggesting the hairs were likely forcibly removed [10]. Even toxicological examinations can be performed on hair to identify drugs and poisons[11].

Furthermore, case reports have also provided evidence of using hair as a matrix for toxicological analysis. In a drug facilitated sexual assault case, quetiapine was detected in a hair sample six months later, with a trace amount found in the last segment (7–9 cm). This suggests the victim consumed a small dose shortly before the assault, illustrating the value of hair analysis when blood and urine tests no longer detect the substance [12]. Hair analysis shows higher precision and consistent recovery (87.2% to 93.0%) of psychedelic drugs, making it a more reliable method for detection in comparison to blood or urine[13].In two metformin intoxication cases, hair concentrations (22.5 ng/mg to 44.8 ng/mg) were significantly higher than therapeutic levels (0.3 to 3.8 ng/mg), suggesting that acute overdose [14]. In fatal pholcodine intoxication cases, hair analysis showed concentrations ranging from 38 ng/mg to 161 ng/mg at the root, 8.54 ng/mg to 41.6 ng/mg at the midshaft, and 0.26 ng/mg to 2.66 ng/mg at the tip. This suggests higher drug levels near the root, indicating recent exposure[15]. In view of its forensic importance, hair analysis relies on microscopy for morphological assessment and spectroscopy for structural insights. This review compiles the major microscopic and spectroscopic techniques employed for the comprehensive examination of human hair.

Methodlogy

Literature Collection

The scoping review search was conducted on Science Direct (https://www.sciencedirect.com/, n.d.)[16] and R-Discovery[17].The searches were performed using the PICO [Population, Intervention, Comparison, Outcome] framework. The population (P) included terms related to various types of hair, such as "hair," "human hair, "scalp hair," "pubic hair," "facial hair," AND "body hair,". The intervention (I) and comparison (C) were not applicable. The outcome (O) focused on several spectroscopy and analytical techniques, including UV-VIS Spectroscopy, ATR-FTIR, IR Spectroscopy, Mass Spectroscopy, NMR, AAS, AES, Raman Spectroscopy, ICP-MS, and others.

The identification phase involved 2,399 articles published between 2014-2025. After removing duplicates, 2,189 records were excluded based on titles and abstracts, leaving 146 reports for full-text review. Of these, 74 were excluded based on P & O criteria, 27 due to unavailable full texts, 1 due to assessable full text, 4 as duplicates, and 1 for lack ofprimary data. In the end, 39 studies were included in the review **(Figure 1).** Each step was assessed by authors, simultaneously usingComputer-Assisted Data Integration and Management for Systematic Reviews(CADIMA), a free web toolfacilitating the conduct and assuring for the documentation of systematic reviews, systematic maps and further literature reviews[18].

Results and Discussion

Microscopy Analysis of Hair

Boyala et al. (2023) found that plasma-treated hair appeared cleaner and had altered surface morphology due to MA-POSS grafting[19]. Cloete et al. (2023) compared body hair from different regions and found that scalp hair had smoother cuticle margins, while nape hair exhibited raised scales, likely from damage[20].Mills et al. (2018) measured human hair diameters, which ranged from 35.4 to 87.6 μm[21]. Richena et al. (2014) used Transmission electron microscopy (TEM) to show that grey hair contained melanin granules, while white hair exhibited nuclear remnants and fragmented granules[22].De Oblitas et al. (2024) examined human hair treated with bleach and cosmetic products, revealing that bleaching weakened hair filaments, while cosmetic treatments helped restore chemically damaged hair[23].Dos

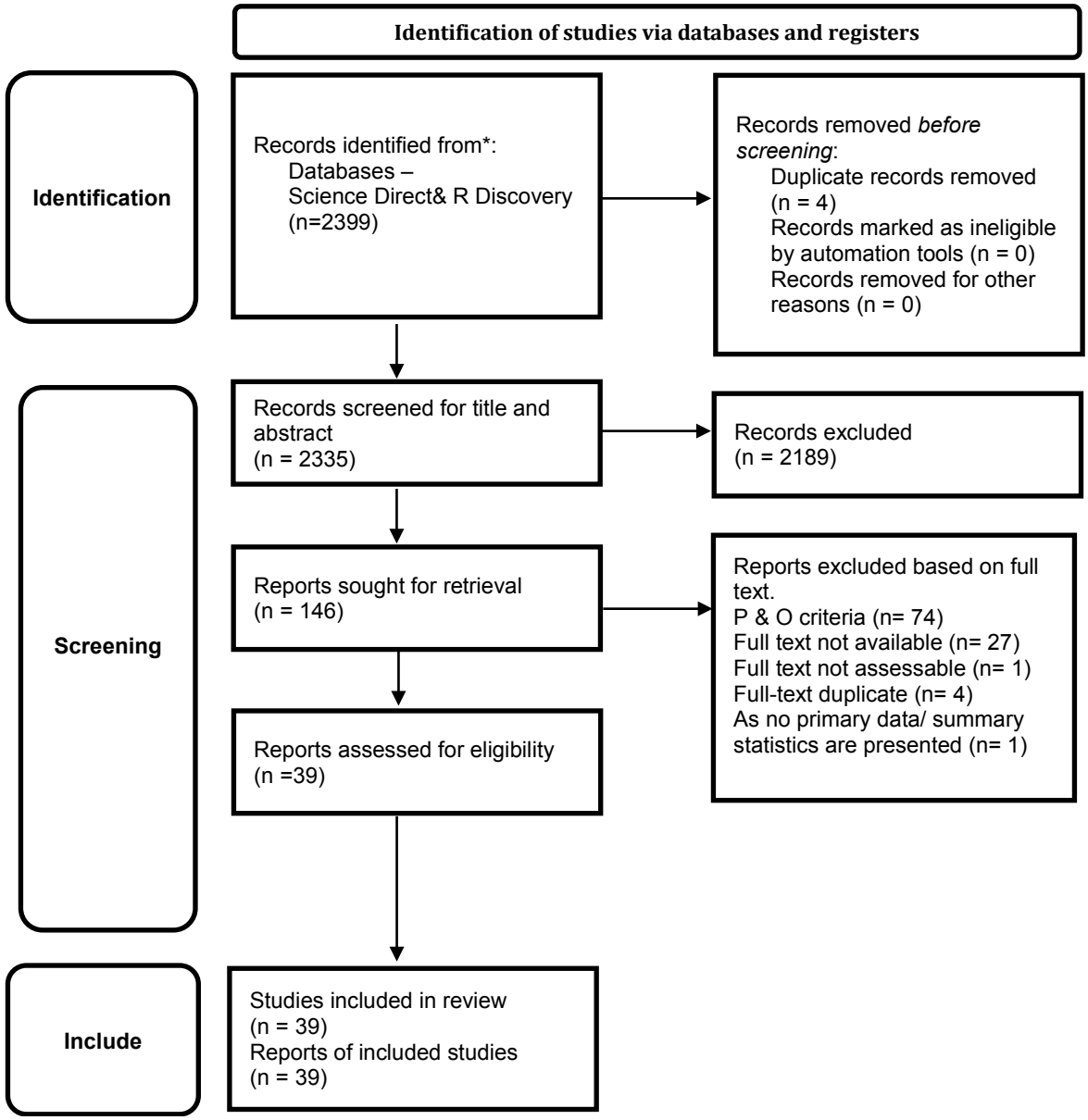


Figure. 1: PRISMA flow chart

Santos et al. (2019) reported that hair treated with a straightening iron showed cuticle damage, while washing products caused detachment and irregularities[24].Fedorkova et al. (2016) demonstrated that Ultraviolet rays irradiation caused increased surface roughness and cuticle damage in white hair[25]. Richena& Rezende (2015) observed small bumps on the cuticle surface of UV-irradiated human hair after prolonged exposure[26].

Spectroscopic Examination of Hair

Ultraviolet-Visible (UV-VIS) Spectroscopy

Richena et al. (2014) reportedλmax of 320 nm for hair samples. Additionally, Diffuse Reflectance Spectrophotometry (DRS) was employed to study the colour changes in grey hair samples. Blended grey hair initially showed a more yellowish tint (bi* ≈ 18-21) compared to single-donor grey hair (bi* ≈ 9-10). After thermal treatment, blended grey hair

experienced significant photobleaching (-5.9 vs. 1.5) and reduced yellowing (3 vs. 9), indicating that thermal exposure had a noticeable effect on the hair's appearance and colour stability[22].

Near-Infrared (Near-IR) and Mid-Infrared (Mid-IR) Spectroscopy

Sharma et al. (2024) employed FTIR to distinguish human hair based on sex and blood group. They observed distinct amide peaks at 1513 cm^{-1} for males and 1517 cm^{-1} for females. Additionally, a peak at 1300 cm^{-1} was found for the AB blood group, while the 1641 cm-1 Amide I peak was absent for the O blood group[27]. Cloete et al. (2023) employed Attenuated Total Reflectance - Fourier Transform Infrared Spectroscopy (ATR-FTIR) to differentiate body hair from various regions (scalp, eyebrow, underarm, pubic, and leg), identifying specific amide and methyl stretching peaks for accurate classification[20].Pienpinijtham et al. (2018) used FT-IR spectroscopy to analyze the effects of different hair treatments on human hair. Straightening creams caused a shift in the Amide II band at 1521 cm^{-1} and introduced new peaks at 1312 cm^{-1} and 1175 cm^{-1}. Permanent wave lotion produced a broad band at 1175 cm^{-1} and a peak at 1042 cm^{-1} due to cysteic acid's S=O stretching. Coloring cream created a new peak at 1175 cm^{-1} and increased the intensity at 1042 cm^{-1}. Split hair showed red shifts in Amide I and II bands and a new peak at 1575 cm^{-1}, linked to N–H scissoring vibrations [47]. Further studies also explored the effects of external treatments and environmental conditions. Boll et al. (2017) compared the FTIR spectra of dyed and non-dyed human hair spectral differences at 662–673 cm^{-1} (Sulfate stretching), 1250–1270 cm^{-1} (Ester stretching), and 1040 cm^{-1} (Oxidation of cysteine to cysteic acid)[1].Additionally, Richena& Rezende (2015) investigated the photodegradation of human hair due to UV exposure and observed increased peak intensity at 1041 cm-1, indicating cysteine oxidation into cysteic acid[26].

Raman Spectroscopy

Dos Santos et al. (2019) examined the effects of cosmetic treatments on white Caucasian and Afro hair, noting spectral changes in decolorized and thermally treated hair. For decolorized hair, they observed peaks at 976 cm^{-1} (cystine oxides) and 1044 cm^{-1} (S-O symmetrical stretch of cysteic acid). In thermally treated hair, they detected a decrease in intensity at 509 cm^{-1} (S-S stretch of cysteine), 665 cm^{-1} and 747 cm^{-1} (C-S bond), and a shift from 1663 cm^{-1} to 1670 cm^{-1}, associated with β-sheet and random coil forms of protein amide groups[24].Fedorkova et al. (2016) studied the effects of photoirradiation on grey hair, observing a decrease in intensity at 440–590 cm^{-1} (S-S bonds of cystine), 630–712 cm^{-1} (C-S bonds of cystine), and 2501–2598 cm^{-1} (S-H bonds)[25].Kurouski& Duyne (2015) studied hair dye effects, identifying distinct peaks for "Ion Blue Sky" dyed hair at 1626–238 cm^{-1} and "Ion Jet Black" dyed hair at 1638–360 cm^{-1}, reflecting differences between semi-permanent and permanent dyes[28]. Holman et al. (2024) studied hair dye degradation under simulated burial conditions, noting significant spectral changes over time, particularly at 1512 cm^{-1}, 1433 cm^{-1}, and 1361 cm^{-1}, which correspond to C–C stretching vibrations of aromatic rings in the colorant compound[29]. Santos et al. (2022) found that cosmetic treatments on Afro-textured hair caused specific spectral changes: Product A (Silicone-based) had a Si-O-Si band at 488 cm^{-1} and reduced cysteic acid at 1044 cm^{-1}. Product B (Water-based) showed increased tyrosine at 852 cm^{-1} and decreased Amide III at 1248 cm^{-1}. Product C (Humectants & Silicones) displayed Si-O-Si and Si-C bands at 489 cm^{-1} and 712 cm^{-1}, with weakened cysteic acid at 1044 cm^{-1}[30]. Juarez & Kurouski (2023) found that household contaminants like soy sauce, molasses, and red wine caused fluorescence interference and peak shifts (1400 cm^{-1} vs 1500 cm^{-1}), while viscous substances like glue and honey weakened the Raman signal[31].

Mass Spectroscopy

Rashaid et al. (2015) found sex differences in scalp hair amino acids, with females having higher levels of valine (9.8 vs. 6.8 mg/mg), proline (12.7 vs. 9.8 mg/mg), and phenylalanine (3.4 vs. 2.3 mg/mg) compared to males[32].Szynkowska et al. (2015) found sex differences in trace elements, with females having higher Co, Cu, and Sr, and

lower Pb, Li, and Cr than males**[33]**. Ha et al. (2019) compared elemental concentrations in hair from 322 individuals, revealing higher calcium and magnesium levels in females across all age groups. Regionally, glycine levels were highest in the valley (5.0 mg/mg), and cysteine was significantly higher in the mountain region (4.1 mg/mg). Smokers had higher Cu levels, and colored female hair showed more Cu and Sr than non-coloured hair[2]. Adav et al. (2023) studied protein differences between sexes and ethnicities, finding higher levels of KRT36, KRT39, KRT82, and KRT84 in males, and KRTAP4-6, 13-1, and 9-9 in females. Filipino females had lower KRT31, 32, 33, and 34, while Indian females had higher KRT31, 81, and 83. Chinese individuals had higher KRTAP3-1, and Indians had higher KRTAP7-1[34]. Mason et al. (2019) used MS to identify 578 unique proteins in human scalp hair, including 29 keratin-associated proteins[35]. Wu et al. (2024) investigated the effects of bleaching on hair, finding that two keratin-associated proteins, KRTAP1-5 and KRTAP1-2, were downregulated in damaged hair[36]. Carelli et al. (2020) used Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) to detect flocoumafen in the hair segment near the root (18 pg/mg) and difenacoum near the tip (140 pg/mg)[37]. Goncalves et al. (2022) confirmed polydrug use in a 26-year-old by analysing hair samples, with no external contamination detected[11]. Poetzsch et al. (2014) compared analytical methods for detecting zolpidem use, showing that LC-MS/MS had higher sensitivity for detecting single-dose exposure, while MALDI-MS (Matrix-Assisted Laser Desorption/Ionization– Mass spectroscopy) was faster but less sensitive, capable of detecting zolpidem usage up to 6-8 weeks prior to collection[38].

Atomic Spectroscopy

Atomic Absorption Spectroscopy

In Rocha et al. (2014), Atomic Absorption Spectrometry were used to assess mercury (Hg) levels in human hair samples from children living in two regions in Brazil. The study found Hg concentrations of 3.57±1.86 mg/kg in hair samples from children in the DeMarcacao area and 6.24±5.89 mg/kg in those from the Gleba do Rio Preto region, highlighting environmental mercury exposure. The results revealed that diabetic rats had significantly higher levels of Cu (158 µg/g vs. 121 µg/g), Fe (87 µg/g vs. 8.34 µg/g), and Na (127 µg/g vs. 0.34 µg/g) compared to their non-diabetic counterparts, providing insights into altered metal metabolism in diabetes[39].

Atomic Emission Spectroscopy

In Zhang et al. (2021), ICP-OES was used to analyze hair samples for the Ca/Na and Mg/Na ratios, revealing 5.6015 ± 0.0383 and 0.3961 ± 0.0012, respectively[40]. In a subsequent study by Zhang et al. (2020), ICP-OES was also applied to measure zinc and copper concentrations in hair, finding zinc levels ranging from 16.3 to 17.8 µg/g and copper levels between 1.16 to 1.78 µg/g[41]. The Rafiee et al. (2020) study revealed significant differences in metal concentrations in scalp hair between male and female participants, with males showing higher concentrations of elements like Cr, Fe, and Ni, while females had higher levels of Ag and Ba. Additionally, smoking had a noticeable impact on the concentrations of Hg and Pb[42]. Kumakli et al. (2017) focused on racial and age-based differences in metal concentrations. They found varying levels of Ca, Mg, and Zn in different racial groups, with Caucasians showing the highest concentrations of Zn and Ca, while African Americans had the highest levels of Na and Ca. Smoker vs. non-smoker groups exhibited lower concentrations of As, Ca, and Mg in smokers. Age trends showed the highest calcium levels in the 25–65-year age group[43].

In the context of Laser-Induced Breakdown Spectroscopy (LIBS), Cherni et al. (2022) used LIBS to compare hair samples from women with osteoporosis, osteopenia, and healthy controls. They observed that Ca intensity decreased with disease severity, while Na intensity increased[44]. Similarly, Zhang et al. (2021) found similar elemental ratios of Ca/Na and Mg/Na in hair samples analyzed with CF-LIBS[40]. Finally, Zhang et al. (2020) utilized Ultrasound-assisted alkali dissolution - LIBS (UAAD-LIBS) for the determination of zinc and copper in hair, confirming Zn concentrations between

16.63 to 18.04 µg/g and Cu levels between 1.14 to 1.80 µg/g[41].

Nuclear Magnetic Resonance (NMR) and Electron Spin Resonance (ESR) Spectroscopy

In Goncalves et al. (2017), NMR Spectroscopy was employed to analyze a hair sample from a 26-year-old polydrug user. The study used proton and carbon NMR to identify signals corresponding to Methoxyphenylpropan-2-amine (MXPr), a drug marker, by detecting 16 carbon and 12 proton signals, which demonstrated the presence of this compound in the hair matrix. In contrast, Cam et al. (2014) applied ESR Spectroscopy to investigate the effects of radiation on human hair samples that were subjected to varying doses of irradiation (5-750 Gy). This study revealed a dose-response curve for radiation-induced changes in hair, with women showing slightly higher radiation effects (0-29 mm/hr) compared to men (0-22 mm/hr)[45].

X-ray Spectroscopy

Boyala et al. (2023) employed X-ray Photoelectron Spectroscopy (XPS) to analyze natural and MA-POSS grafted hair, observing notable differences in the elemental peaks. Grafted hair exhibited additional silicon peaks. For virgin hair, the peaks at 103.3 eV, 154 eV, 284.8 eV, 402 eV, and 532 eV correspond to Si 2p, Si 2s, C 1s, N 1s, and O 1s, respectively. In contrast, MA-POSS grafted hair showed two extra peaks corresponding to Si 2s and Si 2p[19]. Cloete et al. (2023) used Synchrotron Microbeam X-ray Fluorescence to analyse body hair, finding significant variations in elemental concentrations across regions. Underarm and pubic hairs had fewer Al-Mg-Si hotspots. Carbon (C) was highest in the eyebrow, nitrogen (N) in the nape of the neck, oxygen (O) in the leg, and sulfur (S) in the eyebrow. These results highlighted distinct elemental distributions across different body parts[20]. Tintner et al. (2020) used Small-Angle X-ray Scattering (SAXS) to study various animal hairs, observing scattering peaks indicative of inter-filament distances and lipid crystals. Samanta et al. (2016) used X-ray Diffraction and Energy Dispersive X-ray Spectroscopy (EDAX) to analyse bleached, virgin, and treated hair, discovering that treated hair contained Fe-tannin complexes and Fe-oxalates, which were responsible for the color change. While bleached hair may contain oxalates from food or the bleaching process, the color in treated hair was primarily due to the Fe-tannin complex[48].

Conclusion

Microscopic and spectroscopic techniques play pivotal role in forensic hair analysis with each offering strengths for examining morphological, chemical and structural features. Microscopy remains the foundational approach, enabling a detailed assessment of hair morphology and the detection of cosmetic or environmental conditions. UV-VIS spectroscopy and Diffuse reflectance spectrophotometry are useful for evaluating treatments such as irradiation, heat, and bleaching. Near-IR and Mid-IR spectroscopy provide versatile molecular insights, supports species identification, differentiation of body hair types, and identification of cometic and environmental impacts. Raman spectroscopy enhances molecular characterization by detecting subtle structural changes associated with cosmetic products, UV exposure, and contaminants, although fluorescence interference in pigmented hair remains a significant limitation. Mass spectroscopy offers precise biochemical and elemental profiling, detecting metals such as Co, Cu, Sr, Pb, Li, Cr, Ca, and Mg, with applications in assessing environmental exposure, drug abuse, sex differences, and geographic indicators. Techniques like GC-MS and ICP-MS expanded analytical sensitivity but face challenges such as the need for volatile samples or difficulty distinguishing endogenous elements from surface contamination. AAS and AES contribute essential data on elemental concentrations for environmental and psychologicalevaluations. Complementary methods like X-ray spectroscopy, NMR, and ESR provide deeper atomic and molecular insights bit often require high sample purity or complex interpretation. Emerging technologies such as LIBS show considerable promise but requirefurthervalidation. Overall, combing traditional microscopy and

advanced spectroscopic methods guide by case requirement and standardized protocols ensure robust, comprehensive and reliable hair analysis framework.

Declarations

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Consent for Publication: Not applicable

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Scope of CADAVIZ in Training for Forensic Odontology, Anthropology and Radiology as an Adjunct Tool

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Introduction

Forensic sciences train potential scientists for collection, handling and analysis of evidence, supporting investigations in order to solve crimes and maintain law. It is a broad field encompassing psychology, law, statistics, crime scene management, forensic evidences, fingerprints, forensic biology, anthropology, narcotic drugs, forensic ballistics, forensic chemistry, forensic serology, DNA profiling, forensic medicine, toxicology, digital forensics, cyber forensics, forensic pharmacology, pharmaceutical drug analysis, genomics, immunology, proteomics, audio-video analysis, biometrics, etc. Of these, forensic odontology, radiography, imaging and skeletal analysis of bodies covers a heterogenous group of forensic medicine. The application of diagnostic odontology, imaging, radiology, & anthropology, has been employed in the identification and verification of individuals from skeletal remains and decomposed bodies. A forensic autopsy is conducted to address this objective. An autopsy is done in case of any unnatural deaths, suspicious deaths or unexpected deaths as part of the investigation process.[1]

Forensic students require hands-on training on cadavers, to learn the conventional form of autopsy. This autopsy includes training in techniques such as external examination of the body, different types of incisions e.g., *I-shaped incision*, *Y-shaped*, *modified Y-shaped*, *X-shaped incision*, coronal incision for cranial cavity, opening up the thoracic and abdominal cavities, Technique of Virchow (organs removal), Technique of Rokitansky (in-situ dissection of organs), Technique of Ghon (cervical and thoracic organs, organs in the abdomen, and the urogenital system removal as separate organ blocks), Technique of Letulle (removal of the cervical, thoracic and abdominal organs as a single organ block) and other ancillary investigations crucial in forensic education.[1]

Training on cadavers for students of the forensic science program is posed with hassles, given the limited cadaver availability, cost of such training in medical schools, cost of acquiring, maintaining and treating cadavers, and difficulty to revisit completed dissections due to damaged structures, it has become common practice especially after COVID-19 pandemic to study using virtual teaching methods.[2,3]

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Conventional autopsy too faced challenges of body preservation after post mortem, difficulty in acquiring data from decomposed bodies, the risk of infection or exposure to toxins, obtaining data from inaccessible body parts, data acquisition without hurting religious sentiments or ethics, unbiased documentation of evidence, etc. In order to overcome these problems exhibited by conventional autopsy and so as to not default from the objectivity of a post-mortem, a virtual form of autopsy was introduced. Virtual autopsy project or VIRTOPSY was initiated at the Institute of Forensic Medicine and of Diagnostic Radiology of the University of Bern, Switzerland by Dr. Richard Dirnhofer.[4]This was built with the objective to integrate postmortem CT scans (PMCT), postmortem magnetic resonance imaging (PMMR) and image guided biopsies into forensic pathology investigations.[4,5] It has been proposed that, virtually conducted autopsy procedure, will not only aid in quicker, precise and objective documentation, but it will also facilitate in quality control through digital data transfer and archiving. Virtual autopsy is a non-invasive autopsy, which uses digitalized environment for whole body imaging. The use of forensic radiological facilities as compared to traditional autopsy could reduce the danger of infection to the medical staff especially during pandemics such as COVID-19, H1N1 Flu and epidemics like Ebola, tuberculosis etc.[6,7]

Computed tomography (CT) has been used in forensics since the 1970s.[8] Subsequently, modern imaging technologies such as 3D photogrammetry and surface scanning were incorporated[9]along with CT angiography (CTA), magnetic resonance (MR), sonography, ultrasound, ultrasonography, surface scanning, and in recent times, multi detector computed tomography (MDCT) which provides full body multiplanar advanced images.[10,11,12] With the digitalization of imaging modalities, 'virtual autopsy' has become standard practice across many institutes worldwide.[13] This technology can help students train on planning the autopsy process for a more focused approach. It can allow for a wide range of information such as injuries, fractures, physical conditions that can be easily missed by traditional autopsies. The 3D reconstructions could help in understanding the nature of trauma and extent of injuries caused by it. A library of cases can be created and used to train students of trauma cases, to help students identifying bone fractures, ballistic trauma, reconstructing medicolegal cases, gunshot trajectories in ballistics cases, in hospital deaths, helping to determine cause of death and identify potential medical errors.[14, 15] This technology can help plan the autopsy process for a more focused and efficient investigation.

CADAVIZ[16], which is worlds' most advanced virtual human dissection table, can serve dual purpose here. It is a cutting-edge device featuring an 89″electronic table with an ultra-wide intuitive touchscreen, with multiple and simultaneous touch points.It is a pedagogic tool providing a full-scale digital depiction of the human body, enabling observation, manipulation, and virtual dissection of 3D models with detailed anatomical components. Users can navigate through different systems with the help of the visibility menu. The visibility slider enables users to explore life size cadaver dissection, layer by layer for complete understanding. It has an extensive collection of radiology scans and a DICOM viewer (Digital Imaging and Communications in Medicine viewer), enabling users to visualize case data from CT (computed tomography), MRI (magnetic resonance imaging), X-ray, and other imaging modalities and perform comparative studies, when required, to interpret medical images of patients retrieved from the PACS (Picture Archiving and Communication System). It can be used in the study of diagnosis of a variety of conditions. Previous postmortem cases can be uploaded to the system, including data from Virtopsy to present scenarios to students to build on their observational and deductive skills. CADAVIZ is compatible with the available medical imaging data files. These image files can be converted into 3D models for better visualization and be used as a review system for such data both for teaching and diagnosis. CADAVIZ could aid in the learning process of forensic sciences, utilizing 3D reconstructions from CT scan data or other DICOM data.

Aims And Objectives:

1. To understand and review the utilities of CADAVIZ™ instrument in the education and training of forensic medicine students, namely in forensic odontology, anthropology and radiology.

Material and Methods

1. CADAVIZ™ table, Guidelines and manuals of CADAVIZ™ version V1.1.5 (Manual &Notes).[17,18,19]
2. Detailed literature search was performed in Google Scholar, PubMed, and SCOPUS. The search terms virtual autopsy, radiology in forensics, forensic dental data for identification, anthropological data and post mortem were used.

Discussion

CADAVIZ: Use in forensic medicine training to identify individuals from human remains

In spite of the recent technological advances in radiology and forensic imaging, radiology has been a part of forensic sciences since its inception.[20]Radiology modalities are also used in archaeology and anthropology[20]; the increasing demand for the development of accurate and non-invasive methods has led to the evolution of this field.[21] We describe the use of CADAVIZ in the field of forensic odontology, anthropology and radiology, where we make use of radiological data uploaded to CADAVIZ in the form of scans and 3D models.

Forensic Odontology

Dental structures have long been of key importance as the proof of identity in mass fatalities both in natural and man-made disasters. Lack of extensive fingerprint data makes dental identification vitally important. Teeth are strong, durable and resistant to external impacts e.g., fire, explosion, chemicals etc. and hence make them ideal for a postmortem. Teeth can be used to identify the age of an individual, based on factors such as attrition, periodontal disease, secondary dentine formation, and root translucency, resorption of roots, root roughness, apposition of cementum, color change in the crown and the roots which are noticeable with increasing age.[22] Dental restorations can indicate the socio-economic status of an individual. The wear and stain pattern can be an indicator of work or personal habits like smoking etc.[23] Tooth crown morphology can be used in race identification. Anthropologists and odontologists in France and Germany have demonstrated that the number of cuspids of molars differ between the main human races, namely Caucasian, Mongolian and Negroid population. Thus, helping in raceidentification.[24]

A forensic odontology training program requires students to have a practical training component so as to develop a methodological approach in age estimation, bite mark analysis, and dental identification (Image 1). This education relies on hands-on training including head dissections, dental autopsies, exercises on cadavers, on which various dissections are performed, and radiographs.[25] Training on cadavers may be challenging for most dentists, here CADAVIZ could provide with a sustainable learning option for students, doctors and pathologists of forensic sciences. Post-mortem dental and radiological data could be collected and analysed on CADAVIZ. The CT scans could be utilized in 3D reconstructions of the head region, allowing to accurately visualize and analyse with high resolution, the skull, jaws &teeth to identify the reference points for critical measurements. It will also help students to familiarize themselves with the images to understand the basics of standardized collection of dental autoptic parameters.[26]

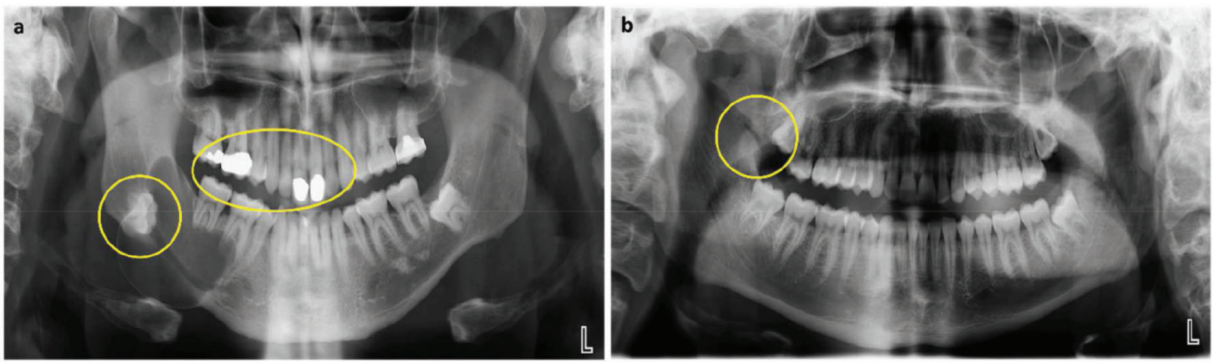


Image1: Radiograph of OPG **(a)** OPG of male patient, showing dental fillings, unerupted molar teeth; **(b)** Male,20 with history of trauma with displaced fracture of the right coronoid process

With the help of 3D reconstructed images on the table, students can visualize the process of dental autopsy, mainly the jaw and teeth. The image can be rotated, zoomed in or out (in proportion) and dissected using arbitrary clipping method. Soft tissues can be hid so as to expose the jaw. Jaws can be dissected to allow detailed visualization of any anomalies, periapical pathologies, dental treatments, analyse missing teeth and alveoli, crown morphologies and dental treatments.[27] With multiplanar reformatting (MPR) function abnormalities at different planes can be located. The multiple presets setting would help to isolate bones, muscles, circulation etc., thereby locating deformities. The table allows to take screenshots of the observed area, which could facilitate the documentation of the case under study.

Forensic Anthropology

Forensic osteometry uses measurements of bones to identify details such as the sex, stature, age, population affinity etc. Bone growth and fusion patterns are specific to different agegroups, the pelvic and cranial structures exhibit gender specific differences, long bones are used in height determination, specific dental and cranial features aid in the estimation of ancestry of individuals.[28,29] Axial skeletons, appendicular skeletons, skull, pelvis and teeth measurements are used for this purpose. The application of osteoma (try for human identification is an important element in the field of forensic sciences.[30] Measurement of dry bones is done by validated instruments such as vernier caliper or osteometric board. Researchers have established landmarks on the bones for the purpose of human identification.[31]

Researchers have used these tools to measure dry bones, however using dry bonesencounters problems; method of bones conservation may be inadequateand this could lead to bone deterioration, wear or alteration of its taphonomicaspects.[32] With time, digital tools have been implemented in forensic anthropology, for identification, forsuperimposition of images, for 3D reconstructions and these tools use diagnostic imaging modalities to obtain more detailed information than that obtained from a dry bone. These images representing the bonescan be stored in centralized databases encouraging multidisciplinary work. The images could be analysed by different software programs to aid in the development of a forensic test method.[33,34] The 3D format would allow students, for macroscopic and morphometric examination, analysing the structure of the bones to facilitate identification of individuals.[30] This application of 3D virtual models within forensic anthropology has led to the new sub-field known as 'virtual anthropology'.[35]

Forensic Radiology

Forensic radiology uses radiological medical imaging techniques to assists pathologists. In this the soft tissues, abdominal viscera, thoracic viscera and osseous skeleton are the prime areas for evaluation.[36] Examination of the osseous skeleton is the key for radiological evaluation. Since long, forensic radiology has been employed for the identification of humans especially in mass disasters and decomposed bodies, in the evaluation of injury and cause of death either accidental or non-accidental, in criminal investigations; in education and research etc[36]. Accidental or inflicted injury can be distinguished by identifying the type of fracture, for e.g., in skull injury, the direction and configuration of the fractures can help determine the impact point, direction, sequence of repetitive blows, shape of the weapon used. Similarly, strangulation, blunt trauma, hanging, etc, is identified by the hyoid bone fractures (Image 2). Other traumas such as intracranial haemorrhage, penetrating wounds, vascular system injuries, etc, can be revealed using contrast media used in CTA.[37]

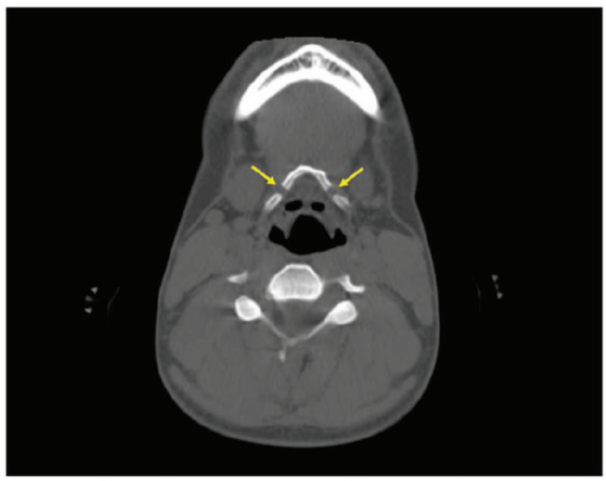


Image 2: Male, 17 with hyoid bone (right cornu) fracture (football tackle)

CT, is mainly used in cases of unnatural deaths related to gunshot injuries and trauma while, MR is used primarily in natural cases related to diseases of the cardiovascular or central nervous system. However, CT is superior to autopsy in

the documentation and visualization of skeletal injuries.[38]The scans obtained from these imaging modalities are available in the DICOM format which can be reconstructed into 3D models. The 3D models can aid in the detection of bone structure, identifying the weapon in case of penetrating wounds (internal haemorrhage), or tracking the direction and extension of the wound[9](Image 3 & 4). The CADAVIZ table is enabled to be viewed with radiology mode and anatomy mode simultaneously on 2 screens. In this module, general and pathological comparisons can be made on two screens placed side by side,at the same time. The multiple presets setting helps to isolate bones, muscles, circulation etc., thereby locating deformities.With multiplanar reformatting (MPR) function user can locate abnormalities at different planes. Adjustment of contrast with quality/shift & shade settings guides for a better view of images. It is equipped with integrated 3D volume rendering tools for high quality 3D visualization and navigation through personalized data and anatomical structures.

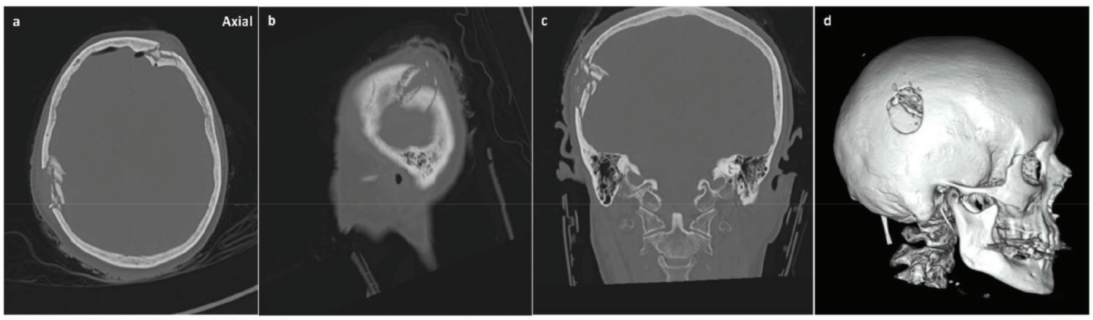


Image 3: (a)(b)(c) - CT scan of a Male, 80 with comminuted, right parietal depressed skull fracture, with internal displacement of the fracture fragments (d) 3D 3D model of the CT image
Source:
Di Muzio B, Depressed skull fractures (hammer). Case study, Radiopaedia.org (Accessed on 24 Apr 2025)

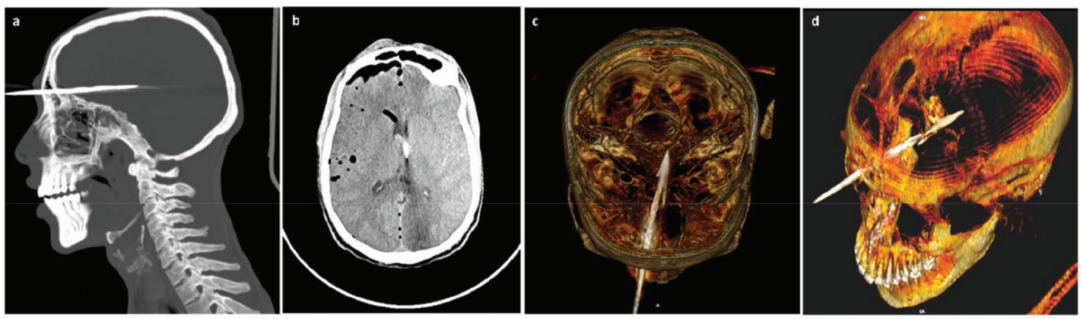


Image 4: (a)(b) – CT scan of Male, 35 Injured by arrow through the forehead, arrow penetrates right frontal sinus and tip terminates near the left basal ganglia. Pneumocephalus and air within the ventricles **(c)(d)** - 3D model of the CT image for better visualization.
Source:
Hartung M, Arrow injury to the brain. Case study, Radiopaedia.org (Accessed on 23 Apr 2025) https://doi.org/10.53347/rID-72101

The digital images can be stored and reviewed again for practice. Thus, training the pathologist to interpret with superior objectivity reducing inconsistencies and biases. This virtual autopsy training method is reproducible and revisitable.

This review does not include comparative studies evaluating the outcomes of CADAVIZ versus cadaver training in the field of forensics. However, similar feedback studies involving MBBS and BAMS students have been previously reported in the subjects of anatomy and dissections [39, 40].

Additionally, a comparative study on teaching MBBS students using CADAVIZ and cadavers is currently under publication.[41] All of these studies positively conclude that while traditional cadaveric dissection is an essential tool for learning human anatomy, combining it with a virtual dissection table provides the best understanding.

Results and Conclusion: Adjuvant to autopsy training

In this paper we refer to examples of blunt force trauma to identify the weapon used, the impact of arrow injury to the head.[42, 43] how dental structures and bone structure analysis can be used to identify individuals especially in mass fatalities.

The CADAVIZ table enables for the conversion and observation of CT scan images into detailed 3D reconstructions (Image 5). These digital images can help train students to identify key findings in an autopsy. We can repeat the training innumerable times, helping in enhancing student assimilation. It eliminates the need for cadavers and is cost effective. CADAVIZ reduces the risk of exposure for trainee students/pathologists doctors, to infection and hazardous chemicals from cadavers/dead bodies.

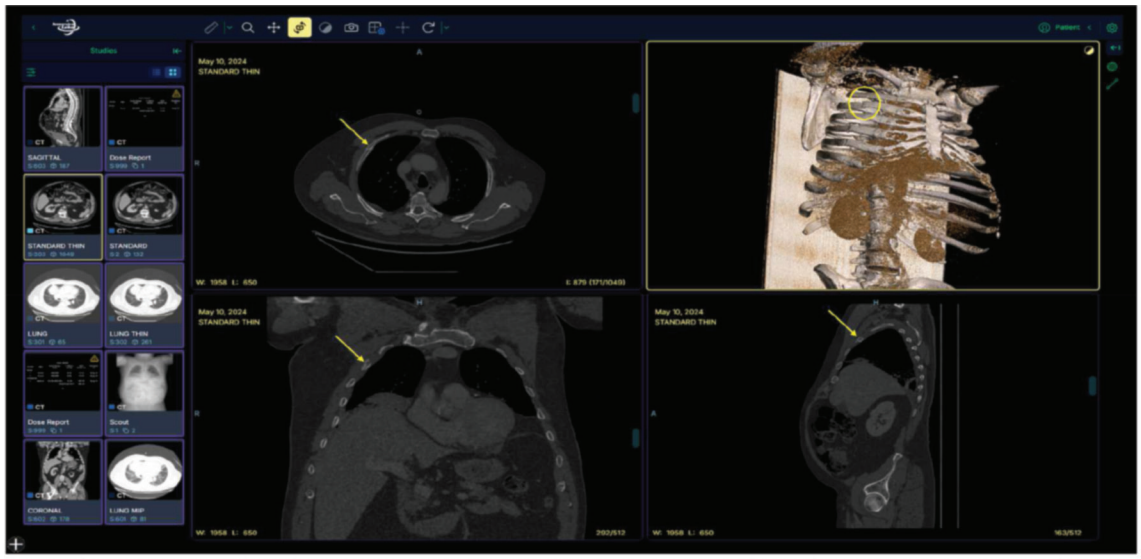


Image 5: CT abdomen and pelvis (trauma), with and without contrast images demonstrating a mildly displaced fracture of the second rib, laterally as seen on CADAVIZ

This imaging technique could help to identify findings that may not be easily identifiable through traditional dissection methods e.g., bony lesions, intra-abdominal lesions, foreign bodies, air embolisms, gross soft tissue abnormalities etc. Since forensic imaging is a non-invasive technique, the bodies are preserved in "as is" state of the body.[44]Hence, we propose that CADAVIZ could be used as an adjunct tool in the training for forensic science graduates.

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Declaration of Conflicts of Interest Statement: NA

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Assessment of the IrisPlex Markers in Order for Eye Color Prediction in Iran (Tehran) Population

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Abstract

Aim: Today usually the crime scene is evaluated by using externally visible characteristics (EVCs) in forensic centers. EVCs such as eye color is a physical trait determined by feature the genetic of the iris. Evaluation of the accuracy of human, iris color prediction based on informative single nucleotide polymorphism (SNPs)can provide a useful intelligence tool to identify the suspect. In our current research, we analyzed a six informative SNP marker (IrisPlex) (rs12896399 rs16891982, rs1393350, rs12203592, rs12913832, rs1800407) prediction of human eye color in 106 individuals of Iranian population with different ethnicity. We hypothesized that IrisPlex marker evaluations could be a considerable tool in eye color prediction. Hence, we performed this assessment for the first time in Iran.

Methods: Peripheral blood samples were collected from 106 unrelated donors with informed consent in ophthalmology clinic of Baqiyatallah hospital. We analyzed a six informative SNP (rs12896399 rs16891982, rs1393350, rs12203592, rs12913832, rs1800407) with single base extension (SBE) assay (SNaPshot). The multinomial regression prediction model was used for these informative SNPs, for this model we calculated sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and the area under the receiver characteristic operating curves (AUC) to increase accuracy for eye color prediction.

Results: By Statistical analysis of logistic regression, the AUC value of IrisPlex markers was evaluated to determine eye color. AUC value for Dark eyes and blue eyeswas 0.99 ± 0.0004 and 0.949 ± 0.0100 respectively and for intermediate eye colors was 0.9505 ± 0.017.

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Conclusion: We conclude that accuracy of IrisPlex predictive SNPs was acceptable in our study. However, the mere IrisPlex markers are not enough and some additional markers should be evaluably to increase the accuracy of eye color prediction.

Keywords: Eye color, SNP, EVCs,IrisPlex, forensic

Introduction

After the introduction and rapid development of FDP (Forensic DNA Phenotyping) in forensic sciences, the ability accurately to predict the human`s characteristics with DNA markers significantly increased[1]. Predictions of human EVCs (Externally visible characteristics) by analyzing informative SNP (single nucleotide polymorphism) in samples suggest a new perspective in the FDPs. Informative genes, including HERC2, OCA2, MC1R, SLC24A5, SLC45A2, TYR, TYRP1, ASIP, SLC24A4, TPCN2, KITLG, and IRF4 have been associated with eye, hair, and skin color in European populations and have been used in the forensic studies to predict eye color in target population[2,3]. The formation of eye color is dependent on eumelanine density in the iris, a with highest concentration of melanin in the dark eye color and the least concentrations in blue eyes[4]. Recent investigations have shown that a significant part of the iris color variation can be explained by SNPs in the human DNA, Containing the conserved region of HERC2 gene and the Proximity OCA2 gene on the chromosome 15. The other genes (SLC24A4, SLC45A2, TYR, and IRF4) involving other variations of eye colors[5]. Since to calculate potential differences may influence the eye color prediction value in various populations, we decided to evaluate the accuracy of the IrisPlex system in an Iranian population sample. Iran is a country in the Middle East.The Iranian population includes heterogeneous ethnic groups including: Persians (51%), Azeris (24%), Gilakis and Mazandaranis (8%), Kurds (7%), Arabs (3%), Lurs (2%), Balochis (2%) and Turkmen (2%)(6). However, the distribution of ethnicity is homologous In Tehran. Today in forensic practice more EVCs is used to identify suspects in crime. This study consists of a multiplex genotyping system for the six informative SNP markers to predict eye color according to previous studies using a statistical prediction model[6,7].

Material and Methods

Sample Collection and DNA Extraction

2 milliliters of venous blood on K2-EDTA anticoagulant were collected from each of 106 (61 males, 45 females) subjects randomly and after medical examination by an ophthalmologist, photographs were taken in macro-mood condition from each iris with a digital camera (Canon EOS.60D, Japan) in the Baqiyatallah DC Clinic. First, we classified pictures into three categories “Dark, Intermediate and Blue” In addition the consent was obtained from all participants. In this study, we performed DNA extraction by RGDE standard methods[8] To determine the concentration of extracted DNA, Nanodrop and the optical density (OD) was used and the ratio of light absorption (260/280) was calculated. The light absorption ratio (260/280) for all 106 samples were on average 1.6 to 1.8 and all samples were checked for contamination with protein.

Multiplex Reaction and SNP Genotyping

In this investigation the Six SNP Primers (HERC2 - rs12913832, OCA2 - rs1800407, SLC24A4 - rs12896399, SLC45A2- rs16891982, IRF4 - rs12203592, and TYR – rs1393350) described in 2011 by Walsh et al were used[2]due to the impending ability to predict externally visible characteristics (EVCs.SNP fragments were amplified in standard PCR reaction with 1µl (0.5-1 ng) extracted DNA from each person in 12 µl total reaction with 5 µl AmpliTaq Gold Master Mix (Applied Biosystems Corp) and 0.5 µl of each primer. PCR programs for this reaction were, according to Walsh et al protocols[9]. The PCR product was purified by USB ExoSAP (Affymetrix, Corp), then SNaPshot step using the SNaPshotTM Multiplex Kit (Applied Biosystems) with six SBE (Single Base Extension) primers, Primer sequences and thermocycling conditions wasshown in table 1.

SNaPshot product was cleaned up with CIP (Takara Japan) and detected by capillary electrophoresis (ABI Applied Biosystems 3130xl Genetic Analyzer, USA). The results of SNaPshot were analyzed by GeneMapper Id ® Analysis Software version 3.7.

Table 1. the 35 cycle thermocycling conditions in standard lab mode (A). Introducing ordered SBE primers and molecular detail for IrisPlex SNP markers to predict eye color in a Tehran's population (B).

A

Temperature	Time
96°C	10 Sec
50°C	5 Sec
60°C	30 Sec

B

SNP	SBE Primer	TM
rs12913832	TGATGATAGCGTGCAGAACTTGACA	64.0 °c
rs1800407	GACTGACTGACTGACTGACTGACTGACGTGGCCAGGCATAC CGGCTCTCCC	91.0 °c
rs1393350	GACTGACTGACTGACTGACTGACTGAGTAAAAGACCAC ACAGATTT	81.0 °c
rs16891982	GACTGACTGACTGACTAGAGGAGTCGAGGTTGGATGTTGGGGCTT	83.0 °c
rs12896399	ttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttTCTTTAGGTCAGT ATATTTTGGG	73.0 °c
rs12203592	ttttttttttttttttttttttttttttttttttttttttttttttttTCCACTTTGGTGGGTAAAAGAAGG	75.0 °c

Eye Color Classification and Quantification

The eye digital photo was obtained from all subjects. To be more accurate for eye color classification, Iris melamine index (IMI) was used for eye color classification used. An IMI scale was defined and classified in three main categories according to with the visual determinations. In our study, brown eye color Values were in the range 1.25-1.65, intermediate eye color values in the range of 1.66-2.32, and blue eye color values in the range of 2.33-3.20[9]. To calculate IMI factor in this study, we decided to design and develop NoorGraphy software for the first time.

The Statistical Prediction Model

We applied statistical prediction model for eye color prediction. this model is based on the results of 3804 Dutch Europeans introduced by Liu et al[6]. Hence, we added the ethnicity and gender of volunteers to the statistical model.The prediction model based on multinomial logistic regression (MLR) model for correcting prediction of eye color by calculating areas under the curve (AUC) for three eye color (Dark, Intermediate, Blue). Classification of IrisPlex marker value and ROC (Receiver operating characteristic) curve. For all statistical analysis using SPSS 22.0.1 for Windows (SPSS Inc., Chicago, USA).

Results

Eye Color Classification and Ethnicity Distribution

The IrisPlex predictive model was 106 Iranian subjects with diverse ethnicity in Tehran population. The distribution of three eye colors (Dark, Intermediate, and Blue) was determined by the ophthalmologist. The IMI index isshown in chart 1 and figure 1, which indicates human accuracy is less than software. By analyzing the questionnaires found that the ethnic distribution was:52.8% Fars, 17.9% Azeris, 13.2% Lurs, 10.4% Kurds, 3.8% Gilaks and 1.9% Mazani.

Eye Color Prediction Accuracy of Irisplex Model

Chart 1. The distributions of eye colors (blue, medium, dark), (A) eye color classification by ophthalmologist. (B) eye color classification by NoorGraphy IMI index.

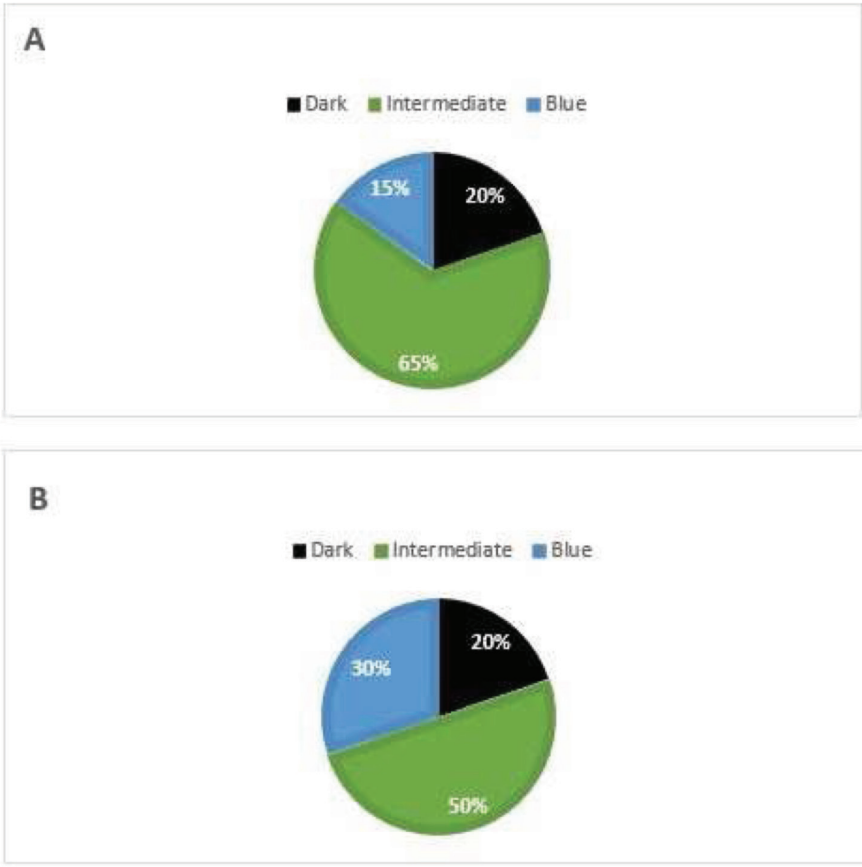


Figure 1: Eye color Images of 16 sample contribute to studies, IMI index was calculated and classify (Blue, Dark, and Intermediate) by NoorGraphy native software.

The SNaPshot reaction for all samples was performed according to the standard procedures with (15 ng) DNA for each SNaPshot reaction[2]due to the impending ability to predict externally visible characteristics (EVCs. The results,after setting and optimizing were acceptable with high quality. Several different results are shown in Figure 2.

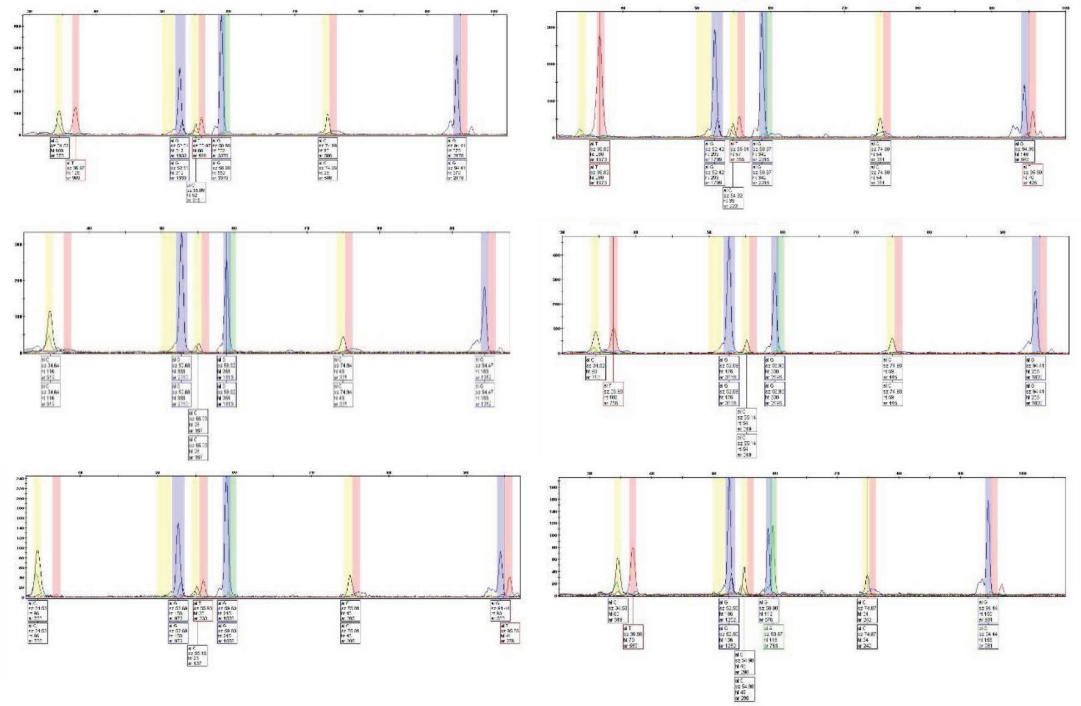


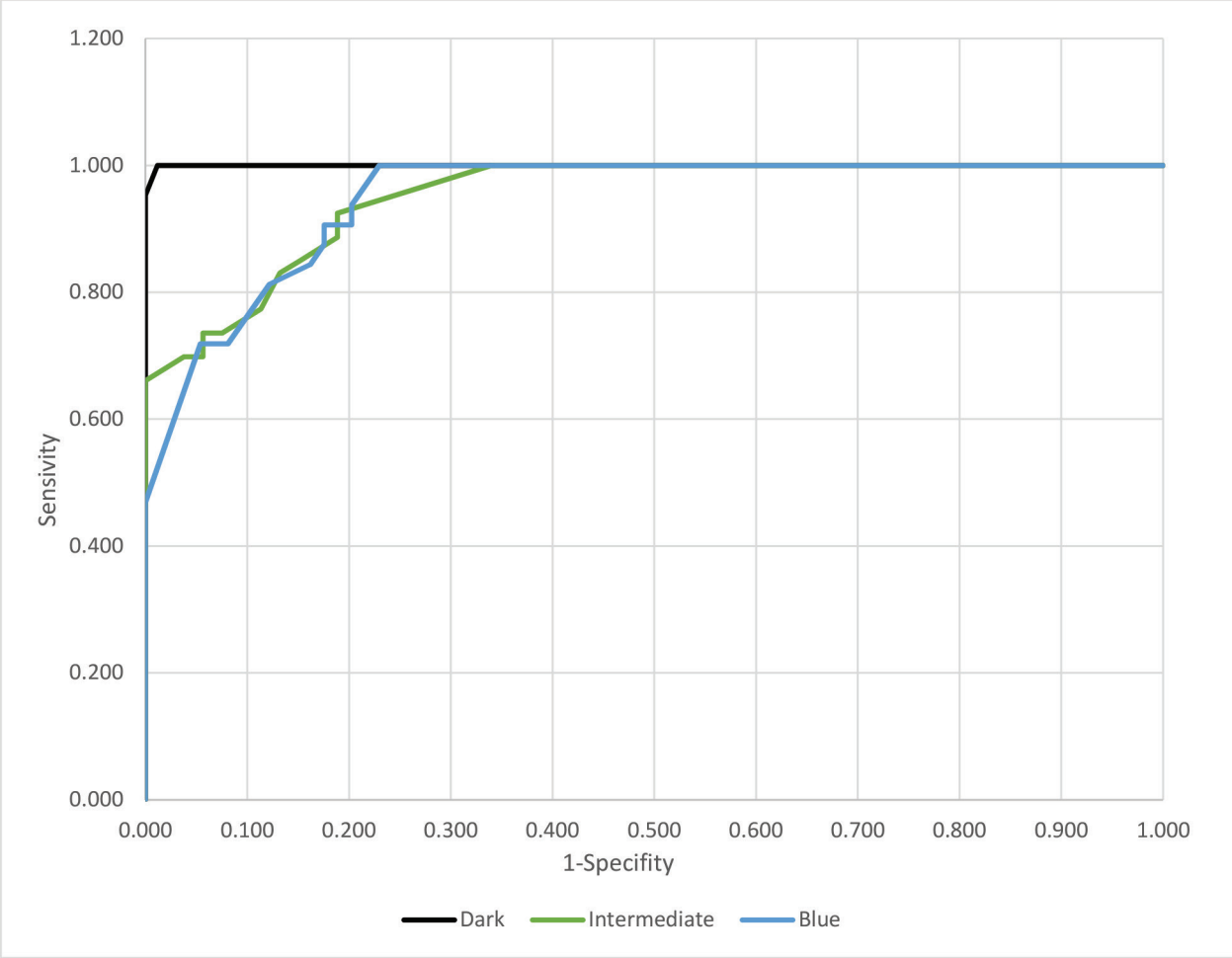
Figure 2: Several SNaPshot results with different eye color and ethnic group

The SNaPshot tests of the multiplex reactions were performed by multinomial logistic regression standard analysis[9]. The output of this study, according to cross-tab test verified that the highest association between dark eye color and Fars ethnic, and high correlation between blue eye color and other ethnicities. In order to have accurate results of eye color we used AUC for each SNPs. As previous studies show rs12913832 SNP marker had the most predictive allele in determining eye colorwith 98.2% correct results. In this study, we used eye color predictive models (The IrisPlex model). In addition, we also used two other variables (sex and ethnicity) along with the main variables (IrisPlex markers) to predict eye color. The AUC, sensitivity, specificity, PPV, and NPV in both tables are reported. The receiver operating characteristic curve for the IrisPlex model is shown in chart 2.

Table 2. The statistical properties of the prediction model with IrisPlex variables for eye color (dark, intermediate and blue).

	Dark	Intermediate	Blue
AUC	0.9997 ± 0.0004	0.9505 ± 0.017	0.949 ± 0.0100
Sensitivity[1]	100	73.06	87.5
Specificity[1]	98.82	92.45	82.43
PPV[1]	95.45	90.69	68.29
NPV[1]	100	77.77	98.84

Chart 2. Receiver operating characteristic curve analysis of all samples with a different ethnic group in Tehran, based on the IrisPlex prediction model.

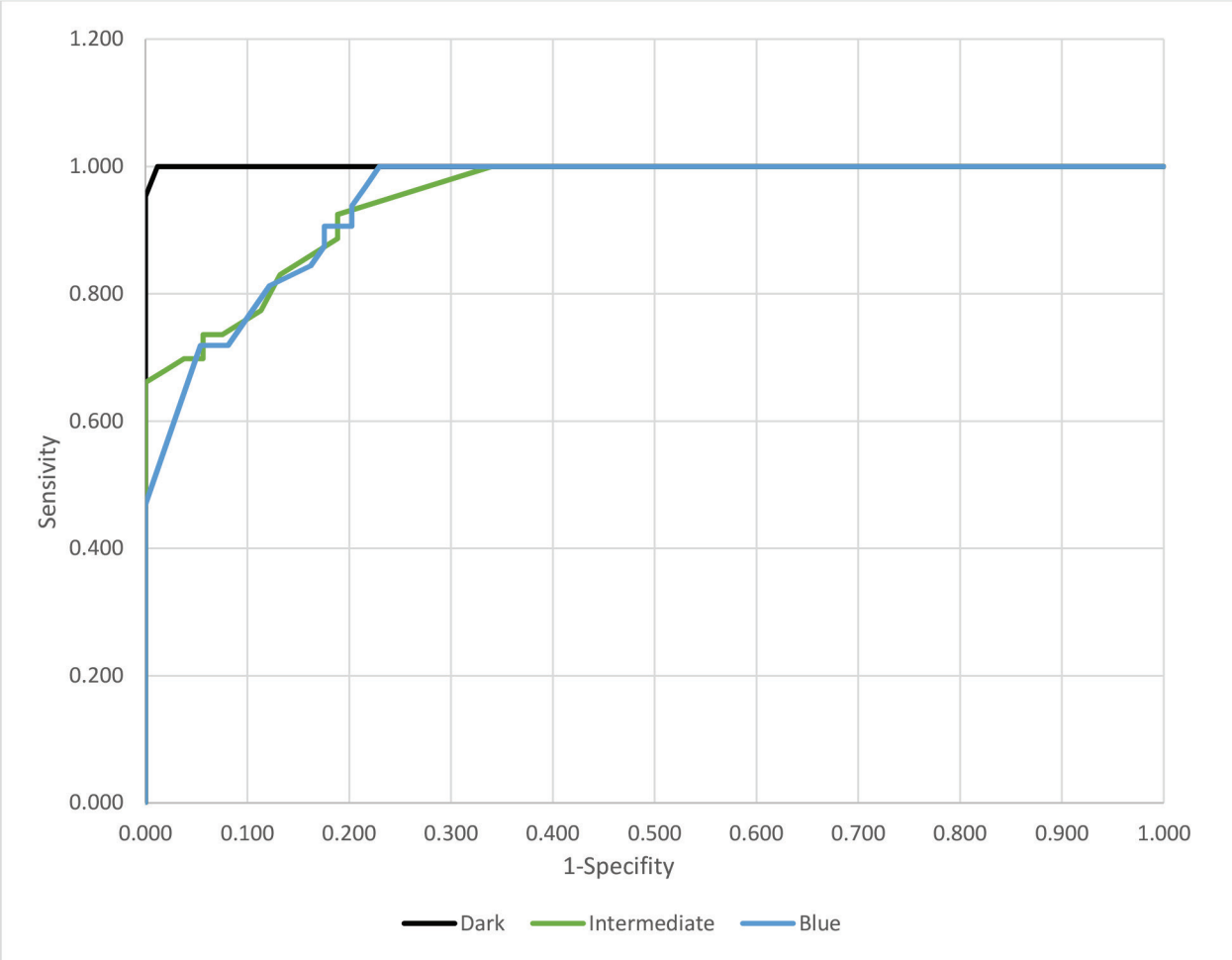


In contrast to in other models, we also used gender and ethnicity in the eye color prediction model. The percent of the AUC, sensitivity, specificity, particularly in dark eye color group was increased. Other characteristics of the IrisPlex model were reported. The receiver operating characteristic curve for the first model is shown in chart 3. In this study, the addition of other factors (gender and ethnicity) improved the specificity and sensitivity of eye color prediction.

Table 3. The statistical properties of the prediction IrisPlex model with gender and ethnicity for eye color (dark, intermediate and blue).

	Dark	Intermediate	Blue
AUC	1 ± 0.00001	0.9505 ± 0.0154	0.9521 ± 0.0179
Sensitivity[1]	100	72.90	87.5
Specificity[1]	100	92.45	85.13
PPV[1]	100	91.30	71.79
NPV[1]	100	81.66	94.03

Chart 2. Receiver operating characteristic curve analysis of all samples with different ethnic group in Tehran, based on Irisplex predictive model with gender and ethnicity.



Discussion

Human eye color is exceptionally polymorphic due to it being governed by multiple genes. This trait has been studied on a genetic level extensively, and some of the studies employed Europeans for genome wide association studies. Conversely, the IrisPlex system is also a commendable set of markers that due to its reliability and across multiple types of geographical locations is being validated to accurately target EVCs [9,10]. When it comes to genetic determinants of EVCs, the IrisPlex system successfully acts as an deterministic model which when combined with the precision of genetic data acts as a highly powerful with high accuracy of EVCs in forensic usage capabilities [9,10]. The said markers aforementioned are inclusive of 6 SNPs that are vital genes that greatly affect the control of pigmentation of the eye. Some of the genes registered as major including HERC2 rs12913832, OCA2 rs1800407, SLC24A4 rs12896399, SLC45A2 rs16891982, TYR rs1393350 and IRF4 rs12203592 eye color determining genes [7]. In the current study, SNaPshot method is deployed to undergo SNP genotyping process. SNaPshot is an ideal technique for this comparison owing to the fact that it has higher sensitivity and specificity during SNP detection. The rs12913832 SNP on the HERC2 gene was previously associated with having the highest predictive power for eye color, and AUC values registering 98.2%, 85.2%, and 87.8% for dark, intermediate, and blue-colored eyes respectively. Such findings were replicated in our population because the data analyses showed that the TT genotype of rs12913832 was strongly associated with dark eyes, similar to other findings made in regions

including Egypt, Turkey, and Iraq [11–13]. In this study, we evaluated the genotype frequency of rs12913832 as TT (47%), CT (30%) and CC (23%). In previous studies, Persians (Fars ethnicities) accounted for about 61% of the total population of Iran [13,14]. The sample that we evaluated had a Fars ethnic distribution of 52.8% . The presence of such a large proportion of Fars people, coupled with their dominance in the population of Iran, indicates that the trend within the GWAS cohort is toward darker eye colors. This finding is consistent with the AUC results of our data analysis, where the most predictive accuracies were found for dark eye color. Results from the studies of other populations assist in validating these. For example, the application of AUC analysis in a similar study in the Netherlands revealed AUC values of 93% for brown eyes, 91% for blue and 73% for mixed eyes [13]. Similarly, work done in Iraq demonstrated 100% accuracy in recognizing dark eye color and 52.2% accuracy for blue eye color[12]. Although the dark and blue markers of the iris tackle their respective target in the eye color model very cohesively, the system fails to perform with equal finesse in regard to the intermediate eye colors. Hence the western population and diverse populations highlight a marked deficiency in the use of the systems in conjunction. This may be focused on modifying the marker set and further including the genetic and demographic components that may smoothen the predictions, especially for the intermediate eye color phenotypes [9]. In this investigation, a large number of participants are reported to be with intermediate eye color. However, there is a consistently high lack of sensitivity in providing a prediction for this category, similarly, guidance is required for development of new prediction systems for the determination of the intermediate type eye color as it is already present in literature. The prediction accuracy of the IrisPlex system can be further enhanced however the addition of new markers is required to improve upon this system. Recently three SNP were discovered that directly relate to the intermediate eye color and have been shown to be potentially effective for use in forensic studies. The IrisPlex system has a number of drawbacks, including the fact this could potentially be a solution to some of those concerns. These markers could also assist not only improving the predictive accuracy to a certain extent, for providing intermediate eye color, but also extend the IrisPlex system functionality [6,9]. In 2020 for example, the HIrisPlex system of hair color prediction was presented, which is an enhancement of the previous methods of predicting EVC for forensic experts, as it employs an amalgamated set of SNP markers for the prediction of hair and eye color. That technique is relatively novel, but is potentially a great leap in terms of forensic genetics. We recommend that in the future scans of Iranian populations, the HIrisPlex markers be used for EVC prediction optimization purposes. An issue that has has troubled researchers conducting FDP is the relative categorization of EVCs. This issue was addressed by use of a native software that was developed with the aim of classifying the eye color. This software lowered human errors significantly and thus increased the accuracy of the predictions made, especially in the case of blue eye color (Chart 1). Such technological advancements are vital in improving the dependability and reproducibility of forensic tools[10,11,15]. Because this research has been the first one ever conducted on EVCs in Iran it is important to explore a range of traits such as hair or skin color in the Iranian populations. Once more, as Iran has a fenestrated and diverse gene pool, our population has significant potential for these types of genetic studies. It is without doubt that interdisciplinary investigations in this discipline will not only broaden scientists' horizon concerning the genetic basis of a variety of phenotypic characters but also provide a basis for the application of these findings in forensic and anthropological investigations.

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This manuscript has been prepared and conducted in accordance with the ethical and publication guidelines of ICMJE and COPE.

Ethics Approval

Ethics this study was approved by the Ethics Committee of Baqiyatallah University of Medical Sciences, this study also had been recorded with IR. Bmsu.REC013950373 id number in the university ethics committee.

Conflicts of Interest

The authors declare that there are no financial, personal, or professional conflicts of interest that could have influenced the results or interpretation of this study.

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Suicidal Trends in Tribals of Bastar-A study of 11-Years

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Abstract

Background: Due tomultiple externalfactors, the lifestyle and cultural factors of the Bastar tribes have undergone a significant shift in the last three decades. Our approach has been to extensively study the tribal communities to find out which Tribe is more vulnerable to suicide, what pattern they follow, and how it can be prevented.

Objective: The objective of the study was to study the pattern of Suicide cases in the tribal communities of Bastar on a Tribe-wide scale so that necessary steps can be taken to prevent Suicides incommunities thathavea high scale of Suicidal cases.

Material and Method: The data of postmortem reports of all cases of Suicidal deaths of tribals of Bastar region, Chhattisgarh, that occurred in 11 years (2011 to 2021), which had been brought to the mortuary of the Forensic Medicine department.

Results: Out of a total of 454 cases evaluated, it consisted predominantly of the male population of the age group 21- 30 years, with a maximum of 154 cases.The maximum number of cases found in the year 2020was 68. Maximum cases occurred in the Months of January/June/December, with 44 cases. Tribe-wise,the Bhatra tribe stood 1st with 105 cases, followed by the MuriaTribe with 60 cases. The maximum number of suicide cases was from Hanging, 248 cases, Poisoning stood 2nd, 170 cases.

Conclusion: In our study, we have found that the Bhatra Tribe, followed by the Muria Tribe, has the leading cases, and the government needs to prioritize to prevent cases from occurring. Suicidal deaths among tribals were mainly due to Hanging, followed by poisoning, mainly consisting of organophosphate and organo-chloride poison; authorities and the government control and monitor the distribution and use of organophosphate and organo-chloride poison.

Keywords: Tribal; Suicide; Hanging; Poisoning; Bastar; Insecticide; Pesticide; Kaner seeds; self-immolation.

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Introduction

India has a total of 104,545,716 scheduled tribes, which constitute 8.6% of the total population (Census 2011)[2]. Chhattisgarh has a 7,822,902 Scheduled Tribe population; of this, 3,873,191 are Males and 3,949,711 are Females. Chhattisgarh stands in 7th position with 7.5% of tribal population[2]. As per the report of NCRB 'Accident Deaths and Suicide in India 2022',56th Edition[5]published by National Crime Records Bureau(Ministry of Home Affairs), Govt. of India[1] in Chapter 2, Suicides in India, page 196: Total number of suicides recorded in year 2022 = 1,70,924. In Chapter 2, Page 197,198: Chhattisgarh state accounted for 8446 cases, a percentage share of 4.9% in the year 2022 standing at 5th position(28.2%), while leading at 1st place was Sikkim(43.1%) in the year 2022 among all states/ Union Territories.

Bastar region is the country of tribes; about 70% of the total population is tribal, representing about 30% of the total tribal population of Chhattisgarh. Bastar is the homeland of numerous tribal groups like AbujhMaria, Muria, Dhurwa, Dorla, Bhatra, Halba, etc. Each tribal Tribe hasits cultural boundary, and within this cultural boundary. The Bhatra are distributed on the eastern and western sides of Jagdalpur district and the eastern sides of the Kondagaon district. Traditionally, they are hunter, gatherers, and cultivators. While their ancestral trades were like making baskets, cultivation, hunting, collection of forest products like bees honey, herbs, tendu patta for beedi making etc, in context to today they have adopted modern practices also like starting of modern small scale industries cashew industry, making of packaged honey, herbal plants based product to domestic and international market, new types of farming methods are now adopted by them to get more benefits.

The Abujh Maria are found in about 237 villages of the Orcha block of Narayanpur and adjacent Bijapur and Dantewada districts; Their number is about 12000[4]. Abujh Maria is the only tribal group in Bastar till date outside of External forces; their mental processes are not unlike those of urban people because of their centuries of isolation[3]. The Muria are mainly distributed in the plain forest area at Narayanpur, Kondagaon, and Antagarh. Their economic life centres around the forest, land, and settled cultivation. The significant issues are poor literacy rates, slow pace development, they have adistinct ethnic entity, limited livelihood opportunities, and high deforestation in recent years, as tribes have a high dependency on land and forest produce, which has led to a significant impact on the mental and social well-being of the tribes of Bastar.

Material and Method

Data of post-mortem reports of all cases of deaths of Suicidal deaths of tribals of Bastar region, Chhattisgarh, have been brought to the mortuary of Forensic Medicine department, Late Baliram Kashyap Government Medical College, Jagdalpur, District Bastar, Chhattisgarh, from 2011 to2021, a total of 14 years have been scrutinized and evaluated. In each case, a detailed examination was done on the body to evaluate the method of suicide used,theTribe that person belonged to, the Manner of death, and the Cause of death was thoroughly evaluated.

Results

The study found that 454 deaths were reported from 2011 to 2021. The following are the findings of the study.

Sex ratio of Suicidal cases among Tribal population: Male- 309 cases, Female-145 cases. In national level, As per the report of NCRB[1] in Chapter 2, Page 203,204: Male: Female Ratio =1,22,724 (71.8%) :48,172(28.2%)

Age group in cases: It was divided into 10- 10 age groups, e.g., 1-10 years,11-20 years, etc. Out of the 454 cases, the maximum number of cases were found in the age group of 21-30 years, 154 cases, and the Minimum number of cases were found in the age

group of 1- 10, 71-80 years,i.e.,2 cases. In national level as par NCRB[1] In Chapter 2, Page 203: The age group (18 -below 30 years) and persons of 30 years - below 45 years of age were the most vulnerable groups resorting to suicide. These age groups accounted for 34.6% and 31.8% of suicides, respectively, in the year 2022[1]. [Figure 1]

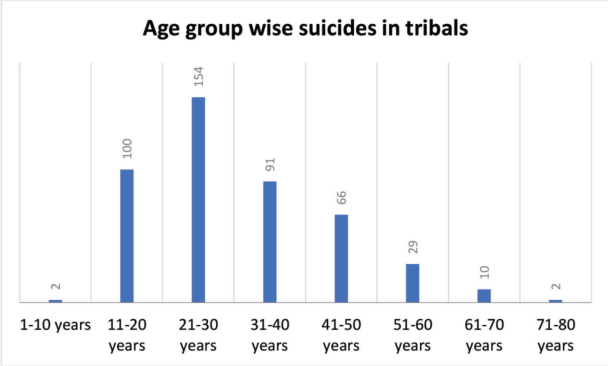


Figure 1

Year of Cases Reported: In a detailed analysis of the cases reported, out of the 454 cases,the Maximum number of cases were found in the year 2020- 68 cases, 2018-61,2019-51,2021-50, 2016-41, 2014 & 2015- 40, 2012- 11 cases.[Figure 2]

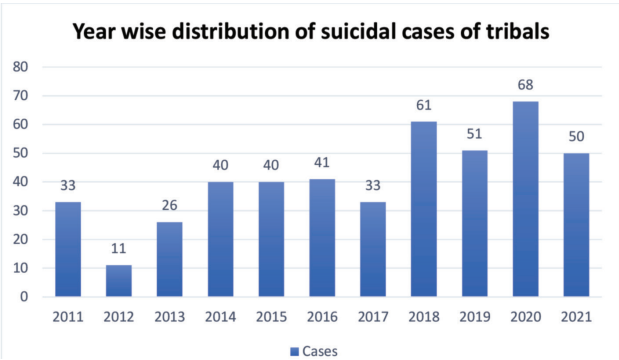
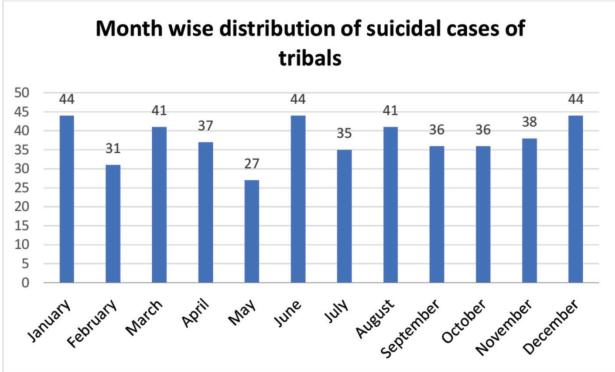


Figure 2

Month of reported cases in the period of 2011-2021: In a detailed analysis of the cases reported, it was found that out of the 454 cases,a maximum number of cases occurred in the Months of January, June, and December, -44 cases followed by March, August, -41 cases, and November-38 cases. [Figure3]



[Figure 3

The pattern of Suicide cases committed by the different tribal Tribes of the Bastar region:

Among the tribal communities of the Bastar region, Out of the 454 cases,

1. The Bhatra Tribeis predominant in the leading suicide cases with a total of 105 cases, maximum by Hanging=58 cases, Poisoning=19, Poisoning(Insecticide/Pesticide/Weedicide poison)=14, Self-immolation (kerosene oil)=6, Poisoning(alcohol)=3, Poisoning (Rat/Ant poison)=2 cases, Poisoning(super vasmol 33 dye)=1, Poisoning(Kaner seed) = 1, Cut throat =1 case.
2. The Muria Tribe stood second with a total of 60 suicidal cases maximum were of Hanging=31 cases, Poisoning=12, Poisoning(Insecticide/ Pesticide/birdpoison)=6, Poisoning(alcohol)=4, Self-immolation (kerosene oil)=2, Poisoning (Rat/Ant poison)=2, Poisoning(mosquito kill poison)=1, Poisoning(Kaner seed) = 2 cases, Cutthroat =1, Jump from tractor=1 case.
3. The Madia Tribe stood third with 53 cases. Maximum by Hanging=18 cases, Poisoning=17, Poisoning(Insecticide/Pesticide/Agriculture/ Bird poison)=12, Self-immolation(kerosene oil)=3, Poisoning (Ratpoison)=2, Poisoning (Ratanjot seed) = 1.
4. The Gond Tribe stood fourth with 51 cases, Hanging = 34 cases, Poisoning, Self-immolation (kerosene oil)=6, Poisoning (Insecticide poison) =4 cases, Poisoning (Rat poison)=1 case.

5. The Kashyap Tribe stood fifth with 33 cases, Hanging = 22 cases, Poisoning(Insecticide/ Pesticide/bird poison) = 3, Self-immolation (kerosene oil)=3, Poisoning=1, Poisoning(super vasmol 33 dye)=2, Poisoning(Kaner seed) = 2 cases. The Baghel Tribe ranked sixth with 30 cases. The maximum incidents involve Hanging with 19 cases, Poisoning with 3, Pesticide Poisoning with 2, Kaner Seed Poisoning with 2, Rat Poisoning with 2 cases, and Self-immolation (using kerosene) oil)=1, Cut by train= 1 case.

6. The Dhurwa Tribe stood seventh with 27 cases. Maximum by Hanging = 19 cases, Poisoning(Insecticide/Pesticide/Bird poison) =4, Poisoning=3, Self-immolation (kerosene oil)=2 cases, Poisoning (alcohol)=1 case.

7. The Nag Tribe stood eighth with 24 cases. Maximum by Hanging = 17 cases, Poisoning cases, Poisoning (Kaner seed) = 1, Poisoning (Rat poison)=1 case.

8. The Halba Tribe stood ninth with 16 cases. Maximum by Hanging = 9 cases, Poisoning (insecticide poison) =3 cases, Poisoning (alcohol)=1, Poisoning (Rat poison) = 1, Drowning=1 case.

9. The Netam Tribe stood tenth with 9 cases. Maximum by Hanging=5 cases, Poisoning=2 cases, Self-immolation (kerosene oil)=1 case, Poisoning(insecticide poison)=1 case.

10. The Markam Tribe had 6 cases. Dhakad Tribehad 5 cases. The NageTribe had 4 cases. Dorla, Kawasi Tribe had 2 cases. Bagh, Chander, Dhadiya, Dhruw, Dhruwa, Dadi, Gadwa, Gadwa(Nag), Hidma, Jeram, Jogi, Kalar, Karai, Karma, Korram, Kunjam, Nag(telga), Nage, Netam, Odel, Podem, Podiyami, Poyam, Samrath, Satnami, Sori, Toti, Urav, UsendiTribe had 1 case of suicide. [Figure 4]

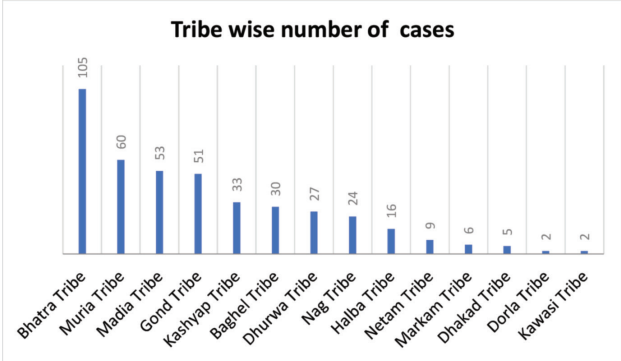


Figure 4

Pattern of Suicide Among the Tribal Population

The Maximum number of a pattern of suicide Out of the 454 cases, was fromHanging = 248 cases followed by Poisoning total 78 cases, followed by Poisoning(insecticide) = 37 cases; Poisoning(pesticide)/Burn injury (kerosene oil)/ Poisoning (Kanerseed) = 12 cases; Poisoning (alcohol) = 10 cases; Poisoning(Rat kill poison)=8 cases; Poisoning(Bird poison)= 5 cases etc. [Figure 5]

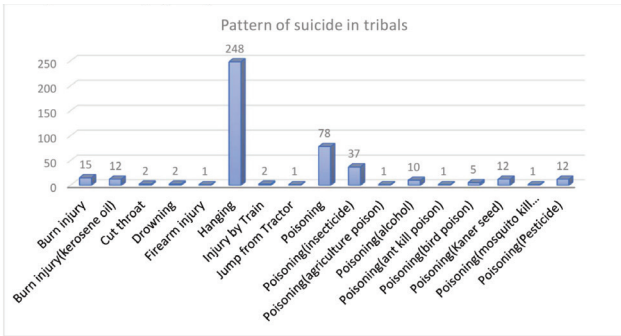


Figure 5

As per the report of NCRB 'Accident Deaths and Suicide in India 2022" 56th Edition[1]published by National Crime Records Bureau(Ministry of Home Affairs) Govt. of India. In Chapter 2, Page 259 Table 2.12 Distribution of Suicides by Means/Mode Adopted during 2022(All India)

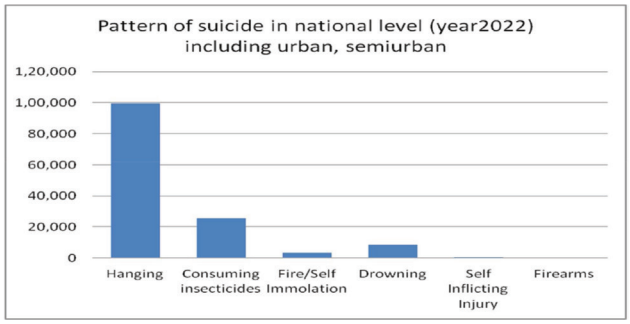


Figure 6

Discussion

Suicide is a multi-factorial issue; multiple initiatives need to be taken by the central and state governments to prevent suicides in the tribes of Bastar. A significant factor is deforestation, as given in the detailed study by Mohanty et.al. study on Lonja Saoras tribe of Odisha describing how the Lonja Saora's tribes had to migrate to urban areas due to deforestation weakening their traditional economy[8].

As per Stone D et al "Suicides Among American Indian or Alaska Native Persons — National Violent Death Reporting System, United States, 2015–2020", published by Centre of Disease Control and Prevention, Morbidity and Mortality Weekly Report[5]; During 2015–2020, a total of 3,397 suicides among AI/AN persons and 179,850 suicides among non-AI/AN persons were recorded in NVDRS(National Violent Death Reporting System, United States): Male: Female ratio=2553(75.2):844(24.8)[5]; Maximum number of suicides were found in the Age group 24-44 years=1593(46.9%); Methods used were Hanging, strangulation, or suffocation=1,594 (46.9%), Firearm=1,261 (37.1%), Poisoning=1,261 (37.1%), Sharp instrument=74 (2.2), Motor vehicle=62(1.8%)[5]. While in our study in the year of 2015-2020 in tribes of Bastar, a total of 294 cases of suicide were found, Male: Female Ratio=204(69.4%): 90(30.6%), Maximum number of suicides cases was found on age group 25-44 years=107 cases, followed by 20-24 years=60 cases, 45-64 years=57 cases. In the method of suicide Hanging= 175(59.6% was predominant method, followed by poisoning=84(28.6%), Sharp instrument=2(0.006%).

A recent study for framing policies to prevent suicides by MSG Mangala et al., "Suicide Prevention Strategy Framework for Madhya Pradesh" by MSG Mangala et al., for the Centre for Social Sector Development[6], Atal Bihari Vajpayee Institute of Good Governance and Policy Analysis (AIGGPA)[6], has proposed provisions and policies for the prevention of suicides.

As par Suicide Prevention Resource Center (SPRC) US federally supported resource center, report of "Recommended CSTE(Council of State and Territorial Epidemiologists) Indicators for Suicide among American Indians and Alaska Natives July 2018" has mentioned use of Tribal Surveillance Systems: Tribally-based surveillance systems have several distinct advantages: access to real-time data that may aid identification of emerging suicide clusters, trends, and characteristics; identification of characteristics and patterns that are unique to that American Indians/Alaska Natives community; and establishment of a vital management role and control for the American Indians/Alaska Natives community, which can foster culturally relevant interventions[7]. Two of such surveillance systems operating in the United States are 1. Maniilaq Association (Alaska): serving 12 federally recognized tribes in remote Northwest Alaska, developed the first-recorded tribal suicide surveillance system in an indigenous region, starting in 1990. 2. White Mountain Apache Tribe (Arizona): In 2001, the White Mountain Apache Tribe in Arizona created the Life surveillance system for reporting suicide death, attempts, ideation, self-injury, and binge substance use. Through a tribal resolution, suicide-related data are routinely collected by community members[7]; In the same way government should make a data set of the particular tribe group which is susceptible to suicides and address their needs and focus on preventable measures, just like in our data we had made a date of which group of tribe is more susceptible in committing suicides, the state

government of Chhattisgarh, can focus on such tribe group more and address their problems, and finding solutions.

Conclusion

In this retrospective study we have evaluated the point to be considered by the government to implement policies to reduce it so that tribes can lead their tradition and cultures; public health programs should be implemented to assess the mental health of tribals monthly. Suicidal deaths among tribals were mainly due to Hanging, followed by Poisoning, mainly consisting of organophosphateand organo-chloride poison. Authorities and governments should control and monitor the distribution and use of organophosphate and organochloride poisons. Research must focus on finding less toxic alternatives like herbal based which if consumed also not produce severe damage to body/cause death to humans/ animals. A monitoring group should be established by the State/Central government for the prevention of the suicides in tribes of India

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Prevalence of Intimate Partner Violence and Associated Factors Among Antenatal Women: A Cross-Sectional Study

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Abstract

Intimate Partner Violence (IPV) is defined as any behaviour pattern observed in an intimate relationship that causes physical, mental, emotional, or sexual harm to one's partner. This study aimed to determine the prevalence of IPV among pregnant women attending antenatal services. A non-experimental, descriptive survey was conducted involving pregnant women attending the OPD services at AIIMS Jodhpur. Data was collected through a self-administered questionnaire, comprising 200 pregnant women, of whom about 5.5% reported experiencing IPV. The majority (81%) reported physical violence and sexual abuse, 72.72% experienced a sense of threat and controlling behaviour from their partner, and 81% reported restricted access to antenatal care services. Further research should focus on identifying key factors affecting women and associated with IPV.

Keywords: Prevalence, Intimate Partner Violence, Gender Based Violence, Antenatal women

Introduction or Background

Antenatal women need support during pregnancy and become physically and psychologically dependent. It gives a whole new meaning to life of her, if her intimate partner provides support and emotional strength during this time. However, current reports have shown that every 1 in 3, that is to say roughly 30% of women worldwide, according to the World Health Organisation, 2024, have been subjected to physical or sexual Intimate Partner Violence and nearly 18% of women aged 15-49 reported Intimate Partner Violence (IPV) in the previous 12 months. [1,2]

IPV is the most prevalent form of Gender-Based Violence. The World Health Organisation addressed

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Intimate Partner Violence (IPV) as behaviour by an intimate partner that causes physical, sexual, psychological harm, including physical aggression, sexual coercion, psychological abuse and control behaviour.

A Systematic review revealed that the prevalence of IPV among the global population is 1-20% & in the Asian population is 4-48%, but in India, it is 21-28%.[3] NFHS V highlighted, 29.3% of women after marriage and 3.1% of pregnant women in India simultaneously 24.3% of women and 2.1% of pregnant women in Rajasthan have been subjected to violence[4] causing a multifaceted impact on physical and mental health of the mother and new born health.

A recent study has shown that certain risk factors increase the prevalence of IPV during pregnancy. These risk factors include a history of violence, low socioeconomic status, poor social support, substance abuse, low level of education, being young age, early age at marriage, being a full-time housewife, and living in a rural area.[5]

Pregnancy-related variables like male dominance in economic and decision-making autonomy in the family are a precursor of IPV. IPV can have serious consequences for the mother and unborn child, & Family. IPV leads to both acute injuries and profound longer term challenges to health and well-being.

In India, women are still socially limited within the bounds of society and essential norms of marriage, as they hesitate to disclose information. A study undertaken in Lok Nayak hospital, Delhi, with 1500 Antenatal participants, prevalence of IPV was noticed to be 29.7% and victims were recognised with 47.2% of poor weight gain during pregnancy, 12.7% had preterm delivery and significantly low birth weight of new-borns (32.1% v/s 22.3%).[6]

Health care providers play a critical role in identifying and interpreting IPV among pregnant women, along with providing support and communicating resources to help them stay safe. There are manoeuvres available for victims of IPV (Intimate Partner Violence), for instance, women's helpline, shelter, and counselling services. We must strive to raise awareness about this issue and join forces to prevent and address intimate partner violence in all.[7]

To encourage the reporting of such events, our study will try to screen the pregnant women visiting our tertiary care hospital for regular antenatal care services to determine the prevalence of Intimate Partner Violence (IPV). It is necessary to educate society, increase awareness, and provide women with the necessary resources to prevent and address intimate partner violence to ensure healthy pregnancies and safe environments for women and their unborn children.

Materialand Methods

Design and Participants

A non-experimental descriptive survey study design was used to meet the objectives of the study. The target population was Antenatal women attending antenatal OPD services at the All India Institute of Medical Sciences, Jodhpur, Rajasthan, India. The convenient sample technique was used, and a total 200 sample size was estimated by power analysis using formula $n=(Z^2\times p\times(1-p))/d^2$), with Z= 1.96, p=0.5 and d=0.07. The calculated sample size was 192 which was approximated to 200). The inclusion criteria consisted of all antenatal women attending the antenatal OPD of the hospital, while those women who were currently not residing with their spouse / Intimate Partner were excluded.

Instruments

Data was collected by a self-structured questionnaire of Sociodemographic data and Intimate Partner Assessment Scale. Sociodemographic includes the participants' information such as age, education and occupation of antenatal women and her husband/partner, religion, duration of marriage, parity, gravidity, period of gestation, and socioeconomic status.The validity and reliability are checked, and the instruments are found to be reliable. Approximately 10-15 minutes were taken to complete the questionnaire. The pilot study was conducted on 20 antenatal women at the Antenatal

OPD in AIIMS Jodhpur, and was found to be feasible to conduct the study.

Results and Discussion

Demographic Characteristics of the Participants

The frequency and percentage distribution of the demographic characteristics of participants in the study and the mean age & period of gestation of participants are presented in Table 1.

Table 1. Socio-demographic variables of participants N=200

Sociodemographic variables	F	(%)
Age (in years)		
≤25	115	57.5
>25	85	42.5
Mean ± Standard Deviation	**25.13 ± 2.99**	
Educational qualification (respondent)		
No formal education	31	15.5
Primary	50	25.0
Secondary/senior secondary	73	36.5
Graduation and above	46	23.0
Educational qualification of partner		
No formal education	27	13.5
Primary	42	21.0
Secondary/senior secondary	72	36.0
Graduation and above	59	29.5
Occupation of respondent		
Homemaker	145	72.5
Govt/private job	55	27.5
Occupation of partner		
Daily wage worker	140	70.0
Govt/private job	60	30.0
Duration of marriage		
<1 yr	60	30.0
1-5yr	109	54.5
>5yr	31	15.5
Religion		
Hindu	184	92.0
Muslim	16	8.0
Monthly family income (in rupees)		
Less than 25000	54	27.0
Between 25000-50000	84	42.0
More than 50000	62	31.0
Gravida		
1	89	44.5
2	77	38.5
3	26	13.0
4	5	2.5
5	3	1.5
Parity		
Nullipara	96	48.0
1	74	37.0
2	25	12.5
3	3	1.5
4	2	1.0
Substance abuse by partner		
Present	50	25.0
Absent	150	75.0
Period of gestation (in trimester)		
First trimester	30	15.0
Second trimester	134	67.0
Third trimester	36	18.0
Mean ± Standard Deviation	**22.65 ± 7.16**	

The mean age of participants was 25.13 ± 2.99 years. Regarding education, 36.5% of respondents and their partners (36%) had completed secondary or senior secondary education. In terms of occupation, the majority of respondents (72.5%) were homemakers, while partners (70.0%) were daily wage workers. More than half (54.5%) of the respondents were married for one to five years. Nearly all (92.0%) of participants were Hindus, and about 42.0% family income was in between ₹ 25,000–₹ 50,000

Looking at the gravidity distribution showed 44.5% were primigravida (Gravida 1), 38.5% gravida 2, 13.0% gravida 3, with smaller percentages for higher gravidity. Parity data indicated 48.0% were nulliparous, 37.0% had one child, and the rest had two or more children. The mean period of gestation was 22.65 ± 7.16 weeks. Substance abuse among partners was reported by 25.0% of respondents, while 75.0% reported its absence.

Prevalence of Intimate Partner Violence

In our study findings regarding prevalence of Intimate Partner Violence was 5.5% (11) in the study population. The majority of respondents, 81% confirmed with physical hurt in the form of slapping, hitting and also sexual abuse from their intimate partner. 72.72% reported with sense of threat and a controlling attitude shown by their partner. The majority (81%) of women reporting IPV were restricted from access to antenatal care services by their intimate partners. (Fig. 1) Among antenatal women confirming IPV majority, 72.72% [8] them tried to acquire help to cease violence towards them. However, negligence of care by the partner was notably seen at 8% of the total participants of the 200 sample.

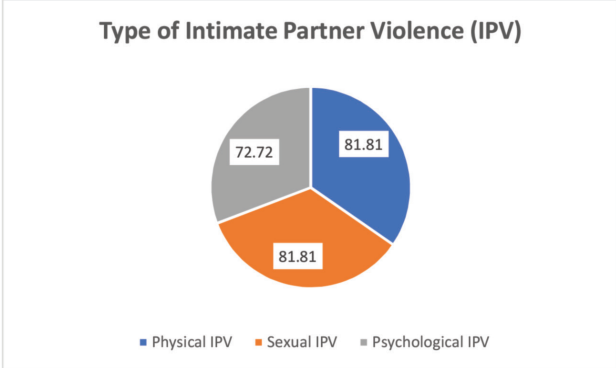


Figure 1: Type of Intimate Partner Violence

Association between Victims and Non-victims of Intimate Partner Violence with selected Socio-demographic variables: Chi-Square Analysis Findings

Table 2 represents the association between Victims and Non-victims of Intimate Partner Violence with selected socio-demographic variables by executing a chi-square test, which is robust as per the categorical distribution. The analysis revealed that age was significantly associated with IPV ($\chi^2 = 11.162$, $p = 0.001$), with a higher proportion of victims (90.90%) being over the age of 25, compared to only 39.68% among non-victims. The correlation strength of 23.6% indicates a moderate relationship.

Table 2. Findings of Chi-Square Analysis containing Intimate Partner Violence association with Victims and Non victims of IPV with socio-demographic variables.

Socio- Demographic Variables	Intimate Partner Violence		χ^2	p	Correlation Value (%)
	Victims (N=11), F(%)	Nonvictims (N=189), F(%)			
AGE (in years)					
≤25	1(9.0)	114(60.3)	11.162	0.001*	23.6
>25	10(90.9)	75(39.6)			

Continue....

DURATION OF MARRIAGE					
Less than 1 yr	0(0)	60(31.7)	10.230	0.006*	15.9
1-5 yr	6(54.5)	103(54.4)			
More than 5 yr	5(45.4)	26(13.7)			
GRAVIDA					
1	0(0)	89(47.0)	65.250	0.000*	57.2
2	3(27.2)	74(39.1)			
3	3(27.2)	23(12.1)			
4	4(36.3)	1(0.5)			
>4	1(9.0)	2(1.0)			
PARITY					
nullipara	0(0)	96(50.7)	45.213	0.000*	27.4
1	3(27.2)	71(37.5)			
2	5(45.4)	20(10.5)			
3	2(18.1)	1(0.5)			
4	1(9.0)	1(0.5)			
SUBSTANCE ABUSE BY PARTNER					
Present	6(54.5)	44(23.2)	5.419	0.020*	16.4
absent	5(45.4)	145(76.7)			

Note: The correlation is significant at 0.05 level. χ2 = Chi-square value, p = Significance level.

The duration of marriage also showed a significant association with IPV (χ^2 = 10.230, p = 0.006), with a correlation value of 15.9%, suggesting a low to moderate association; notably, women married for more than five years experienced elevated rates of IPV. A strong alliance was found linking gravida (number of pregnancies) and IPV (χ^2 = 65.250, p = 0.000), with a high correlation of 57.2%, indicating that IPV was more prevalent among women with higher gravidity. This trend was mirrored in parity (χ^2 = 45.213, p< 0.001), where all IPV victims had at least one child, and most had two or more. It has a moderate correlation of 27.4%, suggesting that having more children increases the risk of experiencing IPV.

Furthermore, a statistically significant association was observed between substance abuse by the partner and IPV (χ^2 = 5.419, p = 0.020) with a correlation of 16.4%, indicating a small but meaningful association.. More than half of the victims (54.54%) reported substance abuse by their partners, compared to only 23.28% among non-victims.

These findings highlight that factors such as older age, longer marriage duration, higher pregnancy and birth rates, and substance abuse by the partner are important correlates of intimate partner violence among women.

Discussion

Intimate Partner Violence remains a persistent and deeply rooted issue, often normalised within the

socio-cultural fabric of Indian society, especially in patriarchal regions. Our study findings suggest the prevalence rate of Intimate Partner Violence among antenatal women was 5.5%. These findings are similar to the descriptive as well as systematic review analysis, where prevalence ranges from 1- 28%.[8,9]

Further, among the IPV Victim participants, about 81% reported experiencing physical violence—including slapping and hitting—as well as sexual abuse (72.72%) from their intimate partners. Their findings were partially consistent with a study where participants had 7.7 to 27.9% had physical violence and 18.1 to 79.8% had psychological violence.[10-12] This high percentage indicates that physical and sexual abuse remains a pervasive issue in intimate relationships and highlights the urgent need for targeted interventions and support systems.

The present study findings showed that age emerged as a significant predictor of IPV, with a higher prevalence among women over the age of 25 ($\chi^2 = 11.162$, $p = 0.001$), and a moderate correlation strength of 23.6% suggests that as women age, they may face increased exposure to IPV. The variables of pregnancy, like gravida and parity, are significantly associated with the occurrence of IPV. They are the strong predictors of IPV, suggesting that women who have experienced multiple pregnancies are more likely to be victims of IPV.

Another significant result showed that women married for more than five years report higher rates of violence. It may be due to repeated exposure to conflict, unmet expectations, or economic stressors may contribute to the development or persistence of violent behaviour in intimate relationships.

Lastly, but important findings present that participants' partners who were abusing substances were abusing their partners. These findings were consistent with many systematic reviews and meta-analyses.[13] It may be because substance abuse impairs judgment, increases impulsivity, lowers inhibition, and leads to aggression.

Strengths and Limitation of The Study

This study is among the first of its kind to explore the prevalence and correlates of intimate partner violence (IPV) among antenatal women in North India, specifically in Rajasthan. By focusing on this vulnerable population, the study fills a critical gap in regional public health and maternal care research.

The study was conducted in a single hospital setting, which may limit the generalizability of the findings to the broader population of antenatal women across North India. Also, Sample size was relatively limited, and the study relied on self-reported data, which may be subject to underreporting due to stigma, fear, or social desirability bias.

Implications

The findings of this study have several inferences for public health practice, maternal healthcare, and policy formulation in India, particularly in Rajasthan.

Primarily initiating early Screening and identification of IPV among antenatal women for prompt implementation, assisting and counselling the antenatal women as well as the community. Enhancing the horizon of Maternal Health Services, considering the psychosocial support through aided guidance from social workers, legal assets, and protective care within the hospital, ensuring holistic needs are met.

Tailored policies, rules and regulations on state and national levels addressing the areas of concern, promoting gender equality, empowering women's education and presenting uniform opportunities to express opinions to women to be furnished.

Collaboration with the community and their active engagement towards maternal needs, besides educational campaigns be beneficial in rural and semi-urban areas to elude men and community leaders confronting their harmful and injurious behaviour.

Considering the study a novice, delving further into larger cohort, multicentric and longitudinal

studies would ensure a better understanding and be effective for locating favourable interventions.

Conclusion

This study explored the prevalence of intimate partner violence (IPV) among pregnant women attending antenatal care services and found notably in antenatal women of Jodhpur, Rajasthan. Crucial factors associated with the cause were marriage duration, parity, and partner's substance abuse appeared highlighting the vulnerability of pregnant women to maternal and fetal health and welfare to bargain. Considering the study to be prime on such a delicate topic, it provides notable insight into identifying exposed women and improving clinical screening for early identification, along with prompt interventions. Emphasising overall community awareness and featuring supportive policies at the root level are necessary to apprise the responsiveness towards antenatal women.

- **Funding Sources***:* Nil

- **Ethical Clearance/Statement of Ethics***:* Ethical approval to conduct the study was obtained from the Institutional Ethics Committee of AIIMS, Jodhpur, with IEC No. - AIIMS/IEC/2023/5543. Informed consent was obtained from Antenatal Women attending the antenatal OPD before data collection, after explaining the procedure. Participants were allowed to leave the study at any moment if they wished to discontinue their participation in the study. Confidentiality regarding the data was assured to get cooperation throughout the procedure of data collection.
- **Conflicts of interest statement : None Declared**

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Efficacy of Thin Layer Chromatography in the Detection and Separation of Various Pyrethroids from Gastric Aspirate Samples

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Abstract

Pyrethroids are synthetic derivatives of the plant toxins pyrethrins and are available in markets as a class of pesticides used in the agricultural, textile and wood industry, day-to-day products such as insect repellents and topical hair lice medication. Pyrethroids are neurotoxic agents and while most are relatively safe for mammals, the easy availability and chronic or excess exposure to it has resulted in an increase in the number of poisoning cases reported in recent years. Quick and accurate detection is therefore a necessary part of identifying and diagnosing pyrethroid poisoning. Our study aimed to determine how effective TLC (Thin Layer Chromatography) is, as a method for analysis of pyrethroid compounds in a biological sample such as gastric aspirate and the best solvent and mobile medium for the same. Thin layer chromatography (TLC) is a chromatographic technique used to separate the components of a mixture using a thin stationary phase supported by an inert backing. TLC functions on the same principle as all chromatography: a compound will have different affinities for the mobile and stationary phases, and this affects the speed at which it migrates. A market study was conducted and pyrethroid containing products which are suitable for the research were chosen.Our results demonstrated that TLC was effective in separating and differentiating between the 8 pyrethroid compounds identified from the 12 products that were tested and the combination of Hexane and Acetone (1:1) was found to be the best solvent system among the two used. Cyclohexane, Acetone and Chloroform (70:25:5) was the best mobile phase from the five tested. We also found that binary solvent extraction was better than single solvent extraction in most of the samples tested.

Keywords: Pyrethroids, Thin Layer Chromatography, Gastric aspirate samples, Cypermethrin, Trans-Allethrin, Prallethrin, Bifenthrin, Lambda-cyhalothrin, Permethrin, Deltamethrin, Transfluthrin

Introduction

Pesticides are the chemicals (natural or synthetic) employed in various agricultural practices to control pests, weeds and diseases in plants. In the process of agricultural development, they have become vital tools for plant protection and for enhancing crop yield. They are an effective and economical

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way to enhance the yield quality and quantity, thus ensuring food security for the ever-growing population around the globe [1]

Pyrethrins are pesticides found naturally in some Chrysanthemum flowers (*Chrysanthemum cinerariaefolium)*. They are a mixture of six chemicals that are toxic to insects. Natural pyrethroids are unstable compounds that quickly decompose under the influence of light, therefore derivatives more resistant to radiation, which are also more toxic to insects, have been developed [2]

Pyrethroids are a class of synthetic insecticides that have been designed and optimized based on the structures of the pyrethrins. Structurally, the six insecticidally active pyrethrins contain a cyclopropane carboxylic acid group and a cyclo-pentenolone group joined by an ester linkage. [3] They are commonly used in crop protection; in the forestry, wood, and textile industries, as well as in medicine and the veterinary field to treat parasitic crustacean infestations. Pyrethroids are recommended for personal protection against malaria and Zika virus by the World Health Organization. [4]

Acute insecticide poisoning is a serious global problem accounting for an estimated 3 million of severe poisoning cases worldwide each year, with approximately 220,000 deaths. More than 90% of these cases are reported from developing countries such as India. In the UK, pesticides are responsible for only about 1% of deaths from poisoning while various studies in India indicate that the figures range from 20% to a staggering 70%.[5]

Mechanism of Action

Pyrethroid insecticides are neurotoxic to both insects and mammals. Most pyrethroids exhibit low to moderately acute oral toxicity in mammals, but the intrinsic toxicity of pyrethroids, which is observed upon direct administration to the CNS, is significant. [6]

Pyrethroids act on voltage-gated sodium channels, which cause an influx of sodium ions into the nerve cells and permanent depolarization. They also influence activities of enzymes, especially in nerve and liver cells. Contact of pyrethroids with the skin, digestive tract, and respiratory tract results in their penetration into the body since they are lipid soluble. [4] Pyrethrins and pyrethroids usually enter the body when people eat foods contaminated by these chemicals, breath air that contains these compounds,through dermal exposure to insecticides that contain pyrethroids or drink water contaminated with these compounds (rare).

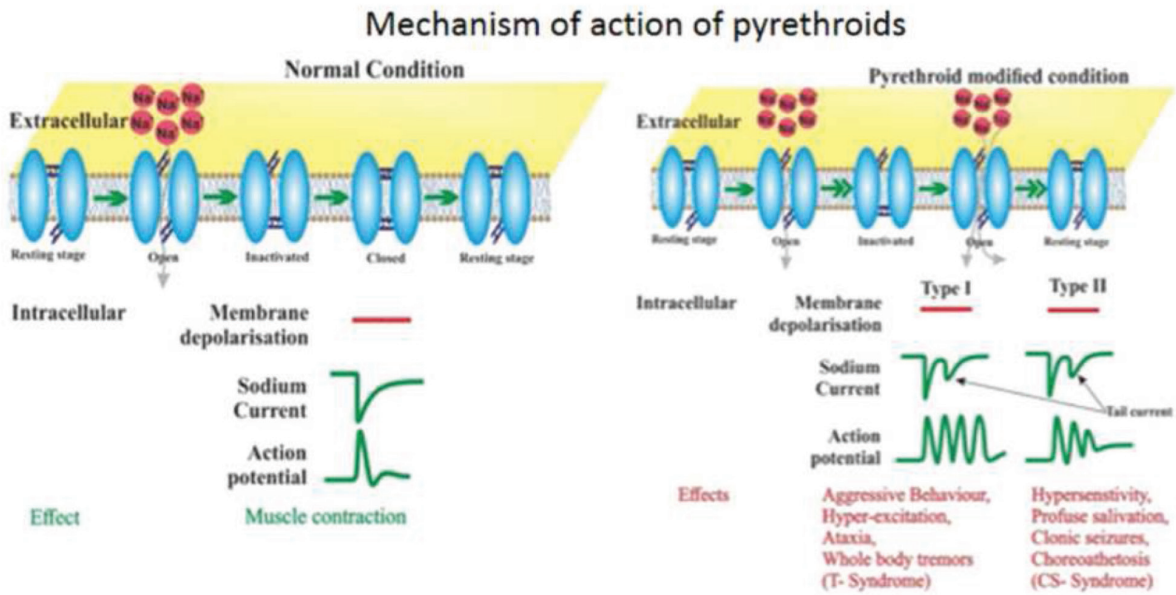


Image 1: Pyrethroid based pesticides – chemical and biological aspects [7]

Pyrethroids that enter the body, leave quickly, mainly through urine, but also in faeces and breath. These compounds are also quickly broken down by the body into other metabolites. Certain types of pyrethroids can also be retained for longer periods of time in the skin and hair. [8] Pyrethrum has an LD50 of over 1gm/kg. However, the minimal lethal dose of pyrethrum is not clearly established, though it is probably in the range of 10 to 100 grams. Most cases of toxicity are actually the result of an allergic reaction. [9]

Pyrethroids are divided into two groups, depending on the type of intoxication symptoms that appear- Type I pyrethroids, including Permethrin, cause the symptoms known as the Tremor type syndrome (T), characterized by tremors throughout the body, hypersensitivity, aggressive behaviour and ataxia. Type II pyrethroids, such as Deltamethrin and Cypermethrin, are associated with the Choreoathetosis-salivation syndrome, in which salivation and muscle dysfunction occur. Children and pregnant women are at risk of faster pyrethroid penetration into the body. It has been proven that pyrethroids and their metabolites can be found in human milk, which poses a risk to newborns. [4]

Currently, pyrethrins are found in over 2,000 registered pesticide products. They are commonly found in foggers (bug bombs), sprays, dusts and pet shampoos.[10] Pyrethroids are often commercially combined with synergist compounds like piperonyl butoxide, which enhance their insecticidal activity, or mixed with fungicides such as 2-phenylphenol or other pesticides like propoxur (a carbamate pesticide).[11] Some formulations registered for use in India under the Insecticides Act, 1968 include Bifenthrin 2.5%, Lambda Cyhalothrin 5%, Allethrin 0.05, Malathion 02% House Hold Spray, Prallethrin 0.8% Vaporizer, Renofluthrin 1.5% w/w Liquid Vaporiser, Transfluthrin 0.08% Aerosol 273, Transfluthrin 0.88% Liquid Vaporiser [12]etc.

Pyrethroid containing products used in households in India include mosquito coils (D- trans-allethrin 0.5% in mortein, Allethrin 00.50 % Coil, Metofluthrin 00.005 %), mosquito mats (Prallethrin 01 %, S-Bioallethrin 02.40 %), household sprays (Malathion 02 %, Pyrethrin 00.20 %, Lambda-cyhalothrin 02.43%) liquid vaporizers (Prallethrin 02.40 %, Transfluthrin 00.88 %, Allethrin 05 %), chalk (Cypermethrin 01 %, Deltamethrin 00.50 %), pesticides (Cyfluthrin 00.025 % + Transfluthrin 00.04 % Aerosol, D-trans allethrin 00.10 % + Permethrin 00.03 % + Imiprothrin 00.02 % Aerosol, Transfluthrin 1 % w/w + Cypermethrin 0.2 %) [13]

Impact

In the largest published series of 573 cases of acute pyrethroid poisoning, 229 were due to occupational exposure and 344 were due to accidental exposure, primarily ingestion.[14] Common reported symptoms included facial paraesthesia, skin itching, skin burning, dizziness, nausea, vomiting, and more severe cases of muscle fasciculations. Hyperglycaemia has been reported to be associated with adverse outcomes in pyrethroid poisoning. In a study on 104 patients with type II pyrethroid poisoning, hyperglycaemia is associated with complications of respiratory failure, acidosis, and hypotension. [15]

Pyrethroids, when combined with organo-phosphorus compounds, are associated with increased toxicity in human poisoning. In a study that compared the clinical profile and outcome of patients with isolated Chlorpyrifos (n = 20) or Cypermethrin poisoning (n = 32) with combination (Cypermethrin–Chlorpyrifos) poisoning (n = 32), patients with organophosphorus–pyrethroid poisoning were found to have lower ventilator-free days compared with patients with either poisoning alone. Mortality was not significantly different. [14]

Pyrethroid exposure can cause oxidative stress, inflammation, and DNA damage. Epidemiological studies, although still limited, have suggested that environmental pyrethroid exposure may impair neurodevelopment, interfere with reproductive health, and increase the risk of major chronic diseases, such as diabetes, cardiovascular disease (CVD), and Parkinson disease. [16]

The conventional methods for determining pyrethroid pesticide residues are often based

on instrumental methods such as GC-ECD (Gas Chromatography with Electron-Capture Detection), GCMS (Gas Chromatography-Mass Spectrometry), and HPLC (High-Performance Liquid Chromatography). In addition to this, UV–Visible spectroscopy and FTIR spectroscopy are also utilized for the same purpose. [17] Relatively few TLC methods are however available for clear separation, leading to ambiguous detection of the new generation synthetic pyrethroids in forensic exhibits.

The aim of our study is to determine how Thin Layer Chromatography (TLC) is beneficial as a method for analysis of pyrethroid compounds in gastric aspirate samples. TLC is a qualitative technique which involves the movement, by capillary action, of a liquid phase (usually an organic solvent) through a thin, uniform layer of a stationary phase (usually silica gel) held on a rigid support (usually a glass, aluminium or plastic sheet). Compounds are separated by a partition between the mobile and stationary phases.

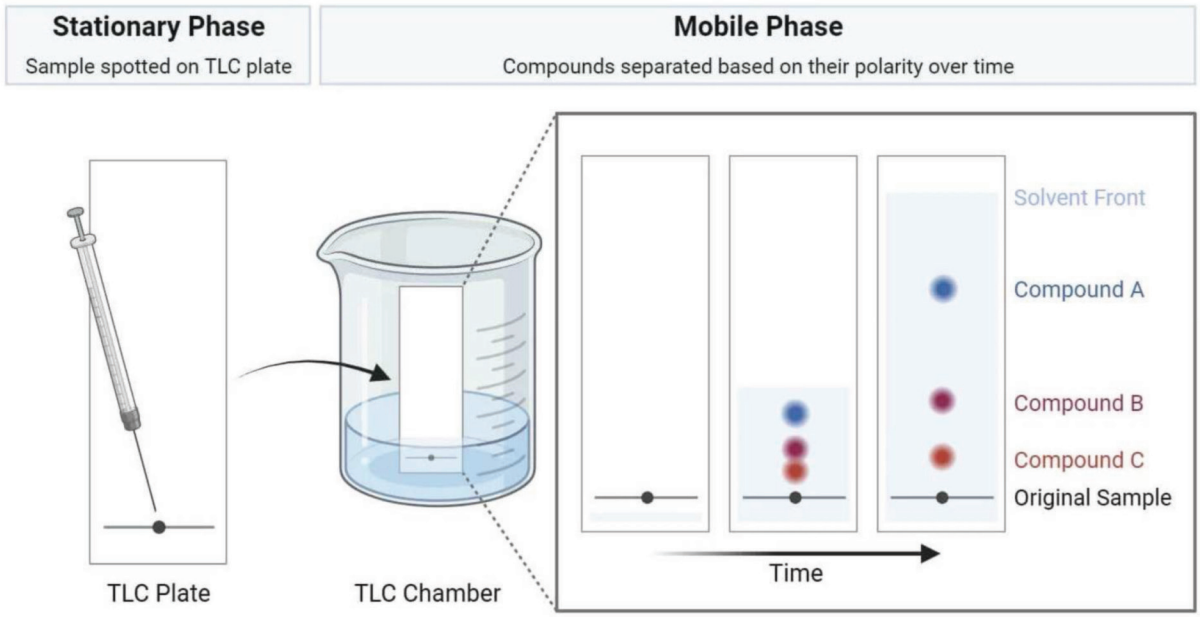


Image 2: Thin Layer Chromatography: Principle, Parts, Steps, Uses [18]

TLC applies to only non-volatile compounds, thus limiting its use. Since TLC operates within the confines of an open system, it is susceptible to being affected by environmental factors such as temperature and humidity, etc. These factors can have consequent effects on the results of the separation on the chromatogram. Compared to other chromatographic techniques like High Performance Liquid Chromatography (HPLC) or Gas Chromatography (GC), TLC may provide lower resolution leading to less accurate separation and identification of compounds. [19] There has been a case study where a new solvent-based, environment friendly and economically viable method was developed to detect Allethrin in blood and urine samples based on a Vortex assisted dispersive liquid-liquid micro extraction method combined with a Thin Layer Chromatography imaging system. This method can help resource- limited laboratories in determining urine and blood samples [20]

Objectives

- To test the efficacy of Thin Layer Chromatography as a detection method for pyrethroid compounds in gastric aspirate samples.
- To identify the most suitable solvent and mobile phase for extraction and identification of particular pyrethroid compounds.

- To evaluate whether binary extractions give better results than single solvent extractions

Methodology

1. Pyrethroids

The pyrethroids were selected based on a market study conducted by our group. The choice of these compounds was guided by their ready availability to the general population in the region where the investigation was carried out, ensuring relevance to the real world exposure patterns.In our survey,we found out that the most widely used and sold pesticides in our area of study include Trans-fluthrin, Transallethrin, Prallethrin, Cypermethrin, Permethrin, Deltamethrin, Bifenthrin and Lambda Cyhalothrin varieties of Pyrethroids. Since these compounds are the most popular choices for use in agriculture, public health and household insect control,they were used in the study.

1. TRANSFLUTHRIN is found in a mosquito repellent named Goodknight Gold Flash at a concentration of 1.6% Transfluthrin. It is present in liquid form.
2. TRANS ALLETHRIN / D-TRANS ALLETHRIN is identified in 2 products by the name Black HIT and Mortein Power Booster. Black HIT, which is in aerosol form, has a concentration of 0.25% Trans-allethrin and is used as an insect repellent. Mortein Power Booster is used to repel mosquitoes which is available as a solid coil form with 0.1% Trans-allethrin.
3. PRALLETHRIN is found in Goodknight Mini Jumbo, which is another mosquito repellent coil of a different brand name and has a concentration of 0.04% Prallethrin.
4. CYPERMETHRIN is found in 4 products by the names Red Hit, Hit chalk, Ant powder and Challenger 10. Red Hit and Hit chalk are used to kill crawling insects, especially cockroaches and ants. Red Hit is in aerosol form and HIT chalk in a solid chalk form with a concentration of 0.20% and 1% Cypermethrin respectively. It is also available in powder form which when applied on surfaces kills ants and comes in a concentration of 0.5% Cypermethrin. Challenger 10 is another product of the same compound with a concentration of 10% and is found in liquid form. It is used on crops as an insecticide.
5. PERMETHRIN is found as a cream which when applied on scalp treats head lice. It is sold under the brand name Medilice and the compound is present at a concentration of 1%.
6. DELTAMETHRIN is obtained from a liquid product named Tagcis Flow. It has a concentration of 2.5% Deltamethrin and is used indoors, to kill mosquitoes.
7. BIFENTHRIN is available as a liquid insecticide named Banner which is useful on crops as an insecticide. It is in liquid form and is available at a concentration of 10%.
8. LAMBDA CYHALOTHRIN is available in 2 products by the names Tag command and Reeva. They are both insecticides used on crops. Tag command is available in liquid form with 5% concentration of Lambda Cyhalothrin. Reeva has 2.5% concentration of the same compound.

2. Sample preparation.

Simulated gastric aspirate samples were prepared by combining water, pulverized food particles (rice), saliva and hydrochloric acid (final pH= 4- 4.3),then spiked with pyrethroid compounds to have an approximate effective concentration of 5 mg/dl. 10 ml of the sample was taken for each extraction.

3. Sample extraction

- Single solvent extraction

  20 ml of the spiked liquid (gastric aspirate) is taken, filtered, if necessary and transferred to a separating funnel. It is then mixed with 20 ml of ether (ER) by gentle shaking. Fumes generated inside the funnel are carefully vented in between the mixing. The ether layer is separated on top of the

aqueous layer. The aqueous layer is then collected/separated through the tail end of the separating funnel into a beaker by opening the stopcock. The remaining ether layer in the separating funnel is allowed to pass through anhydrous sodium sulphate taken in a glass funnel layered with Whatman filter paper and collected in a china dish. The aqueous layer is re-extracted using 20 ml of ether 2 more times. The ether layers are collected/ pooled and evaporated to dryness, keeping it reserved for analysis.

- Binary solvent extraction.

  A mixture of solvents Hexane (ER) and Acetone (ER) in the ratio 1: 1 was prepared and used as the solvent for extraction.

4. TLC technique.

Few drops of methanol were added to the China dish in which the organic solvents were collected and mixed thoroughly. It is used for spotting on TLC plates. A 10 µl of this reconstituted sample extract was spotted on a TLC plate (Silica gel G) along with pyrethroid standards and developed in various solvent systems.

Mobile Phases

Table 1

Sl. No	Solvent System	Ratio
1.	Cyclohexane: Acetone: Chloroform	70:25:5
2.	Benzene	
3.	Cyclohexane: Toluene	70:30
4.	Hexane: Acetone	95:5
5.	Hexane: Chloroform	70:30

The TLC chamber/beaker is pre-saturated with mobile phase at room temperature for 20 minutes. Develop the chromatogram (10-cm run) using a mobile phase (saturated tank), take out and allow it to dry. The separated spots are located and identified by iodine fuming method. Rf values of both single solvent extracted and binary solvent extracted compounds in different mobile phases were calculated using the formula

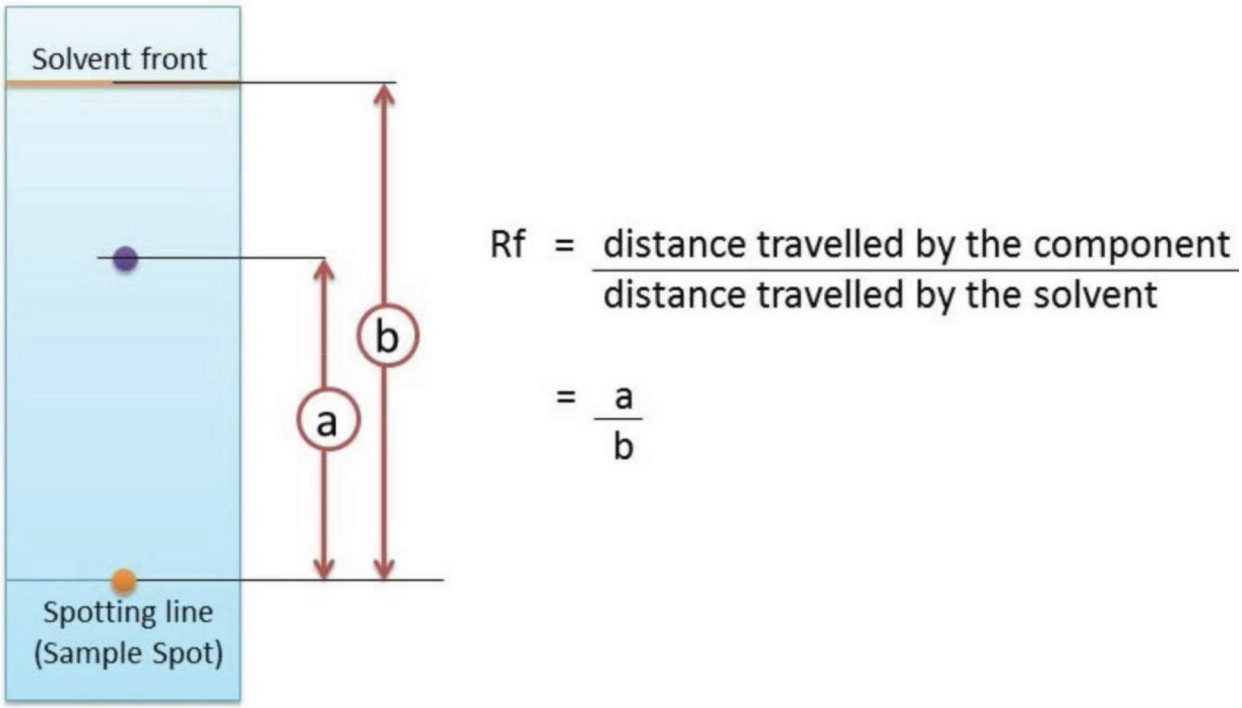


Image 3: Calculating the Retention Factor Value [21]

heightened Retention factor (hRf) = Retention Factor (Rf) x 100

Results

1. CYPERMETHRIN POWDER FORM

Table 2

SL NO	MOBILE PHASE (MP)	SINGLE SOLVENT (S1)	BINARY SOLVENT (S2)	STANDARD
		hRf	hRf	hRf
1.	CYCLOHEXANE: ACETONE:CHLOROFORM	76	76	76
2.	BENZENE	92	92	92
3.	CYCLOHEXANE: TOLUENE	30	30	30
4.	HEXANE: ACETONE	36	36	36
5.	HEXANE: CHLOROFORM	-	60	60

Solvent- S1>S2

Mobile Phases- mp1=mp2=mp3=mp4>mp5

Better results with binary solvent extraction

2. CYPERMETHRIN CHALK FORM

Table 3

SL NO	MOBILE PHASE (MP)	SINGLE SOLVENT (S1)	BINARY SOLVENT (S2)	STANDARD
		hRf	hRf	hRf
1.	CYCLOHEXANE: ACETONE:CHLOROFORM	93	95	90
2.	BENZENE	90	90	90
3.	CYCLOHEXANE: TOLUENE	96	98	91
4.	HEXANE: ACETONE	97	96	92
5.	HEXANE: CHLOROFORM	95	94	90

Solvent- S1 and S2

Mobile Phases- mp2>mp1>mp4=mp5>mp3

3. CYPERMETHRIN LIQUID FORM

Table 4

SL NO	MOBILE PHASE (MP)	SINGLE SOLVENT (S1)	BINARY SOLVENT (S2)	STANDARD
		hRf	hRf	hRf
1.	CYCLOHEXANE: ACETONE:CHLOROFORM	76	76	76
2.	BENZENE	96	94	96
3.	CYCLOHEXANE: TOLUENE	90	90	90
4.	HEXANE: ACETONE	36	36	36
5.	HEXANE: CHLOROFORM	60	64	60

Solvent- S1>S2

Mobile Phases- mp1=mp3=mp4>mp2=mp5

4. TRANS-ALLETHRIN (MORTEIN)

Table 5

SL NO	MOBILE PHASE (MP)	SINGLE SOLVENT (S1)	BINARY SOLVENT (S2)	STANDARD
		hRf	hRf	hRf
1.	CYCLOHEXANE: ACETONE: CHLOROFORM	38	44	44
2.	BENZENE	-	48	48
3.	CYCLOHEXANE: TOLUENE	-	-	-
4.	HEXANE: ACETONE	-	34	34
5.	HEXANE: CHLOROFORM	-	18	20

Solvent- S2>S1

Mobile Phases- mp1>mp2=mp4>mp5 >mp3

Better results with binary solvent extraction

5. TRANS-ALLETHRIN (BLACKHIT)

Table 6

SL NO	MOBILE PHASE (MP)	SINGLE SOLVENT (S1)	BINARY SOLVENT (S2)	STANDARD
		hRf	hRf	hRf
1.	CYCLOHEXANE: ACETONE: CHLOROFORM	76	74	68
2.	BENZENE	34	36	36
3.	CYCLOHEXANE: TOLUENE	-	94	94
4.	HEXANE: ACETONE	16	16	16
5.	HEXANE: CHLOROFORM	96	96	96

Solvent- S2>S1

Mobile Phases- mp4=mp5>mp2>mp1>mp3

Better results with binary solvent extraction

6. PRALLETHRIN

Table 7

SL NO	MOBILE PHASE (MP)	SINGLE SOLVENT (S1)	BINARY SOLVENT (S2)	STANDARD
		hRf	hRf	hRf
1.	CYCLOHEXANE:ACETONE:CHLOROFORM	98	98	98
2.	BENZENE	82	82	82
3.	CYCLOHEXANE:TOLUENE	-	-	-
4.	HEXANE:ACETONE	-	24	24
5.	HEXANE:CHLOROFORM	-	-	-

Solvent- S2>S1

Mobile Phases- mp1= mp2>mp4>mp3=mp5

Better results with binary solvent extraction

7. BIFENTHRIN

Table 8

SL NO	MOBILE PHASE (MP)	SINGLE SOLVENT (S1)	BINARY SOLVENT (S2)	STANDARD
		hRf	hRf	hRf
1	Cyclohexane: Acetone: Chloroform	92	90	94
2	Benzene	98	96	98
3	Cyclohexane: Toluene	94	92	92
4	Hexane: Acetone	90	88	92
5	Hexane: Chloroform	96	96	94

Solvent- S1>S2

Mobile Phases- mp2=mp3>mp5>mp1=mp4

8. LAMDA-CYHALOTHRIN

Table 9

SL NO	MOBILE PHASE (MP)	SINGLE SOLVENT (S1)	BINARY SOLVENT (S2)	STANDARD
		hRf	hRf	hRf
1	Cyclohexane: Acetone: Chloroform	76	82	82
2	Benzene	98	98	96
3	Cyclohexane: Toluene	52	56	58
4	Hexane: Acetone	36	52	54
5	Hexane: Chloroform	40	62	54

Solvent- S2>S1

Mobile Phases- mp2>mp3>mp1>mp4>mp5

9. PERMETHRIN

Table 10

SL NO	MOBILE PHASE (MP)	SINGLE SOLVENT (S1)	BINARY SOLVENT (S2)	STANDARD
		hRf	hRf	hRf
1	Cyclohexane: Acetone: Chloroform	92	94	92
2	Benzene	90	90	92
3	Cyclohexane: Toluene	52	56	52
4	Hexane: Acetone	82	80	80
5	Hexane: Chloroform	70	68	68

Solvent- S1 and S2

Mobile Phases- mp1>mp3>mp4=mp5> mp2

10. DELTAMETHRIN

Table 11

SL NO	MOBILE PHASE (MP)	SINGLE SOLVENT (S1)	BINARY SOLVENT (S2)	STANDARD
		hRf	hRf	hRf
1	Cyclohexane: Acetone: Chloroform	96	96	96
2	Benzene	70	72	72
3	Cyclohexane: Toluene	10	22	10
4	Hexane: Acetone	30	30	30
5	Hexane: Chloroform	14	14	14

Solvent- S2>S1

Mobile Phases- mp1=mp4=mp5>mp2>mp3

11. TRANSFLUTHRIN(BLACKHIT)

Table 12

SL NO	MOBILE PHASE (MP)	SINGLE SOLVENT (S1)	BINARY SOLVENT (S2)	STANDARD
		hRf	hRf	hRf
1	Cyclohexane: Acetone: Chloroform	90	92	96
2	Benzene	90	96	90
3	Cyclohexane: Toluene	96	96	96
4	Hexane: Acetone	94	-	92
5	Hexane: Chloroform	80	80	80

Solvent- S1>S2

Mobile Phases- mp3=mp5>mp2>mp1>mp4

Better results with single solvent extraction

12. TRANSFLUTHRIN(GOODKNIGHT)

Table 13

SL NO	MOBILE PHASE (MP)	SINGLE SOLVENT (S1)	BINARY SOLVENT (S2)	STANDARD
		hRf	hRf	hRf
1	Cyclohexane: Acetone: Chloroform	94	92	92
2	Benzene	98	96	96
3	Cyclohexane: Toluene	96	94	94
4	Hexane: Acetone	94	90	90
5	Hexane: Chloroform	96	96	96

Solvent- S2>S1

Mobile Phases- mp5>mp1=mp2=mp3>mp4

Cypermethrin (chalk) and Permethrin were found to be effectively extracted by both s1 and s2. Cypermethrin(liquid), Bifenthrin and Transfluthrin (Black Hit) were better extracted by s1. Cypermethrin (powder), Trans-allethrin, Prallethrin, Λ-Cyhalothrin, Deltamethrin and Transfluthrin (Goodknight)were better extracted by s2 (Table14), making s2 the superior extraction solvent, overall.

Table 14

Solvents	Samples
S1- Ether	Cypermethrin chalk and liquid, Bifenthrin, Permethrin, Transfluthrin (Blackhit)- (5)
S2- Hexane + Acetone (1:1)	Cypermethrin powder and chalk, Trans-allethrin (Mortein and Blackhit), Prallethrin, Lambda-cyhalothrin, Permethrin, Deltamethrin, Transfluthrin (Goodnight)-(9)

From the 5 different mobile phases used in the TLC process-

Mp1 was found to be the best amongst all 5, separating the individual components in 6 samples (6/12), followed by mp2 (5/12) and mp3, mp4 and mp5 with (4/12) samples each.

Trans-allethrin (Mortein) and Permethrin were better separated by mp1.Cypermethrin chalk and Λ-Cyhalothrin were better separated by mp2. Transfluthrin (Goodnight)was better separated by mp5.

Mp1, mp2, mp3 and mp4 were equally good at separating the sample containing Cypermethrin powder, whereas mp1,mp3 and mp4 were better at separating the sample containing Cypermethrin liquid.

Mp4 and mp5 were equally good at separating the sample containing Trans-allethrin(Blackhit).

Mp1 and mp2 were most effective at separating the sample containing Prallethrin.

Mp2 and mp3 performed equally well at separating the sample containing Bifenthrin.

Mp1, mp4 and mp5 were equally good at separating the sample containing Deltamethrin.

Mp3 and mp5 were both effective in separating the sample containing Transfluthrin (Blackhit) (Table 15).

Table 15

Mobile Phases	Samples
Mp1 - Cyclohexane +Acetone +Chloroform (70:25:5)	Cypermethin powder and liquid, Trans-allethrin (Mortein), Prallethrin, Permethrin, Deltamethrin- (6)
Mp2- Benzene	Cypermethrin powder and chalk, Prallethrin, Bifenthrin, Lambda-cyhalothrin -(5)
Mp3- Cyclohexane +Toluene (70:30)	Cypermethrin powder and liquid, Bifenthrin, Transfluthrin (Blackhit)- (4)
Mp4- Hexane +Acetone (95:5)	Cypermethrin powder and liquid, Trans-allethrin (Blackhit), Deltamethrin- (4)
Mp5- Hexane + Chloroform (70:30)	Trans-allethrin (Blackhit), Deltamethrin, Transfluthrin (Blackhit), Transfluthrin (Goodnight)- (4)

Cypermethrin powder, Trans-allethrin (Mortein and Blackhit) and Prallethrin were found to give better results with binary extraction than with just a single solvent extraction. Transfluthrin (Blackhit) was found to give better results with a single solvent extraction (Table 16).

Table 16

Single solvent extraction	Binary solvent extraction
Transfluthrin (Blackhit)	Cypermethrin powder, Trans-allethrin (Mortein and Blackhit), Prallethrin

Discussion

Investigating the presence of pyrethroid compounds from commercial products in body fluids is crucial for understanding potential human exposure and its associated health risks.

In a case that had been reported from Odisha, a 22-year-old female was successfully treated after drinking one bottle of Good Knight Advance mosquito repellent liquid and had presented with concussions, respiratory distress and altered sensorium. [22] One of the key factors that led to the diagnosis was the awareness and treatment measures, based on previous studies, that Prallethrin is a key ingredient in mosquito repellents. In 2022, Thin Layer Chromatography was used to test visceral samples in a case of a complex suicide. The retention factor values were calculated and had tallied with Chlorpyrifos and Cypermethrin [23] Similarly, a 62 year old male was admitted in All India Institute of Medical Sciences, New Delhi after his relatives had found him unconscious post dinner with pinpoint pupils, increased respiratory rate, bronchi spasm and convulsions but unfortunately passed away after critical care was administered-Transfluthrin was detected in the visceral samples by the Forensic Science Laboratory, where they had used Thin-Layer chromatography, GC-MS and FTIR techniques. [24]

Studies like this one are essential as they help identify the most effective techniques for detecting specific compounds, ensuring accurate and reliable results, especially in India, where pyrethroid poisoning is common. By evaluating different methods, researchers can determine which tests are best suited for particular scenarios, such as environmental monitoring, forensic investigations, or public health assessments.

Ashlesha Bhardwaj, Biswa Prakash Nayak, Gyan Prakash Sharma' and Himanshu Khajuria' conducted a study in India on Thin Layer Chromatographic separation and identification of pyrethroid insecticides in food grain samples and positive results were seen with the pyrethroids (Bifenthrin, Permethrin, Cypermethrin, Deltamethrin) in 7 cereal samples out of 17 samples.[25] In our study, positive results were seen with all 13 of the pyrethroid samples used.

The article on optimization of analytical method for detecting pyrethroids by TLC and GC-MS in forensic sample by Yadav, B; Yadav, B; Tripathi, S; Rohatgi, S in 2020 was conducted to develop an efficient method for simultaneous determination of four Pyrethroid insecticides: Allethrin, Cypermethrin, Permethrin and Transfluthrin. Thin Layer Chromatography (TLC) and Gas Chromatography-Mass Spectrometry (GC-MS) techniques were explored for detection of Pyrethroid insecticides. Four solvent systems including Hexane, Methanol, Ethyl acetate and Acetone, were applied to evaluate the extraction efficiency of Pyrethroids from the samples collected. Hexane: Chloroform (7:3) solvent system proved to be the most efficient for extracting solvent for identification of Pyrethroids from forensic samples. [26] In this study of ours, Hexane+ Acetone(S2) was found to be the better extraction solvent in 9 samples (9/12) and Ether (S1) was found to be the better solvent in 5 samples (5/12).

With biological samples, the most common methods of analysis are GC combined with ECD, FID, or flame photometric detection (FPD) and HPLC coupled with UV detector. [27] The best advantage which TLC offers is saving time as compared to traditional paper chromatography. For instance, chromatographic separations using paper chromatography can take as long as a day. An additional advantage being offered by TLC is the large number of samples that can be run at a single time along a plate. It allows for the identification of the compounds, mainly carried out by comparing the compound separated on the TLC plate with some standard (pure form of the compound being identified) run alongside the experimental separation. It is a cost-effective technique that allows for the separation of multiple samples and better detection without getting diffused into each other. [28]

Given the overlap of symptoms with organophosphate toxicity, cholinesterase measurement may be helpful to delineate the type of exposure further. However, such tests are not rapidly available

and lack the ideal sensitivity and specificity. [29] Cypermethin is frequently encountered in poisoning cases in India. With TLC, the compound can be detected by spraying the plate with 0.2% p-Benzoquinone in Dimethyl Sulfoxide (DMSO). The sensitivity of the test after plate development is 0.25 ug. [30]

Conclusion

A total of 12 different pyrethroid-containing products were collected from various locations within Kochi city, from which 8 types of pyrethroid compounds were identified. All 8 pyrethroids were detectable and separable with TLC. From the 2 solvents used, Hexane + Acetone (1:1) was found to be a better extraction solvent and from the 5 mobile phases used, Cyclohexane+Acetone+Chloroform (70:25:5) was the best. We also found that binary solvent extraction (Cypermethrin powder, Trans-allethrin– both Mortein and Blackhit, and Prallethrin) was superior to single solvent extraction (Transfluthrin- Blackhit).

This study proved that TLC was effective in distinguishing between the various pyrethroids tested in gastric aspirate samples, required simple lab equipments and techniques, with minimal manpower and skills. This renders it as a reliable detection test in cases of pesticide poisonings,when quick, low cost and qualitative results are needed.

The few drawbacks of TLC are that some pyrethroids may not be easily detected due to factors like volatility, weak UV absorption, or insufficient reaction with common visualization reagents. The occurrence of objective errors is notably high, which may significantly impact the accuracy and reliability of the results, potentially leading to skewed or inconsistent findings that could undermine the integrity of the entire process. Future research could explore ways to enhance the versatility of TLC in detecting a broader spectrum of toxins, from other biological samples such as blood and urine. This may include detecting more volatile compounds or improving resolution for more precise separation.

Source of Finding: Not applicable

Ethical Clearance: Not applicable

Conflict of Interest: The authors declare that they have no conflicts of interests related to this work.

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Magnitude and Causes of Sudden Cardiac Death in Ayder Comprehensive Specialized Hospital: A Retrospective Autopsy Study

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Abstract

Background: Sudden cardiac death poses a significant global health challenge; however, its extent and causes are not well documented in sub-Saharan Africa. This study aimed to assess the prevalence and underlying causes of sudden cardiac death at Ayder Comprehensive Specialized Hospital in Ethiopia's Tigray region.

Methods: Retrospective data of autopsy records spanning from January 2014 to December 2023 from the autopsy records was collected using standardized checklist and demographic information, death circumstances, autopsy findings, and underlying causes of death were analyzed.

Results: Among all autopsied cases, 4.91% were identified as sudden cardiac deaths, with a predominance of males and a median age of 49. The leading cause was atherosclerotic coronary artery narrowing, followed by hypertrophic cardiomyopathy (HCM). Atherosclerotic coronary artery disease was the main cause in individuals aged > 35 years, whereas hypertrophic cardiomyopathy was the most common cause in those aged ≤ 35 years.

Conclusion: This study highlights the prevalence and causes of sudden cardiac death in Ethiopia's Tigray region. These findings underscore the necessity for enhanced interventions targeting cardiovascular risk factors, especially coronary artery disease and cardiomyopathy. Additionally, this study highlights the importance of improved autopsy practices and documentation to better comprehend and tackle the burden of sudden cardiac death in resource-constrained settings.

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Keywords: Sudden cardiac death,Cardiovascular disease, Forensic autopsy, Hypertrophic cardiomyopathy, coronary artery disease

Introduction

Sudden cardiac death (SCD) is defined as a sudden, natural, and unexpected death as a resultofa cardiac disease.[1] It is a major global health issuewith a rising incidence in developing countries.[2,3] The incidence of SCD is influenced by numerous factors, such as age, sex, genetic susceptibility, health and nutritional status, socioeconomic status, and lifestyle habits.[4,5] Although the causes of SCD may vary across nations, regions, and demographics, they are classified intostructural and nonstructuralcardiac disorders.[6,7] Generally, coronary artery disease (CAD) is the main cause of SCD in those over 35 years of age, whereas non-atherosclerotic heart conditions are more common in individuals under 35 years of age.[8–10]

However, the extent and underlying causes of SCD in Africa, especially sub-Saharan Africa, are poorly documented and largely unknown, with variable incidences.[11,12] Contemporary autopsy methods, which include gathering essential data, standardized heart examination,histological analysis of cardiac tissues, molecular analyses, thorough inspecting other body parts,toxicology and drug overdose investigation, and microbiological and biochemical analyses, offer a distinct opportunity to reveal the pathological characteristics of SCD.[13–15] This comprehensive evaluation practice aids in gathering accurate data and insights into SCD, which are crucial for crafting effective intervention strategies and enhancing public health outcomes.[16,17] However, the magnitude and cause of SCD remain unexplored in Ethiopia, particularly in the Tigray region, presenting a unique set of challenges.

This study aimed to determine the magnitude and underlying causes of sudden cardiac at Ayder Comprehensive SpecializedHospital (ACSH), by analyzingautopsy records. We expect that this study will reveal specific etiological patterns and magnitude of sudden cardiac death unique to the study population. Such findings will aid in developing targeted interventions to reduce the burden of SCD and provide decision-makers, planners, and researchers with vital information on SCD in the region.

Methodology

We conducted a retrospective review of autopsy records spanning ten years, focusing on all individuals who succumbed to SCD and autopsied at ACSH between January 01, 2014, and December 30, 2023by experienced forensic medicine specialists, each performing more than 100 autopsies per year, using contemporary guidelines.[18] Located in the Tigray Region of Ethiopia and overseen by Mekelle University-College of Health Sciences (MU-CHS), the ACSH is the sole facility in the region that possesses an autopsy facility since 2012. In Tigray region, medico-legal autopsies are compulsory for cases involving suspicious, unnatural, sudden, or unexpected deaths. Medico-legal autopsies were performed in accordance with updated SCD autopsy protocol except genetic analysis.[19] Written informed consent for the publication of findings was obtained from the deceased's bereaved family member or other legal guardians of each case

SCD was characterized as a sudden, natural, and unexpected death as a result of cardiac origin that occurred within one hour of symptom onset if witnessed or within 24 hours if unwitnessed, provided that the individual was last observed in a stable health condition and the cause wascardiac origin.[20] The Research and Community Service Council Ethical Review Board of MU-CHS, ACSH granted ethical approval for this study. Authorization was obtained from the hospital's management, and an overview of the study's objectives, anticipated advantages, study methods was provided. Confidentiality was maintained, with only data collectors having access to details about the deceased that were unrelated to the study focus. Any labeling information in the collected data was anonymized by removing the labels and coding the data during transcription. We included all cases who are at the age of one year or above and passed

awaydue to SCD.[20] We excluded cases involving any known cardiac or other chronic diseases or congenital anomalies, deaths occurring during or after surgery, individuals with identifiable noncardiac causes of death or signs of active substance abuse, deaths in which toxicology and drug analysis were not conducted, deceased who were pregnant or in the postpartum period, individuals hospitalized within the past 30 days for a noncardiac illness or surgical procedure, unknown cases, cases with signs of decomposition, those aged below one year, cases under police investigation or pending prosecution, those with incomplete records, or cases still under review. Different standardized checklists were reviewed and customized for the study, and data collectors were trained to extract information from the charts based on the developed checklist. Data collection was conducted under the investigator's continuous follow-up and supervision of the investigator. Retrospective data were reviewed using a standardized checklist abstracted from autopsy reports, autopsy documentation, medical records, medical certificates, police investigation results and autopsy requests. Prior to the analysis, the accuracy of the data was verified by entering the data twice. Some outliers and missing values were cross-checked. Data entry was performed using SPSS version 25. Descriptive statistics, such as the mean and standard deviation, were used to describe the variables.

Results

Demographic Profiles and Circumstance of Death

During the study period, 3,054 medicolegal autopsies were performed, of which 150 (4.91%) were identified as SCD (Table 1). Among the SCD victims, 92% (n=138) were Tigray region residents and 8% (n=12) were residents of the neighboring regions of Amhara and Afar. Residences were categorized as urban for 42 % (n=63), rural for 54% (n=81), and unspecified for six 4% (n=6) patients. Males accounted for 86% (n = 129) of the patients. The patients' age ranged from 17 to 85 years, with a median of 49 years. The 41–50-year age group had the highest proportion of cases 28% (n=42), and 86% (n=129) of the cases were over 35 years of age, as shown in Figure 1. Death occurred in the morning in 30% (n = 45) of the cases, and the time of death was unknown in 34% (n = 51) (Table 2). Of the 150 patients, 54% (n=81) died within 24 h of being last seen alive and well, whereas 46% (n=69) of the deaths were witnessed. Among the witnessed deaths, 56.5% (n = 39) experienced unspecified chest discomfort lasting less than an hour, whereas 43.5% (n = 30) showed no prior signs or symptoms of illness. Of the patients who died without prior signs of illness, 30% (n=9) were at rest, 10% (n= 3) engaged in strenuous physical activity, 10% (n=3) slept, 20% (n=6) drank alcohol, and 30% (n=9) had an unknown activity status before death. The majority of deaths, 90% (n=135), occurred outside medical facilities, whereas 4% (n=6) occurred in hospitals.

Table1. Time of death of SCD cases autopsied in ACSH, January 01, 2014- December 30, 2023

R. N0	Time of day of death	Number of cases	Percent (%)
1	Morning	45	30
2	Afternoon	24	16
3	Night	30	20
4	Unknown	51	34

Autopsy records of all cases lacked information regarding the deceased's occupation, prior medication use, dietary habits, socioeconomic status, physical exercise patterns, and psychosocial conditions. Among the SCD patients, 38% (n=57) had a history of unspecified amount of alcohol intake, 52% (n=78) had no history of alcohol use, and 10% (n=15) had an unknown history of alcohol intake. Regarding smoking habits, 36% (n=54) of participants had a history of unspecified cigarette use, 24% (n=36) had no history of smoking, and 40% (n=60) had an unknown smoking history. Additionally, 12% (n=18) of the participants reported a history of unspecified khat chewing, 40% (n=60) had no history of khat use, and 48% (n=72) had a history of unknown khat consumption. In this study, 22% (n = 33) of the patients had an unknown past medical history, 4% (n = 6) had a history of unspecified heart disease, 2% (n = 3) had a history of untreated systemic

hypertension, and 72% (n = 108) had no history of chronic medical disease. Of these, 28% (n=42) had a family history of unspecified heart disease, and the family history of SCD was unknown in all cases. None of the patients underwent cardiovascular intervention.

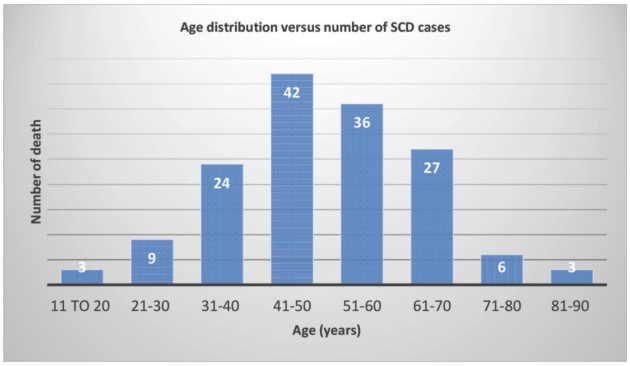


Figure 1: Age distribution and frequency of in 150 SCD cases autopsied in ACSH, January 01, 2014- December 30, 2023

Autopsy Findings

Upon external examination of the corpses, 4% (n = 6) showed disarranged clothingand 14% (n = 18) displayed petechial hemorrhage in the conjunctiva. Rigor mortis was present inextremities of all cases and none of the cases showed any signs of putrefaction. Abrasions of approximately 4 cm × 3 cm were found in the forehead and front of the lower extremities in 24% (n=36) of the cases. The remaining external examination findings were unremarkable in all cases. Body weight and height were not recorded in all cases. The pericardial cavity showed > 200 ml of blood with a ruptured infarcted left ventricular wall in 14% (21) of cases. Pericardial membrane hyperemia was noted in 2% (n = 3) of the cases. The major cardiac blood vessels were normal in all instances. Petechial hemorrhage and hyperemia were observed on the external surface of the heart in 64% (n=96) of cases and the heart size was enlarged in 6% (n=9) of the cases. Transverse sections made at 3-mm intervals along the main epicardial arteries of the heart and their branches were made in all cases, and 68% (n=102) of the cases revealed atherosclerotic coronary artery narrowing (ACAN) in which > 75% of the coronary artery lumenwas occluded in all of the cases, as determined by visual assessment (Figure 2).The myocardium exhibited various conditions: hyperemia in 50% (n=75) of cases, mottling in 12% (n=18), softening in 14% (n=21), and scarring in 4% (n=6). No morphological abnormalities were observed in any cardiac valve. The mid-cavity free wall of the left ventricle exceeded 02 cm in thickness in 44% (n=66) of cases, whereas the right ventricular mid-cavity free wall was greater than 6 mm thick in 34% (n=51) of cases. In 18% (n=27) of the cases, the interventricular septal thickness to mid-cavity free-wall left ventricular thickness ratio was greater than 3:1. As shown in Figure. 3, 46% (n=69) of the patients had a heart weight of > 400 g. All of the cardiac chambers showed dilatation in 6% (n=9) of the cases. Atheromatous plaques were identified in the aortic root in 12% (n=18) of the cases. All the other examinations yielded normal results.

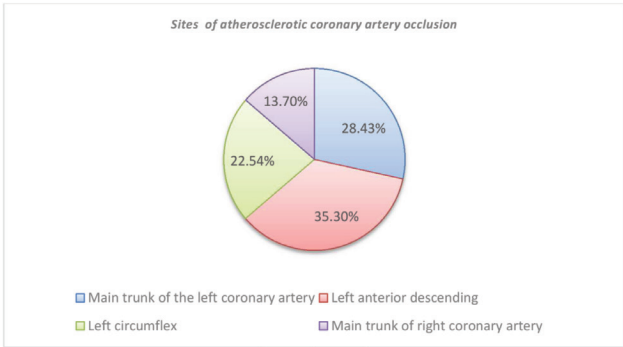


Figure 2: Site of atherosclerotic coronary artery occlusion in SCD cases autopsied in ACSH, January 01, 2014- December 30, 2023

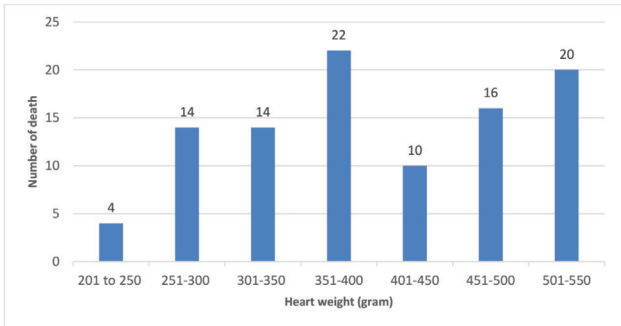


Figure 3; Heart weight of SCD cases autopsied in ACSH, January 01, 2014- December 30, 2023

Ancillary Investigations

Elevated serum troponin I levels (>0.1ng/ml) were observed in 98% (n=147) of cases whereas serum Troponin T levels were not measured in all of the cases. In 92% (n=138) of the cases, serum CK-MB levels exceeded 10ng/ml. Cardiac histopathology was performed in all of the cases and revealed features of coronary atherosclerosis and myocardial infarction in 68% (n=102) of the patients, hypertrophic cardiomyopathy (HCM) in 18% (n=27), myocardial fibrosis in 6% (n=9), dilated cardiomyopathy (DCM) in 6% (n=9), and myocarditis in 2% (n=3). Other supplementary investigations, including toxicology, drug overdose, and drug abuse screening, were negative in all the cases. Molecular and genetic analyses were not performed in all of the cases.

Cause of Death

The underlying causes of death were as follows: ACAN (68%, n=102), obstructive HCM (18%, n=27), old myocardial scar (6%, n=9), DCM (6%, n=9), and unspecified acute myocarditis (2%, n=3). Among the patients who were above the age of 35 years (n=129), the cause of death was ACAN in 76.74 % (n=99) of cases, HCM in 11.63% (n= 15) of cases, myocardial fibrosis in 6.98% (n=9) of cases, DCM in 2.3% (n=3), and unspecified myocarditis in 2.3% (n=3) of cases, whereas the cause of death was HCM in 57.1% (n=12), DCM in 28.6% (n=6), and ACAN in 14.3% (n=3) of the cases for those who were at the age of ≤ 35 (n=21). Among the patients who died due to ACAN (n=102), 2.94% (n=3) were aged ≤ 35 years, while 97.06% (n=99) were older than 35 years. In addition, among the patients who succumbed to HCM (n=27), 44.44% (n=12) were aged below 35 years, while 55.56% (n=15) were above the age of 35 years, with ages ranging from 17–58 years. Furthermore, of all individuals who died due to DCM (n=9), 66.7% (n=6) were younger than 35 years, whereas 33.3% (n=3) were olderthan 35 years.

Discussion

SCD is a global public health issue that is expected to have a more significant impact on low- and middle-income nations.[21] SCD is frequently underreportedin Sub-Saharan areas, particularly in Tigray region of Ethiopia.[22] This studywill have insights into the causes and magnitude of SCD in the region.

Understanding the global epidemiological framework of SCD is challenging due to variations in documentation practices.[2,23] Nevertheless, it is anticipated to account for approximately 15–20% of all deaths, with a presumed higher impact in developing countries.[24,25] In our study, we found that 4.91% of all deaths were due to SCD, which seems to be lower than the global estimates, most likely attributable to our study's methodology.The autopsy rate is usually low in resource limited areas which likely results in an underreporting of autopsy-based SCD registration.[26,27] Furthermore, SCD caused by inherited genetic disorders and coronary spasms might have been overlooked in our study, as the autopsy protocol in the study area did not incorporate molecular analysis in their autopsy practice.[28]

Our research revealed that the male-to-femaleratio of SCD cases was 6.1:1, with 86% of these cases occurring in individuals over the age of 35 years. The highest incidence was observed in the 41-60 years age groups.In developing nations, SCD predominantly affects adults in their middle and late years, with the highest incidence occurring between 40 and 69 years of age, which is consistent with our study findings.[5,24] In contrast, the incidence of SCD is typically below 1% for those aged < 35 years in developed countries but increases significantly with age, particularly after 50 years, with a sharp rise in risk for individuals in their 70s.[12,29] These variations are mainly due to the demographic attributes of the population paralleling the different cardiovascular risk factors.[30,31] The overall male-to-female ratio of SCD is approximately 2:1 across all age groups,with the disparity most pronounced among middle-aged individuals.[32,33] While the likelihood of SCD increases with age in both men and women, men face a lifelong risk of approximately 10.9%, whereas it is 2.8% in women.[34,35] This is primarily due to the fact that men face a higher risk of cardiovascular diseases, particularly

CAD.36–38 The significant variations in SCD related to age and gender underscore the need for targeted intervention strategies, especially for high-risk individuals.[39]

Our study also demonstrated that ACAN was the cause of SCDin 68% of cases, followed by HCM. Additionally, ACAN was the cause in 76.74% of individuals aged > 35 years, whereas HCM was responsible for 57.1% of cases in those aged ≤ 35 years. Globally, for individuals over the age of 35 years, CAD is the primary cause of SCD, whereas for those under 35 years, HCM is the most common cause.[40,41] This aligns with our study findings and emphasizes the growing burden of non-communicable diseases in low- and middle-income countries.8 Furthermore, our study results showed that 97.06% of the patients who died due to ACAN were above the age of 35 years, and the age of the patients who died due to HCM ranged from 17 to 58 years, with 55.56% being above the age of 35 years. This also consistent with concurrent study findings highlighting the need for enhanced screening and treatment of cardiovascular diseases.[2,42] Besides, trivial injuries can occur as a result of SCD, most commonly due to accidental falls or collisions with nearby objects during collapse, which mainly affect prominent body partsor may also be caused by cardiopulmonary resuscitation efforts.[43,44] However, it is crucial to differentiate these injuries from commotio cordis, in which a blunt chest impact causes electrical disruption, leading to SCD.[45,46] This study revealed that 24% of cases exhibited minor injuries to the forehead and prominent areas of the limbs, which aligns with trivial injuries.

This study faces several limitations, mainly due to its retrospective nature, which may have introduced bias from incomplete or inconsistent records. Additionally, the medical and social histories of each case were generally collected by police investigators, who often missed case-specific information pertinent to SCD. This lack of data likely arises from limited medical expertise of the investigators, which hampers our study's capacity to link these factors with the underlying cause of death. To address this issue, it is recommended that the forensic pathologist responsible for the autopsy collects a detailed history specific to the case before beginning the procedure. This will help obtain precise medical information, thereby supporting justice and ensuring the integrity of public health records. Another limitation is that the findings are based on a single-center study, with autopsy cases mainly involving suspected or confirmed violence, which may restrict the generalizability of the results to other populations. Despite these limitations, this study provides crucial baseline data that can guide future research and inform public health strategies aimed at reducing the burden of SCD. However, this study has strengths, mainly its reliance on autopsy records, which highlights the critical importance of postmortem examinations in understanding the causes of SCD and remains a valuable tool for identifying otherwise undiagnosed conditions and providing an accurate cause of death.[15,47–49]

We investigated the prevalence of SCD in the area by analyzing autopsy records and related circumstantial information while also considering its current global context and emphasizing its strengths and weaknesses.Additionally, we examined the autopsy characteristics and causes of SCD, emphasizing the importance of early identification and management of cardiovascular risk factors, better access to healthcare services, and increased community awareness of SCD. Moreover, we investigated the underlying causes in relation to age, sex, and other cardiovascular risk factors,highlighting the study's limitations and suggesting potential mitigation strategies, such as adopting a multidisciplinary approach involving healthcare providers, policymakers, and the public, to tackle the increasing burden of SCD and enhance cardiovascular health outcomes in resource-constrained settings.

Conclusion

SCD is a major global health issue affecting individuals, families, and healthcare systems. The research revealed that ACAN and HCM are the most common causes of SCD in ACSH, underscoring the need to address this using a holistic approach in the region. This should include healthcare professionals, policymakers, and the public, focusing on the

early detection and management of cardiovascular risk factors, better access to healthcare, and increased community awareness of SCD. This multidisciplinary strategy is crucial to combat the global burden of SCD and improve cardiovascular health in similar contexts.

This study also emphasized the role of autopsies in detecting SCD, highlighting the study limitations. To improve SCD registration, enhancing autopsy capacity and training of experts should be prioritized. In addition, it is highly suggested that forensic doctors collect detailed case-specific data that will help obtain quality evidence. Despite the limitations, findings of this study provide valuable baseline data to guide future research and inform public health strategies to reduce the SCD burden. Hence, collaborative efforts are essential for implementing effective SCD prevention and management strategies in the Tigray region and other similar contexts.

Abbreviations and Acronyms

SCD- sudden cardiac death

ACSH- Ayder Comprehensive Specialized Hospital

MU-CHS- Mekelle University of College of Health Sciences

ACAN- atherosclerotic coronary artery narrowing

CAD- coronary artery disease

HCM- hypertrophic cardiomyopathy

DCM- dilated cardiomyopathy

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Statements and Declarations

Ethical Approval: The outlined study protocol for this research obtained ethical approval from the Research and Community Service Council Ethical Review Board of MU-CHS, ACSH.

Informed Consent: This study is a research article and informed consent for the study was obtained from the family of the deceased as a routine autopsy.

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

- Funding Sources Open access funding was provided by the Mekelle University College of Health Sciences Ayder Comprehensive Specialized Hospital. This study received no specific grant from any funding agency, commercial or not-for-profit sector.

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 is no other relationships or activities that could appear to have influenced this study.

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Knowledge, Attitude and Practice of Medical Ethics among Medical Graduates and Post Graduates in a Tertiary Care Teaching Hospital in Navi Mumbai

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Abstract

Introduction: This study aimed to assess the knowledge, attitude, and practices of medical ethics among the medical graduates and post-graduates of a tertiary care teaching hospital.

Materials & Methods: A cross-sectional study was conducted after approval from the ethics committee. A structured questionnaire containing 25 multiple-choice questions relating to medical ethics was validated, pre-tested, and distributed using Google Forms. A total of 262 students participated in the study. The data was entered into an EXCEL sheet and statistical analysis was performed with the help of SPSS version 21.0 and SAS version 9.0.

Results & Conclusion: The study covered 262 students, of whom 52.3% were post-graduates and 47.7% were interns, with a female predominance. The survey revealed a notable deficiency in doctors' understanding of medical ethics, indicating potential gaps in their knowledge of ethical principles. Encouragingly, the survey also demonstrated a positive attitude among doctors towards the importance of medical ethics, suggesting their willingness to engage constructively with moral concerns.

The study concluded that a lack of adequate knowledge of medical ethics and afavourable disposition toward medical ethics. Addressing their insufficient knowledge is imperative to enable them to effectively navigate ethical challenges and deliver the highest quality of care to their patients.

Key Words: KAP Study, Medical Ethics, Ethical dilemmas, Paternalism, Consent in Medicine

Introduction

Ethics has been defined as "the moral principles that govern a person's behaviour or how an activity is conducted" and medical ethics as "the branch of knowledge in medicine concerned with moral principles".[1] The application of ethics to medical practice dates back to ancient civilization as even

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today, all medical graduates must swear symbolic adherence to the Hippocratic oath.[2] Presently, the medical profession is governed by a Code of Ethics and Etiquette and laws regulating the profession which the National and State Medical Councils and the World Medical Association lay down.

There has been growing public awareness regarding the ethical conduct of medical practitioners, and complaints against physicians appear to be escalating. This may reflect an increase in unethical practices done by doctors or increasing public and media awareness about medical facts and fallacies, professional accountability, and rights of patients in terms of information, decision-making, and assessing outcomes.[3] In medicine, professionalism connotes not only knowledge and skills, but also character, especially compassion and ethics.[4] It is a commitment to subordinate our self-interest to the interest of patients and it is the foundation of trust upon which our social contract as physicians rests.[5]

Medical students are taught various subjects to tackle medical problems, but are they taught ethics to solve the moral quandaries that they are likely to face in their practice in the future [6].Medical ethics are integral to all clinical encounters and public health interventions, and a foundation in medical ethics is essential for students to become virtuous doctors.[7]Despite the presence of the AETCOM module, its implementation and effectiveness across institutions remain uncertain and largely unassessed. Moreover, there is a deficiency of published data from Indian medical colleges, especially in the Navi Mumbai region, assessing the knowledge and application of medical ethics in real-world scenarios. This study was conducted to assess the knowledge, attitude and practice of medical ethics and professionalism among the medical graduates and post-graduates of D Y Patil School of Medicine, Navi Mumbai to provide insights that can guide curriculum refinement and promote ethical clinical practice.

Materials and Methods

A cross-sectional study was conducted at D Y Patil School of Medicine after approval from the ethics committee. Consent was taken from the participants before the beginning of the study. Confidentiality was maintained.

Sample size: 262

The sample size was calculated by taking 42.1 as the percentage of people with adequate knowledge in the population based on a similar study conducted in 2015.[8] The confidence level will be taken as 95% with a 5 % margin of error and a total population of medical graduates being close to 800 in number.

Study Period: A period of 3 months, from 30th August to 30th November 2023.

A structured questionnaire containing 25 multiple-choice questions relating to knowledge, attitude, and practices of medical ethics was devised, validated, and pre-tested using Google Forms. The first part of the questionnaire consisted of the demographic characteristics of the respondents including age, gender, and educational qualification. The second part consisted of nine questions on their knowledge of ethics and professionalism. A score of either 0 or 1 was assigned to each question and summed up with a maximum score of 9. In the third part, respondents were required to state if they agreed or disagreed with nine statements concerning ethical conduct, confidentiality, informing patients about wrongdoings, informed consent, abortion, etc. The responses will be provided on a Likert scale ranging from 1 to 3 (1-agree, 2-disagree, 3-not sure). The final part consisted of seven questions on their practices of ethics and professionalism.

Data Analysis

The data was entered intoan EXCEL sheet for statistical analysis. Analysis was done with the help of SPSS version 21.0 and SAS version 9.0. The collected data was interpreted using appropriate statistical methods like percentages and proportions. The chi-square test was used for comparison across the groups, and a probability value of 0.05 was considered significant.

Results

The study covered 262 students at D Y Patil School of Medicine, Navi Mumbai, of which 52.3% were post-graduates and 47.7% were interns with a female predominance.

Most respondents (140, 53.4%) had not read the Code of Ethics (Figure 1). Around 75.2% (197) of the respondents said that they were taught about medical ethics in their undergraduate curriculum and 24.8% (65) could not remember whether or not it was taught. 47.4% (124) felt that the undergraduate medical ethics curriculum was inadequate while 47% (123) thought it was inadequate. 5.7% (15) of the respondents gave a neutral opinion. In a multiple response query, the majority (132, 50.4%) responded that they obtained their knowledge of ethics from their undergraduate curriculum, 34.7%(91) said that they acquired knowledge of ethics through experience at work, whereas 15.1% (39) stated that they got it through attending lectures, seminars, workshops or continuing medical education (CME) and own reading.

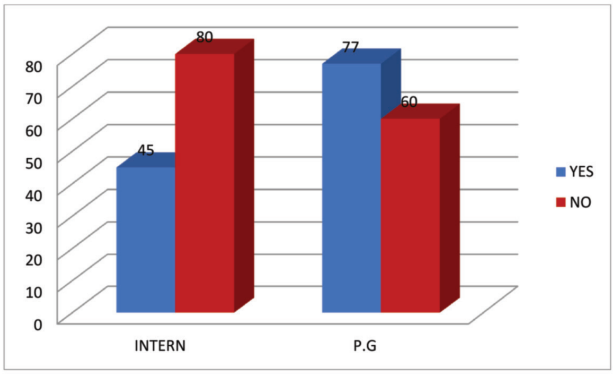


Figure 1: Knowledge of Code of Ethics by Education/ Designation

Only 108(41.2%) of the respondents correctly answered the question regarding the guidelines on the required hours of participation by medical professionals in a CME session in a year. 101(38.5%) responded correctly when asked how long records for indoor patients were to be maintained, and 102 (38.9%) could give the correct response regarding the period within which a physician has to produce the records when asked by patients or legal authorities and 221 (84.4%) responded correctly regarding the requirement of consent for tests and medications.

Among the postgraduates and interns, 49.6 % agreed with the statement doctors know the best irrespective of patients' opinions, while 5.3 % remained neutral, indicating a paternalistic attitude. Most postgraduates and interns agreed with the statements that close relatives should always be informed of the patient's condition, patient autonomy should always be respected, and children should never be treated without parental consent. Over 80 % of participants concurred with these statements. There was a statistically significant difference between the opinions of senior and junior doctors regarding the statements "Patients should always be informed of wrongdoing by anyone involved in their treatment" ($p = 0.022$) and "Doctors should refuse to treat uncooperative/violent patients" ($p = 0.031$) (Table 1). Most postgraduates agreed with these statements, while most interns disagreed. Although a higher proportion of postgraduates agreed with the statements, there were no significant differences in the opinion regarding the statement "If the law allows abortion, doctors cannot refuse to do abortions" with 70.1 % of postgraduates and 68.0 % of interns disagreeing with the statement.

Table 1. Attitude on certain issues of medical ethics by education/designation

ISSUES	EDUCATION/ DESIGNATION	DISAGREE NO (%)	AGREE NO (%)	CHI-SQUARE VALUE	P-VALUE
Q11: Doctors know the best irrespective of patients' opinion	INTERN POST GRADUATION	59 (47.2) 61 (44.5)	62 (49.6) 68 (49.6)	1.096	0.578
Q12: Patient should always be informed of wrongdoing by anyone involved in their treatment	INTERN POST GRADUATION	63 (50.4) 46 (33.6)	59 (47.2) 87 (63.5)	7.630	0.022**

Continue....

Q13: Close relatives should always be told of the patient's condition	INTERN POST GRADUATION	1 (0.8) 2 (1.5)	124 (99.2) 130 (94.9)	3.268	0.118
Q14: Children should never be treated without the parent's consent	INTERN POST GRADUATION	18 (14.4) 9 (6.6)	106 (84.8 124 (90.5)	0.917	0.079
Q15: Patient autonomy should always be adhered to	INTERN POST GRADUATION	9 (7.2) 11 (8.0)	104 (83.2) 114 (83.2)	0.109	0.947
Q16: Doctors should refuse to treat uncooperative/ violent patients	INTERN POST GRADUATION	20 (18.0) 17 (12.4)	98 (78.4) 110 (80.3)	5.671	0.031**
Q17: If the law allows abortion, doctors cannot refuse to do an abortion	INTERN POST GRADUATION	85 (68.0) 96 (70.1)	27 (21.8) 25 (18.2)	1.771	0.621

**Significant difference between interns and post graduates for q12 and q16 ($p<0.05$).

Encountering an ethical dilemma once a week was reported most frequently, at 50.8 %. When asked whom they approach for help in case of such a problem, most respondents answered their immediate senior (41.7%), followed by the head of the department (37.6%). There was a statistically significant difference between the opinions of postgraduates and interns regarding the statements "I spend enough time to explain the nature, purpose, and possible consequences of treatment or procedure when obtaining informed consent from patients" (Table 2) and "I engage in Continuous Medical Education (CME) activities" ($p = 0.001$). 95.1 % of the participants chose written consent as the ideal method of obtaining consent. When asked whether they report instances of professional misconduct to the medical council, 61.7 % of respondents answered "sometimes," while 24.2% answered "often."In the given situation, where a male doctor needs to examine a female patient and no female attendant is available, 40.8 % of respondents felt it is not ethically right to refuse the patient, 28.7 % believed it is right to refuse, and 30.6 % were unsure.

Table 2. Opinion of postgraduates and interns regarding the statements "I spend enough time to explain the nature, purpose, and possible consequences of treatment or procedure when obtaining informed consent from patients"

VARIABLE	PRACTICE OF ETHICS			CHI-VALUE	P- VALUE
	Very Often	Often	Sometimes		
Intern	47 (37.6%)	58 (46.4%)	20 (16%)	14.933	0.001**
Post Graduate	78 (56.9%)	53 (38.7%)	6 (4.4%)		

**Significant difference between interns and post-graduates for q20 ($p<0.05$).

Discussion

Postgraduates (52.3 %) dominated the sample with females outnumbering the male doctors. A significant portion (53.4%) of the respondents had not read the Code of Ethics. This is concerning, given that the code is fundamental to medical practice. Despite the majority (75.2 %) indicating that they

were taught about medical ethics during their undergraduate training, there is a notable disparity in the adequacy of this training. 47.4 % of respondents felt the curriculum was inadequate, while 47 % felt it was adequate. This suggests a divide in the perceived quality and depth of ethical education.

The study also shows that most respondents rely on their undergraduate curriculum (50.4 %) for ethical knowledge, with only a smaller portion acquiring it through work experience or other methods such as lectures and personal reading (15.1 %). This may be because 47.7 % of the participants were interns, with less clinical exposure compared to their postgraduate counterparts. We recommend that more continuing medical education (CME) and guest lectures including seminars should be conducted imparting knowledge of ethics and consequences of infamous conduct by medical practitioners. Only 41.2 % of the participants correctly answered the guidelines on required CME hours, 38.5 % knew the correct duration for maintaining indoor patient records, and 38.9 % knew of the period within which records should be produced upon request.

More than half (140, 53.4%) of the respondents lacked adequate knowledge of the code of ethics. Though statistical significance was lacking, knowledge of ethics was higher among the postgraduate respondents with 56.2% (77) respondents showing positive results. This may highlight the need for more integrated and continuous ethics education throughout medical training including compulsory AETCOM questions.

Postgraduates demonstrated higher knowledge and more favourable attitudes towards certain ethical practices compared to interns. The statistically significant differences observed in opinions about informing patients of wrongdoing ($p = 0.022$) and refusing to treat uncooperative patients ($p = 0.031$) highlight the changing perspectives on ethics with experience. However, no significant difference was found regarding the ethical stance on abortion, with strong adherence to legal boundaries regardless of experience level.

The study also revealed an inclination towards a paternalistic attitude, with 49.6 % of respondents agreeing that doctors know best, irrespective of the patient's opinion. These findings are consistent with the study by Aacharya et al[8]. Over 80 % agreed with principles such as informing close relatives of a patient's condition, respecting patient autonomy, and obtaining parental consent for treating children. This suggests strong adherence to fundamental ethical principles, although it is in contrast with the paternalistic attitude observed.

The study highlights that encountering ethical dilemmas is a common experience, with 50.8% of respondents facing such issues weekly. In these situations, the majority seek guidance from their immediate senior followed by the head of the department. Similar findings have been reported by Aacharya et al, Hariharan et al[11], and Walrond et al[12]. This pattern reflects the importance of mentorship and senior oversight in navigating such issues. The significant difference in opinion on obtaining informed consentand engagement in CME activities may reflect the varying levels of experience and responsibility between the postgraduates and interns. Postgraduates being further along in their training may be more accustomed to situations regarding informed consent and better understand its importance. They also recognize the importance of CME in staying updated with the latest a vancements. Enhancing training in patient communication and a greater emphasis on the importance of CME could help bridge the gap between postgraduates and interns in these areas.

Conclusion

This study identified key areas for improvement in the understanding and application of medical ethics among the study participants.It revealed

that over 50 % of participants lacked adequate knowledge of the Code of Ethics, and a paternalistic attitude was evident among some respondents. These findings are surprising despite the introduction of the AETCOM module by the National Medical Commission in 2019.

An unexpected finding was the difference in ethical awareness between graduates and postgraduates, which may indicate that exposure alone may not be sufficient and that continuous structured training is required throughout their medical education. Everyone should have a strong consensus on fundamental ethical principles, reflecting a solid commitment towards their core moral values. It also gives an insight into the need for formal guidance in handling ethical dilemmas. This study emphasizes the need to strengthen medico-legal education and ethical decision-making, especially regarding consent, confidentiality,and documentation.

Limitations of the study include a relatively small sample size, single-institution design, and reliance on self-reported data, which may be subject to bias. Future research should focus on the long-term implications of ethical training modules on clinical practice. Additionally, comparative studies across government, semi-government, and private medical institutions could identify systemic weaknesses to improve ethical training.

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Ethical Approval: IEC approval reference is taken institution of ethics committee IEC BH

Reference No: from 2023/182, the DY institutional Patil School Medicine, Navi Mumbai.

Conflict of Interest: None to declare.

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Drowning Patterns in Saurashtra Region- a Retrospective Study

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Abstract

Drowning is one of form of mechanical asphyxia. It is major contributor of deaths in India and worldwide. Total 38,503 people died in India due to drowning in 2022. Gujarat reported 1959 deaths due to drowning in 2022. According to WHO approximately 300000 people died in year 2021. **The study was conducted in one year duration from January 2024 to December 2024 in forensic medicine and toxicology department of Shree M. P. Shah Government medical college and Guru Gobindsingh Government Hospital, Jamnagar, Gujarat, India. Total 64 cases of drowning were reported. In this study, male deaths were 84.37%;** Age group of the 21-30 years contributing 25% of total cases; **60.94% cases were married; maximum cases belonged to Hindu community and rural area; most of cases reported in rainy season.**

Key words: Drowning; Asphyxia; Types; Age group; rainy season

Introduction

Water is life source of all living beings; humans, animals and trees alike. Water is literally the life. However the vice versa is also true when water become the reason of death.

Drowning is a form of asphyxia due to aspiration of fluid into air-passages, caused by submersion in water or other fluid. Complete submersion is not necessary, for sufficient period can cause the death from drowning. About 150,000 people die from drowning each year around the world.[1]

Drowning is classified into following types[2]

- Typical or wet drowning - fluid or water is inhaled in lungs
- Atypical drowning - there is very little or no inhalation of water
    - Dry drowning – water dose not enter in lungs due to laryngeal spasm
    - Immersion syndrome – syncope resulting from cardiac dysrhythmia or sudden contact with cold water
    - Near drowning/ secondary drowning – survival beyond 24 hours, death by complications or sequelae.
    - Shallow water drowning – alcoholics, drugged, children and unconscious person may die due to drowning from fall in shallow water or pit.

According to national crime record bureau's report accidental deaths and suicides in India 2022; 38, 503 people died due to drowning in India contributing 9.1% of total accidental deaths and suicides. In which 31056 were male, 7443 were female and 4 were transgender. From which 1959 people died due to drowning in Gujarat. From 1959 total, 1692 were male and 267 were female.[3]

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According to WHO (world health organisation) there were an estimated 300000 drowning deaths in 2021 – this is equivalent to more than 30 people losing their lives to drowning every hour of every day. Almost half of these fatalities were under the age of 29, and one quarter under the age of five.[4] According to WHO's latest Global Health Estimates (2021) reveal that drowning is the fourth leading killer of children aged 1–4 years and the third leading killer of children aged 5–14 years. Among adolescents and young adults aged 15–29 years, drowning is the 12th leading cause of death. [4]

During the autopsy, finding of ante mortem drowning must be confirmed. In some cases, body is removed after some days after the death leading to decomposition of the body. Diatom test must be done to confirm drowning. Diatom test may have some limitation and questions on its accuracy. But still it is easily available method to confirm drowning.

Accidental drowning is very common in India. Suicide by drowning is also seen in many cases, however homicidal drowning occurs less commonly. It may vary from place to place; Homicidal drowning accounts for 20% of all U.S. killings. Amongst the homicidal drowning, most cases are children drowned by their parents, or wives drowned by their husbands in a bathtub.[5]

In the drowning cases, it is needed to acurately determine the cause of death and manner of death, especially in the complex scenarios where it is defficult to determine manner of death. Additionally, research into drowning prevention, such as installing barriers and teaching water safety, is essential to reduce mortality and morbidity.

The research gap in drowning cases includes inconsistent data collection, underreporting of cases, lack of targeted prevention stratergies and gender differences.

Aims & Objectives

Aim of this study was to know the pattern and profile of drowning in Jamnagar and its surrounding regions, Gujarat, India and to study the demographic parameters like age, sex, residential area and socio economic status affecting the deaths due to drowning.

Materials & Methods

This study was conducted in the forensic medicine and toxicology department of Shree M. P. Shah Government medical college and Guru Gobindsingh Government Hospital, Jamnagar, Gujarat, India. The study was conducted in one year duration from December 2024 to January 2024. Total 64 cases of drowning were reported during this period. These 64 cases were studied and all data was compiled and categorised from the documents preserved in the Department of Forensic Medicine & Toxicology, after the prior permission taken from the Head of Department. Detailed history taken from police, relatives and eye witnesses were also noted. History was cross checked with each other and from documents. All details were recorded from police documents like post mortem request letter, inquest panchnama, maranottar form and refer letter etc. In cases of decomposed drowning where the signs of drowning were unclear; bone was preserved for diatom examination and viscera preserved for chemical analysis and sent at forensic science lab. All classes of drowning in unidentified dead bodies were excluded in this study.

Result

Total 1226 autopsy was conducted during this one year of period in the department of forensic medicine department, Shri M. P. Shah government medical college, Jamnagar. Out of all these cases, total 64 cases were opined as drowning as a cause of death. It was 5.22% of total autopsy.

All unidentified dead bodies were excluded because lack of data contribution in the study like age, residential area, socio economic status and religion etc.

Out of total 64 cases, 54 cases were male and 10 were female. In which male contributed 84.37% of total cases followed by female contributing 15.63% of total cases of drowning. This shows male dominances in cases of drowning.

Table No. 1

Age group	Male	Female	Total
0-10	1	2	3
11-20	10	1	11
21-30	12	4	16
31-40	12	0	12
41-50	6	1	7
51-60	6	0	6
61-70	4	1	0
71-80	3	1	4
Total	54	10	64

As seen in table no. 1 age group of 21-30 years contributes maximum cases; 16 out of 64 – 25% of total cases. Following 21-30 years age group 31-40 years provide 12 cases, 18.75% of total cases.

Table No. 2

Marital status	Male	Female	Total
Married	33	6	39
Unmarried	21	4	25
Total	54	10	64

Out of 64 cases, 39 (60.94%) cases were married and 25 (39.06%) cases were unmarried. Among the male 61.11% were married and 38.89% were unmarried. Among females 60% were married and 40 % unmarried.

Total 35 bodies out of 64 cases were in fresh condition and 29 dead bodies were in decomposed condition. Fresh bodies contribute 54.69% and decomposed bodies contribute 45.31%.

Total 43 cases out of 64 were from rural area contributing 67.19% of total cases. Maximum number of cases belongs to lower socio economic status. In this study 58 cases belonged to Hindu religion and 6 cases from Muslim religion.

Total 35 cases registered in monsoon season while 17 cases registered in summer and 12 cases registered in winter; making monsoon season biggest contributor of cases.

Discussion

Drowning cases contributes 5.22% of total autopsy; this makes drowning one of the leading risk factors towards death. In this study male contributes 84.37% and female contributes 15.63%; showing clear male dominance in the study. Study of Pondurthi Shrinivas Rao (2021)[6] and Sujan Kumar Mohanty (2016)[7] also found similar results in their study. They found 88.23% and 82.2% of male cases in their study. Reason behind the male dominance in the drowning could be the high exposure of the water like fishing, irrigation and swimming.

Age group of the 21-30 years contributes 25% of total cases. This age group contains high risk of drowning because this age group contains high energy, less maturity and carelessness. Also the addiction and drunkenness also leads to drowning. This result is similar to study of Laxman Gangadhar Phad (2018)[8] and K. Supriya (2022)[9].

In this study 60.94% cases were married which outnumbered unmarried cases. Study of Pondurthi Shrinivas Rao (2021)[6,] Laxman Gangadhar Phad (2018)[8] and Raut S M (2021)[10] also found married persons more in number than unmarried. In some cases marital stress and responsibility leads to suicidal drowning.

In this study rural area (67.19%) contributes more cases than urban area. This may be because of occupation like fishing and irrigation; also the youngsters swimming in the river and lakes like a sport in villages. Pondurthi Shrinivas Rao (2021)[6] and Raut S M (2021)[10] also found rural area cases are more than urban cases.

In this study 58 cases (90.62%) were belong to Hindu religion; contributing maximum numbers of cases in the study. This may be due to Hindu population is more in the study area. Laxman Gangadhar Phad (2018)[8] also found Hindu religion cases dominance in their study.

Monsoon season contributes 54.69% of total deaths due to drowning. Laxman Gangadhar Phad (2018)[8] and K. Supriya (2022)[9] also found in their study that rainy seasons contribute maximum number of cases. This may because flood and increased water levels in water area increase chances of drowning.

Conclusion

In this study we found that male are more prone to drowning than female. 21 to 30 years age group contributes maximum number of cases followed by 31 to 40 years age group; which make these age groups more at risk than others. Deaths due to drowning are seen more in married people than unmarried people. Drowning deaths are prominent in rural area than urban area. Deaths due to drowning are seen in maximum cases of Hindu religion followed by Muslim religion. Rainy season contribute maximum numbers of deaths due to drowning.

Recommendations

Preventive measures against the drowning must be taken. Possible sites of drowning must have some notice or sign board around the vicinity. During the rainy seasons and flood warning must be issued in advertisement, news and awareness programme.

In case of drowning in children parents must be aware and keep prevention against the drowning and keep surveillance over the kids.

If possible lifeguard must present at susceptible places. Make Life jackets mandatory on the boating activity.

Ethical Approval

No specific approval from ethical comiittee was taken. The approval from the Head of Department, Forensic Medicine & Toxicology was taken for conducting the restrospective study and accessing the documents related to the research.

Conflicts of Interest: None

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Studies on Diatom Diversity of Various Water Bodies of The State Tripura (India) for Forensic Analysis

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Abstract

Diatoms are unicellular algae with unique silica frustules found in diverse aquatic and terrestrial ecosystems. In forensic science, their presence in distant vital organs aids in determining whether drowning occurred ante-mortem and helps identify the possible site of drowning. In Tripura, a small hilly state in the north-east India, there is a significant annual incidence of drowning cases with no existing diatom database. A recent study conducted across four out of eight districts of Tripura aims to establish a comprehensive diatomological database to aid forensic experts in drowning investigations. The study identified 73 species of diatoms belonging to 34 genera and 22 families under 11 orders of the class Bacillariophyceae across different districts with some species common among all districts, while others are exclusive to specific regions showcasing a diverse range of diatoms. Among them, there are 11 species from both the Bacillariaceae and Naviculaceae families, 8 from Gomphonemataceae, 7 from Fragilariaceae, 5 from Pinnulariaceae, 4 each from Achnanthidiaceae and Cymbellaceae, 3 from Sellaphoraceae along with 2 species each from Aulacoseiraceae, Catenulaceae, Diadesmidaceae, Pleurosigmataceae, Stephanodiscaceae and Rhopalodiaceae and 1 species each from the families Achnanthaceae, Brachysiraceae, Eunotiaceae, Mastogloiaceae, Melosiraceae, Neidiaceae, Stauroneidaceae and Surirellaceae. The West district recorded the highest diversity with 36 species, followed by Sepahijala with 30 species, Unakoti with 16 and Gomati with 14 species. Interestingly two species are of common occurrence among all the four districts, while some are exclusive to certain regions. Twenty-two species were exclusively found in the West district, where as 17 species were restricted only to the Sepahijala district. Likewise, 10 species were limited to the Unakoti district and 7 species were found solely in the Gomati district. Certain species are unique to specific region underscoring notable variations in diatom distribution across different water bodies. Moreover, this study adds 72 species to the previously recorded 21 species of diatoms in Tripura. The diverse distribution of diatoms across water bodies aids in linking drowned individuals to specific sites helping identify drowning locations and solving cases.

Key words: Diatom, Drowning sites, Silica frustule, D-Map, Diatom diversity, Diatom database.

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Introduction

Diatoms, the microscopic, unicellular algae classified under Bacillariophyceae of the kingdom Protista, are known for their silica-based frustules which display intricate patterns earning them the title 'jewels of the plant world'. A study indicates that over 200 genera and approximately 200,000 diatom taxa exist worldwide[1]. In the Indian subcontinent, around 14,700 taxa of diatoms are estimated including freshwater, brackish and marine varieties[2]. They occur in diverse aquatic habitats such as rivers, lakes, ponds, brackish and marine waters as well as in terrestrial environments such as mosses, wet rocks and soil[3,4,5]. Diatoms contribute about 25% of atmospheric oxygen and 43% of oceanic primary production significantly influencing global carbon cycling[6].

Diatoms exhibit a remarkable diversity in shapes and sizes ranging from circular to elongated and even triangular forms with dimensions typically between 2µm and 2mm, most commonly 10-80µm. In freshwater ecosystems, they act as key primary producers and serving as food for zooplankton and small fishes. They are also recognized as reliable bio-indicators for assessing water quality due to their environmental sensitivity. Moreover, their silica frustules have forensic significance especially in drowning investigations for determining drowning type, location, time since death and evidence of asphyxia.

Diatom communities vary widely among water bodies due to differences in nutrient and light availability with factors such as temperature, pH, mineral content and water stratification influencing their abundance and diversity[7,8,9,10]. Studies have shown that low pH reduces diatom richness, whereas circum-neutral pH supports higher species richness and diversity[11]. Research also highlights the forensic significance of diatoms as distinct freshwater and marine species help determine drowning sites. Diatom testing in organs and bone marrow aids such investigations. Some studies in France and Delhi revealed site-specific and seasonal variations respectively, assisting in drowning analysis[12,13].

Diatoms were first recognized in lung fluids by Hoffman in 1896, but their forensic relevance emerged when Hoffman and Rovenstorff used them as evidence in a drowning case in 1904. Later, Incze (1942) detected diatoms in blood and organs and Tamasaka (1949) in bone marrow. In India, Ehrenberg first reported diatoms from the Nilgiris mountains, Ganges and Southern coasts in 1845 using light microscopy[2]. Pioneering studies on freshwater diatoms were conducted by H.P. Gandhi[15], Sarode and Kamat[16]. Between 2011 and 2012, many studies have been made on diatom diversity; Pareek *et al* studied 24 diatom species in Galta Kund, Jaipur, Huifang Su *et al* examined the Pearl and Yangtze rivers (China), Marbaniang and Paul identified 22 species in Meghalaya, Singh *et al* reported 22 genera from the Ganga, and Saini and Kushwaha found 39 species in Haryana including 6 new species[17,18,19,20,21,22,23,24], while Gupta *et al*, Luthra *et al*, Chawla and Patel, and Akhila *et al* explored Himachal Pradesh, Punjab, Madhya Pradesh and Andhra Pradesh revealing ecological and forensic significance of diatoms[25,26,27,28]. Diatomological maps could potentially identify drowning sites when locations are unclear as supported by various researchers[8,29,30,31,32,33]. Thakur and Singh created a Diatomological map of Punjab's water bodies to assist drowning investigations[34]. Singh *et al* created a diatom database for Jaipur identifying rare site-specific diatoms[35].

In Tripura, a small hilly state in the north-east India, with numerous drowning incidents annually, there is currently no comprehensive diatom database for comparison with tissue samples from victims. Although Das *et al*[36] studied algae in Tripura from three subdivisions, a thorough investigation of diatom diversity across all eight districts remains pending. This study aims to survey at least two water bodies from each of four districts namely West Tripura, Sepahijala Tripura, Unakoti Tripura and Gomati Tripura districts and to establish a foundational database for future research on drowning cases.

Materials and Methods

Study Area

A survey was conducted during January-July, 2024 in four districts of Tripura (22°51′-24°32′N, 90°10′-92°21E; area:10,491sq.km) namely West, Sepahijala, Gomati and Unakoti districts (Figure 1). The state experiences summer (April-June), monsoon (July-October), winter (November-February) and a brief spring (March) with an average annual rainfall of approximately 2000mm.

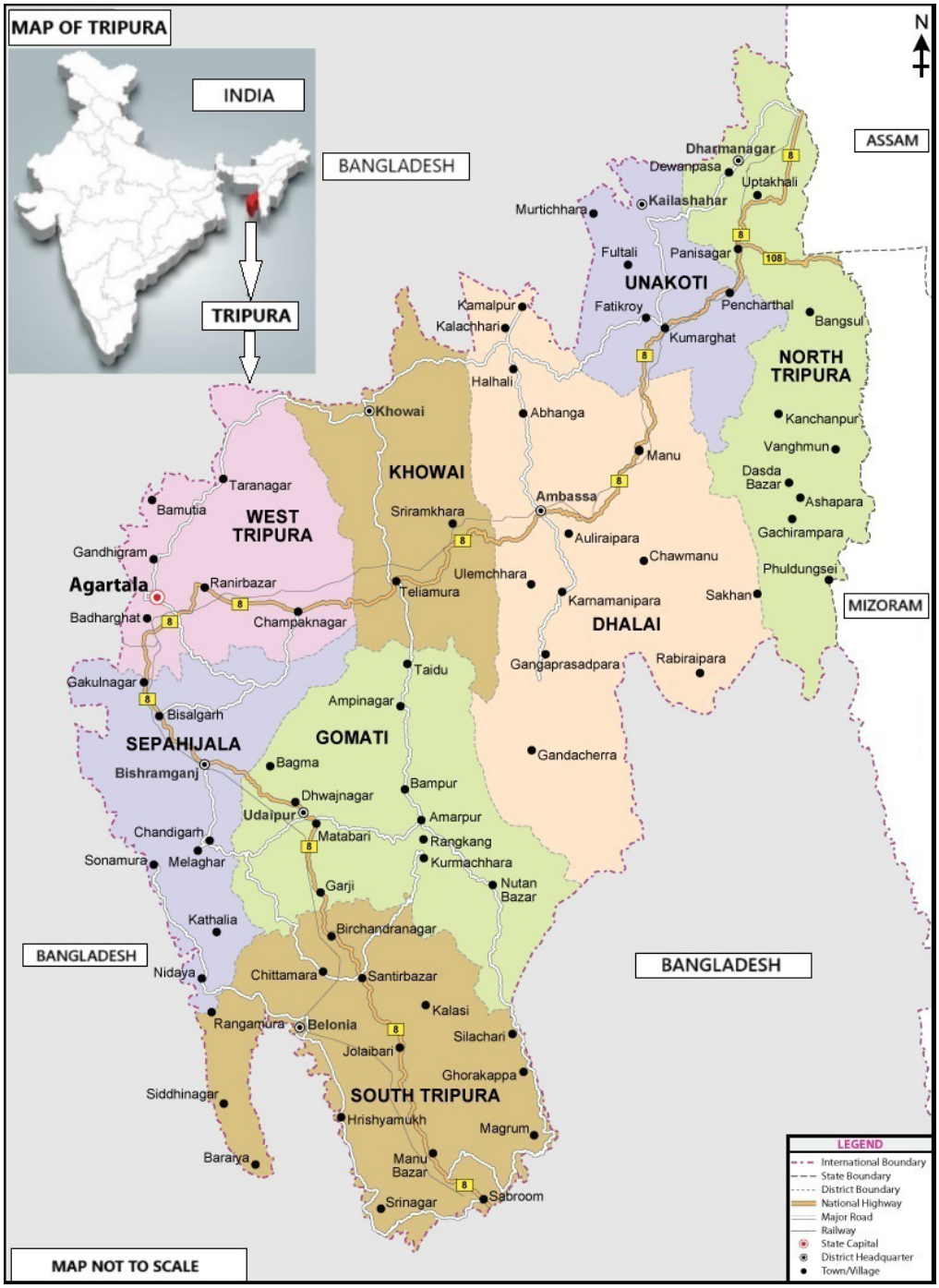


Figure 1: Map of Tripura showing its eight districts [courtesy: www.mapsofindia.com].

Study Sites

Study sites selected for diatom survey include two types of water bodies-ponds and lakes, across four districts in Tripura. A minimum of two water bodies were selected from each district for this study.

Sample Collection

Approximately 100ml of water was collected in clean plastic bottles by filtering 30liters of water through a 20µm mesh plankton net at four sites per pond/lake. The bottles were sealed and labeled with site, date and time, and were transported to the laboratory and refrigerated.

Sample Preparation

In a 100ml water sample, 2-3 drops of 2% formalin were added and was left overnight. Half of the sample was then discarded and the remaining portion was vigorously shaken and centrifuged at 3000rpm for 10minutes. The supernatant was discarded reducing the volume by half. Approximately 10-12ml of the residual pellet was transferred to a test tube, treated with 2ml of 50% H_2O_2 and 5ml of concentrated HNO_3 and left undisturbed for about six hours for digestion. The digested sample was transferred to a labeled centrifuge tube, centrifuged again at 3000rpm for 10minutes and washed thrice with distilled water to eliminate residual acids. Finally, two drops of distilled water were added to the pellet, stored at room temperature and one drop was mounted on a slide using DPX for microscopic examination[35,37]. The slides were observed under a compound microscope at varying magnifications (100X, 400X, 1000X) and the images were captured.

Identification of Diatoms

The diatoms were identified through a review of existing literature including resources such as works by Charles E. Bessey[38], the online database by P.A. Sims[39] and the database compiled by Karthick *et al*[2]. Their identification was based on various morphological characteristics.

Results and Discussion

A total of 73 species of diatoms were collected from four of the eight districts in Tripura, representing 34 genera and 22 families under 11 orders of the class Bacillariophyceae of the kingdom Protista (Table 1). Among these, there are 11 species each from the families Bacillariaceae (*Bacillaria paradoxa, Denticula* cf. *kuetzingii, Nitzschia agnita, N. amphibia, N. gracilis, N. hantzschia, N. intermedia, N. palea, N.* cf. *pura, N. taylorii, Hantzschia amphioxys*) and Naviculaceae (*Navicula angusta, N. arenaria, N. cryptocephala, N. cryptotenella, N. erifuga, N. heimansioides, N.* cf. *leistikowii, N. notha, N.* cf. *radiosa, N. rostellata, N. schroeteri*). Additionally, there are 8 species from Gomphonemataceae (*Gomphonema affine, G. angustatum, G. exilissimum, G. lagenula, G. parvulum, G. pseudoaugur, G. pseudosphaerophorum, Gomphonema* sp), 7 from Fragilariaceae (*Diatoma vulgaris, Fragilaria crotonensis, Pseudostaurosira tenuis, Ulnaria acus, U. delicatissima, U. ulna, Ulnaria* sp), 5 from Pinnulariaceae (*Caloneis aequatorialis, Pinnularia acrosphaeria, P. amabilis, P. viridis* and *Pinnularia* sp), 4 species each from Achnanthidiaceae (*Achnanthidium minutissimum, A. saprophilum, Achnanthidium* sp, *Planothidium rostratum*) and Cymbellaceae (*Cymbella kolbei, C. parva, Oricymba japonica, Placoneis molestissima*) and 3 species from Sellaphoraceae (*Sellaphora alastos, S. bacillum, Sellaphora* sp). Moreover, 2 species each belong to the families Aulacoseiraceae (*Aulacoseira herzogii, A. nivaloides*), Catenulaceae (*Amphora copulate, A. pediculus*), Diadesmidaceae (*Luticola cf. kotschyi, L. mutica*), Pleurosigmataceae (*Gyrosigma eximium, Pleurosigma* sp), Stephanodiscaceae (*Cyclotella atomus, Cyclotella* sp) and Rhopalodiaceae (*Epithemia adnata, Rhopalodia musculus*). Lastly, there is 1 species each from the families Achnanthaceae (*Lemnicola hungarica*), Brachysiraceae (*Brachysira microcephala*), Eunotiaceae (*Eunotia minor*), Mastogloiaceae (*Mastogloia smithii*), Melosiraceae (*Melosira varians*), Neidiaceae (*Neidium productum*), Stauroneidaceae (*Stauroneis acuta*) and Surirellaceae (*Surirella capronioides*). Among the diatom species recorded, 36 species were found in the water bodies of West district, followed by 30 species in Sepahijala district, 16 species in

Unokati district and 14 species in Gomati district. Interestingly, *Amphora copulate* and *Gomphonema parvulum* are of common occurrence among all the districts. Twenty two species namely *Nitzschia amphibia, N. hantzschia, Navicula angusta, N. arenaria, N.* cf. *leistikowii, N. notha, N. schroeteri, Gomphonema* sp, *Achnanthidium minutissimum, Cymbella kolbei, C. parva, Oricymba japonica, Sellaphora alastos, S. bacillum, Aulacoseira herzogii, A. nivaloides, Amphora pediculus*, *Luticola* cf. *kotschyi, Rhopalodia musculus, Mastogloia smithii*, *Melosira varians* and *Denticula* cf. *kuetzingii* were exclusively found in West district; where as, seventeen species such as *Bacillaria paradoxa, Nitzschia gracilis, N.* cf. *pura, Navicula cryptotenella, N. rostellata, Gomphonema pseudosphaerophorum, Pinnularia acrosphaeria, P. amabilis, P. viridis, Pinnularia* sp, *Achnanthidium* sp, *Gyrosigma eximium, Pleurosigma* sp, *Epithemia adnata, Brachysira microcephala*, *Eunotia minor* and *Stauroneis acuta* were restricted only to Sepahijala district. Likewise, ten species namely *Nitzschia agnita, N. palea, Fragilaria crotonensis, Caloneis aequatorialis, Achnanthidium saprophilum, Planothidium rostratum*, *Placoneis molestissima, Luticola mutica*, *Neidium productum* and *Surirella capronioides* were found only in Unakoti district; and seven species namely *Nitzschia intermedia, Hantzschia amphioxys, Navicula erifuga, Ulnaria ulna, Sellaphora* sp, *Cyclotella* sp and *Lemnicola hungarica* were unique to the Gomati district. The findings reveal distinct inter-water body diatom distribution patterns, demonstrating unique site-specific assemblages that enable precise drowning location identification through hydrological fingerprinting.

Table 1: Distribution of diatom species in different water bodies under four districts of the state Tripura.

Sl. No.	Family and Species	West Tripura								Sepahijala Tripura			Gomati Tripura		Unakoti Tripura	
		S-1	S-2	S-3	S-4	S-5	S-6	S-7	S-8	S-9	S-10	S-11	S-12	S-13	S-14	S-15
1	**A. ACHNANTHACEAE** *Lemnicola hungarica* (Grunow) Round et Basson	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
2	**B. ACHNANTHIDIACEAE** *Achnanthidium minutissimum* (Kützing) Czarnecki	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
3	*Achnanthidium saprophilum* (Kobayasi & Mayama) Round & Bukhtiyarova	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
4	*Achnanthidium* sp	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
5	*Planothidium rostratum* (Østrup) Lange-Bertalot	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
6	**C. AULACOSEIRACEAE** *Aulacoseira herzogii*	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	*Aulacoseira nivaloides*	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	**D. BACILLARIACEAE** *Bacillaria paradoxa* Gmelin	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
9	*Denticula* cf. *kuetzingii* Grunow	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
10	*Nitzschia agnita* Hustedt	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-

Continue....

11	*Nitzschia amphibia* Grunow	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
12	*Nitzschia gracilis* Hantzsch	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
13	*Nitzschia hantzschia* Rabenhorst	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
14	*Nitzschia intermedia* Hantzsch	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
15	*Nitzschia palea* (Kützing) Smith	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
16	*Nitzschia* cf. *pura* Hustedt	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
17	*Nitzschia taylorii* Alakananda, Hamilton & Karthick	+	+	+	-	-	-	-	-	+	-	-	-	-	-	+
18	*Hantzschia amphioxys* (Ehrenberg) Grunow	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
19	**E. BRACHYSIRACEAE** *Brachysira microcephala* (Grunow) Compère	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
20	**F. CATENULACEAE** *Amphora copulate* (Kützing) Schoeman & Archibald	+	-	-	-	+	+	-	+	+	-	+	+	+	+	+
21	*Amphora pediculus* (Kützing) Grunow	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
22	**G. CYMBELLACEAE** *Cymbella kolbei* Hustedt	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
23	*Cymbella parva* (Smith) Wolle	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
24	*Oricymba japonica* (Reichelt) Jüttner, Cox, Krammer & Tuji	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
25	*Placoneis molestissima* Metzeltin, Lange-Bertalot & Garcia-Rodriguez	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
26	**H. DIADESMIDACEAE** *Luticola* cf. *kotschyi* Grunow	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
27	*Luticola mutica* (Kützing) D.G. Mann	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
28	**I. EUNOTIACEAE** *Eunotia minor* (Kützing) Grunow	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
29	**J. FRAGILARIACEAE** *Diatoma vulgaris* Bory	-	-	-	-	-	+	+	-	-	-	-	-	-	+	-
30	*Fragilaria crotonensis* Kitton	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

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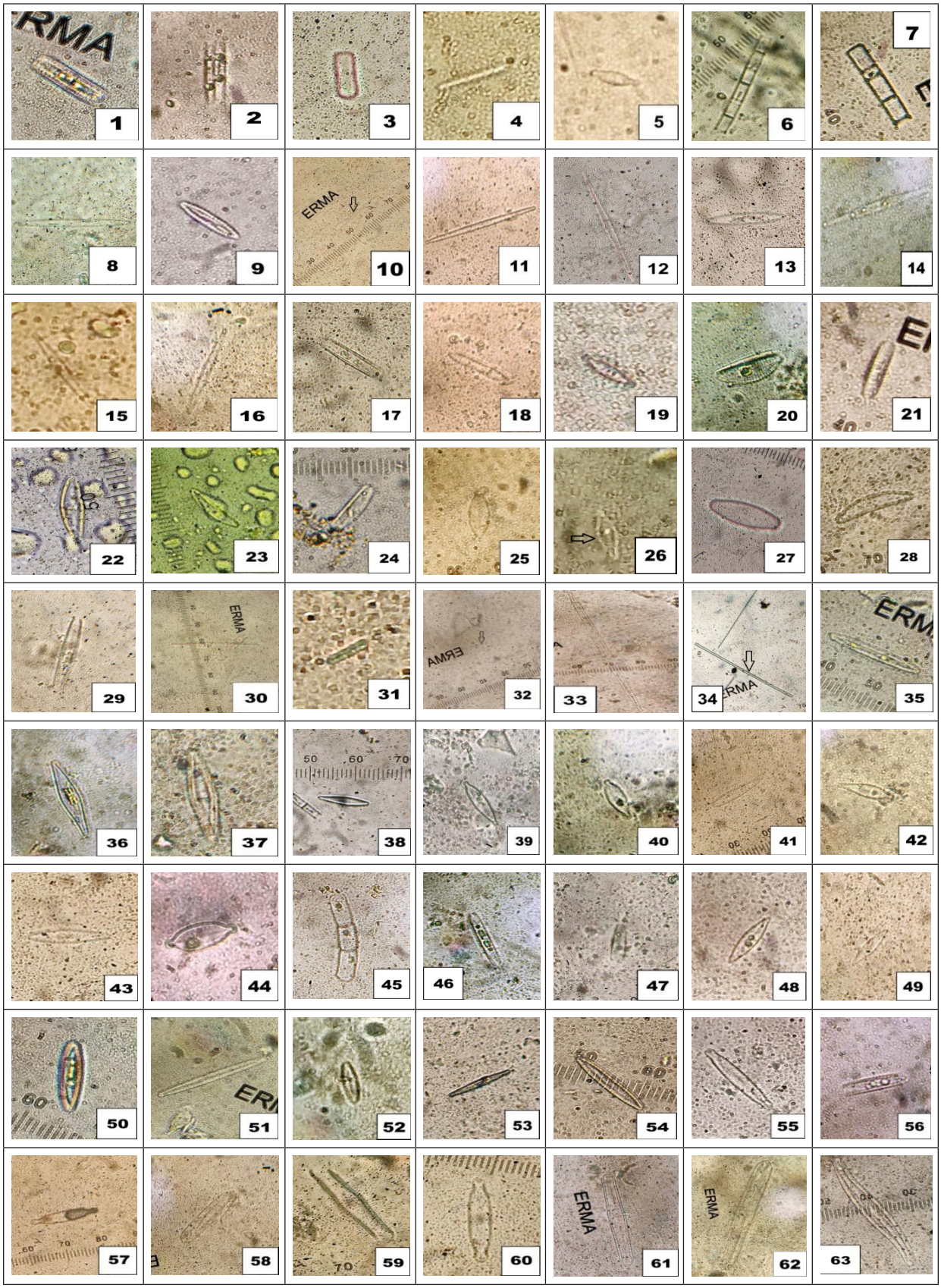
31	*Pseudostaurosira tenuis* Morales & Edlund	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
32	*Ulnaria acus* (Kützing) Aboal	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+
33	*Ulnaria delicatissima* (Smith) Aboal & Silva	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-
34	*Ulnaria* sp	+	+	-	+	-	-	+	-	-	+	+	+	+	-	-
35	*Ulnaria ulna* (Nitzsch) Compère	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
36	**K. GOMPHONEMATACEAE** *Gomphonema affine* Kützing	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-
37	*Gomphonema angustatum* (Kützing) Rabenhorst	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
38	*Gomphonema exilissimum* (Grunow) Lange-Bertalot & Reichardt	+	-	+	-	-	-	-	+	-	-	+	-	-	-	-
39	*Gomphonema lagenula* Kützing	-	-	-	+	+	-	-	-	-	-	-	-	+	-	-
40	*Gomphonema parvulum* (Kützing) Kützing sensu stricto	-	-	-	-	+	-	-	-	+	-	-	-	+	-	+
41	*Gomphonema pseudoaugur* Krammer	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+
42	*Gomphonema pseudosphaerophorum* Ehrenberg	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
43	*Gomphonema* sp	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
44	**L. MASTOGLOIACEAE** *Mastogloia smithii* Thwaites	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
45	**M. MELASIRACEAE** *Melosira varians* Agardh	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
46	**N. NAVICULACEAE** *Navicula angusta* Grunow	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
47	*Navicula arenaria* Lange-Bertalot	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
48	*Navicula cryptocephala* Kützing	-	+	-	-	-	-	-	+	-	-	+	-	-	-	-
49	*Navicula cryptotenella* Lange-Bertalot	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
50	*Navicula erifuga* (Müller) Bory	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
51	*Navicula heimansioides* Lange-Bertalot	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-
52	*Navicula* cf. *leistikowii* Lange-Bertalot	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-

Continue....

53	*Navicula notha* Wallace	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
54	*Navicula* cf. *radiosa* Kützing	-	+	-	+	-	-	+	+	-	-	-	+	-	-	-
55	*Navicula rostellata* Kützing	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
56	*Navicula schroeteri* Meister	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
57	**O. NEIDIACEAE** *Neidium productum* (Smith) Hustedt	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
58	**P. PINNULARIACEAE** *Caloneis aequatorialis* Hustedt	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
59	*Pinnularia acrosphaeria* Smith	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-
60	*Pinnularia amabilis* Krammer	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
61	*Pinnularia* sp	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
62	*Pinnularia viridis* (Nitzsch) Ehrenberg	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
63	**Q. PLEUROSIGMATACEAE** *Gyrosigma eximium* (Thwaites) Boyer	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
64	*Pleurosigma* sp	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
65	**R. RHOPALODIACEAE** *Rhopalodia musculus* (Kützing) Müller	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
66	*Epithemia adnata* (Kützing) Brébisson	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
67	**S. SELLAPHORACEAE** *Sellaphora alastos* (Hohn & Hellerman) Lange-Bertalot & Metzeltin	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
68	*Sellaphora bacillum* var. *jogensis* Gandhi	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
69	*Sellaphora* sp	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
70	**T. STAURONEIDACEAE** *Stauroneis acuta* Smith	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
71	**U. STEPHANODISCACEAE** *Cyclotella atomus* Hustedt	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-
72	*Cyclotella* sp	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
73	**V. SURIRELLACEAE** *Surirella capronioides* Gandhi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

'+' = present; '-' = absent.

Distribution and abundance of diatoms are influenced by a number of physical and biological factors. In different water bodies in the present study, diatoms experienced water temperature ranging from 21.5-36.0°C and a pH from 5.0-7.0. Das *et al* made some studies on algae of Tripura where they found 21 species of diatoms[36]. Of those, only one species, *i.e., Nitzschia intermedia* Hantzsch, is common to the 73 species found in the present study (Figure 2) thus contributing 72 more species to the previous record. Thus a total of 93 species have been identified in the state to date.


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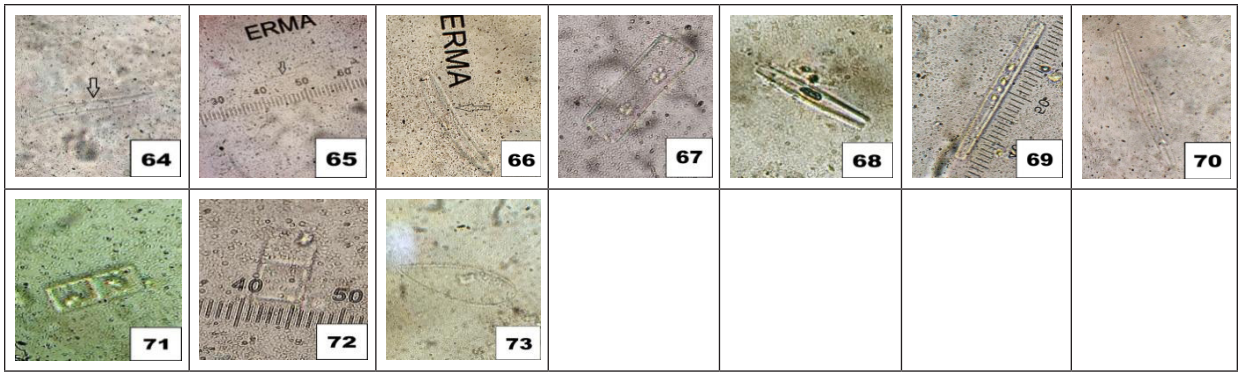


Figure 2: Diverse diatom species identified in various water bodies across four districts of the state Tripura: (1) ***Lemnicola hungarica*** **(Grunow) Round et Basson, (2)** ***Achnanthidium minutissimum*** **(Kutzing) Czarnecki, (3)** ***Achnanthidium saprophilum*** **(Kobayasi & Mayama) Round & Bukhtiyarova, (4)** ***Achnanthidium*** **sp, (5)** ***Planothidium rostratum*** **(Østrup) Lange-Bertalot, (6)** ***Aulacoseira herzogii*****, (7)** ***Aulacoseira nivaloides*****, (8)** ***Bacillaria paradoxa*** **Gmelin, (9)** ***Denticula cf. kuetzingii*** **Grunow, (10)** ***Nitzschia agnita*** **Hustedt, (11)** ***Nitzschia amphibia*** **Grunow, (12)** ***Nitzschia gracilis*** **Hantzsch, (13)** ***Nitzschia hantzschia*** **Rabenhorst, (14)** ***Nitzschia intermedia*** **Hantzsch, (15)** ***Nitzschia palea*** **(Kutzing) Smith, (16)** ***Nitzschia cf. pura*** **Hustedt, (17)** ***Nitzschia taylorii*** **Alakananda, Hamilton & Karthick, (18)** ***Hantzschia amphioxys*** **(Ehrenberg) Grunow, (19)** ***Brachysira microcephala*** **(Grunow) Compère, (20)** ***Amphora copulate*** **(Kützing) Schoeman & Archibald, (21)** ***Amphora pediculus*** **(Kützing) Grunow, (22)** ***Cymbella kolbei*** **Hustedt, (23)** ***Cymbella parva*** **(Smith) Wolle, (24)** ***Oricymba japonica*** **(Reichelt) Jüttner, Cox, Krammer & Tuji, (25)** ***Placoneis molestissima*** **Metzeltin, Lange-Bertalot & Garcia-Rodriguez, (26)** ***Luticola*** **cf.** ***kotschyi*** **Grunow, (27)** ***Luticola mutica*** **(Kützing) D.G. Mann, (28)** ***Eunotia minor*** **(Kützing) Grunow, (29)** ***Diatoma vulgaris*** **Bory, (30)** ***Fragilaria crotonensis*** **Kitton, (31)** ***Pseudostaurosira tenuis*** **Morales & Edlund, (32)** ***Ulnaria acus*** **(Kützing) Aboal, (33)** ***Ulnaria delicatissima*** **(Smith) Aboal & Silva, (34)** ***Ulnaria*** **sp, (35)** ***Ulnaria ulna*** **(Nitzsch) Compère, (36)** ***Gomphonema affine*** **Kützing, (37)** ***Gomphonema angustatum*** **(Kützing) Rabenhorst, (38)** ***Gomphonema exilissimum*** **(Grunow) Lange-Bertalot & Reichardt, (39)** ***Gomphonema lagenula*** **Kützing(40)** ***Gomphonema parvulum*** **(Kützing) Kützing sensu strict, (41)** ***Gomphonema pseudoaugur*** **Krammer, (42)** ***Gomphonema pseudosphaerophorum*** **Ehrenberg, (43)** ***Gomphonema*** **sp, (44)** ***Mastogloia smithii*** **Thwaites, (45)** ***Melosira varians*** **Agardh, (46)** ***Navicula angusta*** **Grunow, (47)** ***Navicula arenaria*** **Lange-Bertalot, (48)** ***Navicula cryptocephala*** **Kützing, (49)** ***Navicula cryptotenella*** **Lange-Bertalot, (50)** ***Navicula erifuga*** **(Müller) Bory, (51)** ***Navicula heimansioides*** **Lange-Bertalot, (52)** ***Navicula*** **cf.** ***leistikowii*** **Lange-Bertalot, (53)** ***Navicula notha*** **Wallace, (54)** ***Navicula*** **cf.** ***radiosa*** **Kützing, (55)** ***Navicula rostellata*** **Kützing, (56)** ***Navicula schroeteri*** **Meister, (57)** ***Neidium productum*** **(Smith) Hustedt, (58)** ***Caloneis aequatorialis*** **Hustedt, (59)** ***Pinnularia acrosphaeria*** **Smith, (60)** ***Pinnularia amabilis*** **Krammer, (61)** ***Pinnularia*** **sp, (62)** ***Pinnularia viridis*** **(Nitzsch) Ehrenberg, (63)** ***Gyrosigma eximium*** **(Thwaites) Boyer, (64)** ***Pleurosigma*** **sp, (65)** ***Rhopalodia musculus*** **(Kützing) Müller, (66)** ***Epithemia adnata*** **(Kützing) Brébisson, (67)** ***Sellaphora alastos*** **(Hohn & Hellerman) Lange-Bertalot & Metzeltin, (68)** ***Sellaphora bacillum*** **var.** ***jogensis*** **Gandhi, (69)** ***Sellaphora*** **sp, (70)** ***Stauroneis acuta*** **Smith, (71)** ***Cyclotella atomus*** **Hustedt, (72)** ***Cyclotella*** **sp, and (73)** ***Surirella capronioides*** **Gandhi.**

Diatomological Maps provide valuable information on the presence and distribution of common, seasonal, rare and site-specific diatom species. By presenting qualitative and quantitative data across different seasons for specific water bodies, D-Maps greatly assist forensic scientists in identifying drowning sites making their creation crucial for targeted water body investigations.

Conclusion

A comprehensive study of diatom diversity across four districts of Tripura revealed 73 species, expanding the state's known diatom record from 21 to 93 species with an addition of 72 new ones. About 70% of the species exhibited site-specific distribution with *Amphora copulata* and *Gomphonema parvulum* found ubiquitously across all districts, while 56 species showed district-wise confinement making them effective hydrological markers. The significant diatom diversity across habitats highlight Tripura's understudied aquatic ecosystems as reservoirs of microbial diversity and provides a framework for forensic limnology applications. The study established a foundational diatomological database for forensic use in drowning investigations providing reference data for linking diatom assemblages in body tissues to potential drowning sites. Future objectives include expanding sampling to all districts, continuous seasonal monitoring and developing a state-wide diatomological map to enhance medico-legal investigations and align with global forensic standards.

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Cross-sectional Study of Unnatural Deaths in BTGH KALABURGI.

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Abstract

This study was conducted in department of forensic medicine and toxicology,Basaweshwar teaching and general hospital attached to M R Medical College Kalaburgi to determine the incidence,pattern, modes and manner of unnatural deaths. Total number of unnatural deaths studied were 230. There were 144(62.6%)cases of unnatural deaths in case of males and 86(37.39%) in females.Maximum no. of unnatural deaths was seen in the age group of 21-30yrs(38.2%), followed by 31-40yrs(18.69%).Majority of the cases were accidental 123(53.4%) cases followed by suicidal 79(34.34%)cases and homicidal 28(12.17%) cases. There were 109(47.39%)cases of road traffic accidents, 54(23.47%) cases of burns &47(20.43%) cases of poisoning. Unnatural deaths in females were mainly suicidal, where as in case of males deaths were accidental. Majority of unnatural death victims were from rural area (57.82%).

Keywords: unnatural deaths, Road traffic accidents, burns, poisoning.

Introduction

As the incidence of unnatural deaths have increased, but the modes and manner of deaths have changed. Unnatural deaths are those deaths which are not due to disease conditions or aging process, but due to accidents, suicides & homicides[1]. It has been observed among all unnatural deaths, accidents top the list followed by suicides and homicide. The increased number of fast-moving vehicles, unskilled and drunken drivers, contribute to increase in number of road traffic accidents[2]. Even though accidents top the list it is suicide and homicides, which pose more challenges to the investigations.The important objectives of present study were to determine the epidemiology of unnatural deaths. This study attempts to know the incidence, causes, patterns & to suggest the legal & preventive measures of unnatural deaths.

Materials and Methods

Study of medico-legal autopsies for 5 years from 2018 to 2022conducted by Department of Forensic Medicine, M.R. Medical College. Total number of cases studied were 230. Detailed information and data pertaining to cases were collected from following sources (1). Inquest Report (2). BriefHistory (3).Postmortem Report.After receiving all the details, the PME was conducted. Case-sheet

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summary was obtained to know the details. All the external injuries were noted from head to toe. All the three cavities – cranial, thoracic and abdomen were opened to study the injuries to the organs. Findings tabulated in Proforma.

Ethics Committee Approval: Approval was taken from institutional Ethics committee with reference no: HKES/MRMCK/IEC 190303 (RPA201847).

Observation and Results: Total number of cases of unnatural deaths studied was 230.

Table No. 1. Yearwise distribution of cases of unnatural deaths.

Year	Total no. of victims	Male	Female
2018	56	35	21
2019	57	37	20
2020	51	33	18
2021	28	19	09
2022	38	20	18
Total	230	144(62.6%)	86(37.39%)

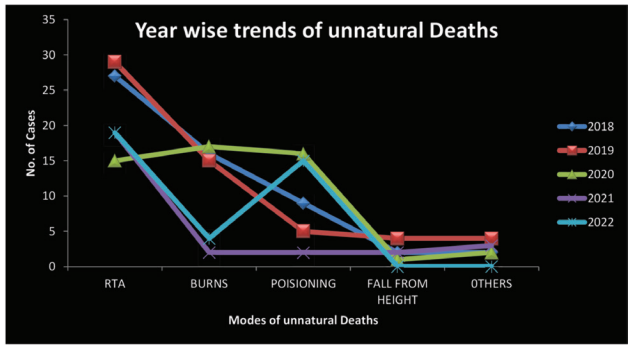


Figure No. 1

Table-2. Age&sex- wise Distribution of unnatural deaths.

Age group	Males	Females	Total
0-10	05	6	11(4.7%)
10-20	08	16	24(10.4%)
21-30	57	31	88(38.2%)
31-40	31	12	43(18.69%)
41-50	28	5	33(14.3%)
51-60	09	7	16(6.9%)
>60	06	7	13(5.6%)
Total	144	86	230

Age-wise analysis of victims of victims of unnatural deaths showed a maximum number of deaths in age group of 21-30 years(38.2%), 31-40 (18.69%) and 41-50 (14.3%) with minimum deaths in age group of 0-10 and >60 years.

Table 3. Manner of unnatural deaths.

Manner	No. of victims	Percentage
Suicide	79	34.34%
Homicide	28	12.17%
Accidental	123	53.4%
Total	230	

In the distribution of manner of unnatural deaths, it was observed that maximum number of victims died due to accidents followed by suicides and homicide

Table 4. Modes of unnatural deaths.

Modes of unnaturaldeaths	Total no.	Percentage
RoadTraffic accidents	109	47.39%
Burns	54	23.47%
Poisoning.	47	20.43%
Fall From Height	9	3.91%
Others (snake bite, railway, assault (etc)	11	4.78%
Total	230	

There were maximum no. of deaths due to RTA109 (47.39%), mainly in males. Other causes of unnatural deaths was due to burns(23.43%), poisoning, fall from height and snake bite etc.

Table 5. Distribution of unnatural deaths according to domicile.

Domicile	Total (percent)
Urban	97(42.17%)
Rural	133(57.82%)

Discussion

Depending upon sex, there was a male preponderance with males constituting 144 (62.6%) cases and females 86 (37.39%) cases. Our findings were similar to the study conducted in Dhaka

Medical College, Dhaka, Bangladesh, where 73.32% were males and 26.68% of the cases were females[2]. This finding is similar to the findings of Kumar Awdhesh- male (68.6%) female (31.4%)[3].Anjanamma T C et al.found male (64.4%) and female (35.6%)[4]. This finding is due to males being more exposed to the environment and having a more physically active lifestyle than females. Males accept more difficult tasks and may be involved in violent acts.

In our study, unnatural deaths due to accidents were 53.4%, suicide 34.34% and homicide 12.17%. Ourfindings are similar to the study conductedat PGIMER, Chandigarh, where 79.3% of deathswere due to accidents, 13.9% due to suicide and6% of deaths were due to homicide [5].

There were a maximum number of deaths due to RTA 109 (47.39%), mainly in males. Other causes of unnatural deaths were due to burns (23.43%), poisoning, fall from height, and snake bite, etc. This finding is quite similar to the studies of Yousufani G. M., Memon Muhammad[6], Rathod S. N., Bharatwaj R. S.[7], and Kumar Awdhesh[8]. Apart from this, congested and overcrowded roads and the increase in the number of vehicles have resulted in more accidents even within the city.

This study reveals that the leading cause of unnatural deaths is from rural areas, accounting for 57.82% of cases. Rural India, home to 65% of the population, often suffers from limited access to healthcare, insufficient mental health services, and economic challenges, all of which are known risk factors for unnatural deaths.[9] This finding is consistent with the studies of Verma A. that highlight the high rate of suicide in rural India.[10]

When we see the trend of unnatural deaths over time and compare the findings with earlier studies that reported higher proportions of burns and poisoning, in earlier Indian studies, burns and poisoning constituted a larger share of unnatural deaths, particularly among women, due to kerosene stove explosions, dowry-related violence, and pesticide ingestion. For instance, Sharma et al. (2004)[11] reported burns at 36% and poisoning at 27% of unnatural deaths, surpassing RTAs. Similarly, Santosh et al. (2013)[12] found suicidal burns as the predominant mode in rural Karnataka.

However, the present study from Kalaburagi demonstrates a clear epidemiological shift, with RTAs contributing nearly half (47.4%) of unnatural deaths, surpassing burns (23.5%) and poisoning (20.4%). Year-wise trend analysis (2018–2022) reinforces this observation, showing accidents consistently outnumbering other causes (Fig 1). This reflects increased motorization, congested roads, poor enforcement of traffic rules, and risk-taking driving behaviors. But in the year 2020 there was reduction of RTA cases due to lockdown.

The decline in burn-related fatalities can be attributed to improved access to LPG and decreased reliance on kerosene, reducing domestic stove explosions. Similarly, greater awareness and regulation of pesticides may explain the slight decline in poisoning deaths.

The numbers of deaths due to poisoning and burns are influenced by a number of factors like the geographic area of study and the cultural and traditional background of the people.

Conclusion & Suggestions

Among all deaths, the cases of unnatural deaths are increasing. Maximum number of unnatural deaths was seen in the age group of 21–30 years, followed by 31–40 years. Majority of the cases were accidental cases followed by suicidal and homicidal cases. Majority of the cases were of road traffic accidents, followed by burns and poisoning. Unnatural deaths in females were mainly suicidal, whereas in case of males, deaths were accidental. Majority of unnatural death victims were from rural areas. However, in an attempt to at least try to decrease its toll, the following suggestions are made:

- Strict implementation of traffic rules, with special attention to be paid to drunken drivers, unskilled or semi-skilled drivers, use of mobile phones and smoking while driving, use of high beam lights and music systems at high volume while driving within the city limits, reckless and rash driving, etc.

- Encroachment of roads by shopkeepers and hawkers should be dealt with strictly. Parking at unauthorized places should be penalized heavily.

This study reinforces the need for targeted interventions to reduce unnatural deaths, including the implementation of mental health awareness campaigns, improved access to healthcare, and enhanced road and workplace safety programs.

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Applicability of Suchey–Brooks Method of Age Estimation from Pubic Symphysis in a North Karnataka Population: A Prospective Study

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Abstract

Background: Age estimation is a critical component of forensic identification. The Suchey–Brooks method, widely used for age estimation from the pubic symphyseal surface, is based on a population sample from North America. Its applicability to the Indian population, particularly in North Karnataka, requires validation.

Objective: To evaluate the applicability and reliability of the Suchey–Brooks method in age estimation from the pubic symphysis in individuals from the Belagavi region.

Methods: This prospective study was conducted on 175 cadavers brought for medicolegal autopsy at the Department of Forensic Medicine, Belgaum Institute of Medical Sciences, Belagavi, from 01/01/2018 to 31/12/2018. Pubic symphyses were harvested from cadavers meeting defined inclusion and exclusion criteria. Age was estimated using the Suchey–Brooks phase method and compared with the known chronological age of the deceased. Morphological parameters such as relief of the symphyseal surface, delimitation, presence of a symphyseal rim, and pubic tubercle development were assessed.

Results: All six phases of the Suchey–Brooks method were represented in the study sample. A good correlation was observed between the estimated and actual chronological ages in both males and females, particularly in phases 1 to 4. Accuracy diminished in phases 5 and 6, where significant overlap in age ranges was noted. The method was most reliable in individuals up to 30 years of age. Sex-specific application showed comparable performance across both sexes, with minor variability in morphological features.

Conclusion: The Suchey–Brooks method is applicable and reliable for age estimation in the North Karnataka population, particularly in individuals younger than 30 years. While it can be used in both sexes, caution should be exercised when applying it to older age groups (phases 5 and 6), where morphological changes show less correlation with chronological age.

Key Words : Age estimation, symphysis pubis, Identification

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Introduction

"Bones never lie; they carry the silent truth of identity and age." Among skeletal structures, the pubic symphysis stands out as one of the most reliable indicators of age-at-death due to its predictable age-related morphological changes. In forensic anthropology, age estimation is a cornerstone for constructing the biological profile of unidentified human remains. The pubic symphysis has been historically recognized as a key anatomical site due to its predictable age-related morphological changes. The Suchey–Brooks method, developed from North American autopsy samples, has become a cornerstone in age estimation worldwide. Hence, the applicability of this method in regional populations, including those in India, requires critical assessment.[1]

However, regional variations in bone development demand population-specific validation. In the Indian context, especially in under-researched regions like North Karnataka, such evaluation is essential. Accurate age estimation forms the backbone of biological identification—an indispensable tool in the field of forensic anthropology for solving medicolegal puzzles.

This study aims to evaluate the reliability and applicability of the Suchey–Brooks method in a sample of the North Karnataka population by comparing morphological age estimates with known chronological ages of deceased individuals brought for autopsy.

Materials and Methods

AProspective observational study was conducted at the, Department of Forensic Medicine, Belgaum Institute of Medical Sciences, Belagavi from January 1, 2018 to December 31, 2018 A total of 175 cases (males and females) was included in the study. The Inclusion Criteria consisted of cadavers aged 18 years and above with documented date of birth or valid age proof. Subjects who had skeletal trauma involving the pelvic region, cases with congenital pelvic anomalies, extensive decomposition of the symphyseal area or pathology affecting the pubic symphysis were excluded from the study.

Methodology

Bilateral pubic bones were dissected during autopsy and symphyseal surfaces were cleaned and preserved.Each pubic symphysis was independently assessed and classified into one of the six phases described in the Suchey–Brooks method based on morphological characteristics: surface texture, ventral rampart, dorsal plateau, ossific nodules, symphyseal rim, and pubic tubercle.Estimated phase-specific age ranges were compared with known chronological ages.Data were stratified by age group and sex. Statistical analysis was conducted to evaluate the accuracy and reliability of age estimation.

Results

The study sample was constituted by 175 cases brought for autopsy to Department of Forensic Medicine, Belgaum Institute Of Medical Sciences, Belagavi. The study sample consisted of 107 males and 68 females accounting for 62 % and 38%, respectively. The same is depicted in Figure 1.

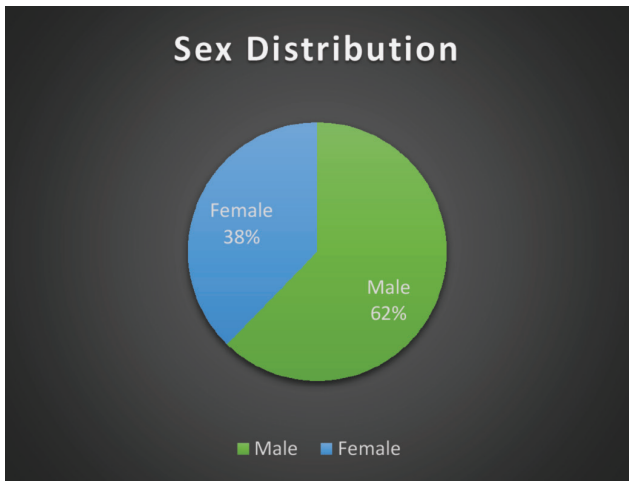


Figure 1: Distribution of gender Among Study Population

The study results showed the maximum number of samples were of Phase 3 in males (27) and in females (15) the maximum samples belonged to Phase 2. The

same is depicted in **Table 1**. After applying Suchey-Brooks method to our study samples, the mean age of males and females were noted as described in Table 2. The standard deviation was highest for phase 3 (5.80) and lowest for phase 1(1.41) for males and highest for phase 4 (7.48) and lowest for phase 1(1.71) for females. **The same has been depicted in Table 2 and Table 3.**

Table 1. Phasewise Distribution Of Study Samples

PHASE	MALE	FEMALE	TOTAL
Phase 1	15	13	28
Phase 2	24	15	39
Phase 3	27	10	37
Phase 4	19	13	32
Phase 5	10	10	20
Phase 6	12	7	19
Total	**107**	**68**	**175**

Table 2. Descriptive Statistics of Various Phases in Present Studyamong Males

PHASE	N	MEAN	S.D.	RANGE
1	15	19.00	1.41	17 – 21
2	24	23.46	3.15	19 – 32
3	27	29.70	5.80	22 – 42
4	19	37.53	4.03	34 – 46
5	10	45.70	4.52	38 – 50
6	12	45.83	4.76	37 – 50

Table 3. Descriptive Statistics of Various Phases Inpresent Study Amongfemale

PHASE	N	MEAN	S.D.	RANGE
1	13	19.54	1.71	17 - 22
2	15	25.60	3.56	19 - 32
3	10	30.30	4.67	25 - 39
4	13	38.00	7.48	26 - 48
5	10	40.00	5.23	34 - 48
6	7	43.14	4.71	36 - 48

The mean values of our current study were compared with Suchey-Brooks, all phases except Phase 6 had high which had high deviation in males and all phases except Phase 5 and Phase 6 had high which had high deviation in females. The mean values of Phase 1, Phase 2, Phase 3 and Phase 4 were consistent with the mean values obtained by Suchey Brooks method for male and females respectively. The same has been depicted in Table 4, Table 5, Figure 2 and Figure 2.

Table 4. Comparison of Mean Between Present Study and Suchey -Brooks Method Among Males

PHASE	PRESENT STUDY	SUCHEY-BROOKS
1	19.00	18.5
2	23.46	23.4
3	29.70	28.7
4	37.53	35.2
5	45.70	45.6
6	45.83	61.2

Table 5. Comparison of Mean Between Present Study and Suchey - Brooks Method Among Female

PHASE	PRESENT STUDY	SUCHEY-BROOKS
1	19.54	19.40
2	25.60	25.00
3	30.30	30.70
4	38.00	38.20
5	40.00	48.10
6	43.14	60.00

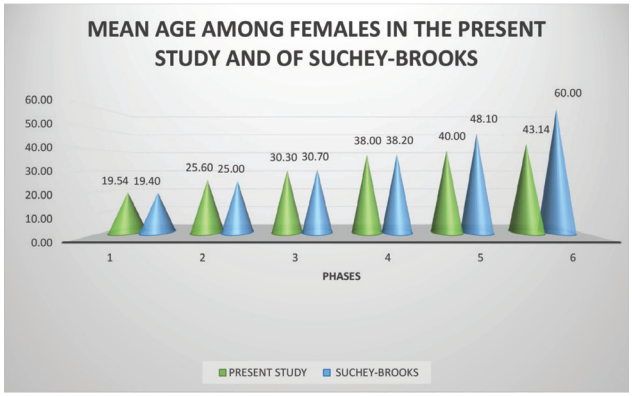


Figure 2

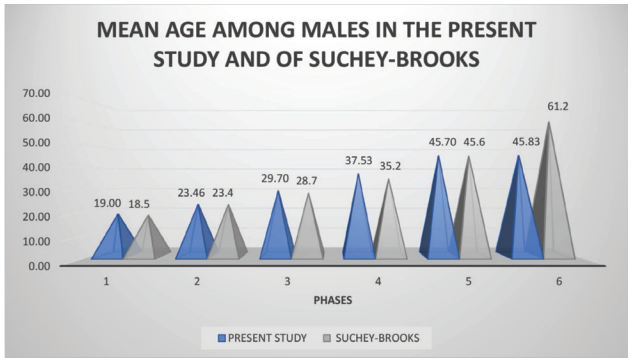


Figure 3

The age ranges obtained our current study were compared with Suchey-Brooks, all phases except Phase 2 and Phase 4did not align with our study population in males and all phases except Phase 2 had high deviation in females. The age ranges of Phase 1, Phase 3, Phase 4,Phase5, and Phase 6for our study population did not align Suchey Brooks method for male and females respectively. The same has been depicted in Table 6 and Table 7.

Table 6. Comparison of Age Ranges Between Present Study And Suchey – Brooks Method Among Female

PHASE	PRESENT STUDY	SUCHEY-BROOKS
1	17 - 22	15 -24
2	19 - 32	19 – 40
3	25 - 39	21 – 53
4	26 - 48	26 – 70
5	34 - 48	25 – 83
6	36 - 48	42 – 87

Table 7. Comparison of Age Ranges Between Present Study And Suchey-Brooks Method Among Males

PHASE	PRESENT STUDY	SUCHEY-BROOKS
1	17 - 21	15- 23
2	19 - 32	19 – 34
3	22 - 42	21 – 46
4	34 - 46	23 – 57
5	38 - 50	27 – 66
6	37 - 50	34 – 86

Table 8. Summary of Comparison

Study	Location	Sample	Key Findings	Comparison with Present Study
Brooks & Suchey (1990)	USA	1012 (both sexes)	Phases 5–6 unreliable	Matches: caution for phases 5–6
Kanchan et al. (2011)	Mangalore, India	103 males	Accurate till 40 yrs	Matches: Reliable till ~30 yrs
Kim et al. (2007)	Korea	186 cadavers	Overestimation in youth, underestimation later	Matches partially: late phases less reliable
Saxena & Jeyaseelan (2007)	Chennai, India	Unknown	Less accurate >40 yrs	Matches: Inaccuracy in older phases
Rissech et al. (2006)	Spain	Spanish autopsy sample	Variable changes across phases	Matches: Environmental/genetic impact seen
Sahni et al. (2012)	Punjab, India	120 males	Earlier phase transitions	Matches: Earlier degenerative changes observed

To summarize the results of our study, All Suchey–Brooks phases were represented in our study.Strong correlation was found between morphological phase and chronological age in early phases (1 to 4).In phases 5 and 6, significant overlap of age ranges was observed.Estimated age matched the actual age within ±5 years in 78.6% of cases aged ≤30 years whileamong individuals >30 years, this concordance dropped to 61.2%.The method showed equal applicability in both sexes, though females demonstrated slightly earlier onset of degenerative changes.Morphological features such as the development of the symphyseal rim and relief of the articular surface were consistent and reliable indicators in younger age groups.

Discussion

The findings of this study reinforce the utility of the Suchey–Brooks method for forensic age estimation in Indian populations, specifically in the North Karnataka demographic. The early phases of pubic symphyseal metamorphosis displayed predictable patterns that were reliably matched to the actual age. However, as with other skeletal aging methods, degenerative changes in older individuals (phases 5 and 6) introduced variability due to individual differences in lifestyle, occupation, and health status.

Previous studies conducted in Indian and Asian populations have reported similar results, emphasizing the method's reliability in younger adults but cautioning against sole reliance on it in older individuals. The present study supports these conclusions and highlights the necessity for multi-method approaches in age estimation, especially in forensic cases involving elderly skeletal remains.

The present study conducted at the Belgaum Institute of Medical Sciences demonstrates that the Suchey–Brooks method is largely applicable to the North Karnataka population, especially among individuals aged up to 30 years, and across both sexes. However, it also highlights the decreasing reliability of this method in older age groups (phases 5 and 6). These findings are consistent with several studies conducted in both Indian and international populations. Brooks & Suchey (1990, USA)[1,2] the pioneers of this method in their seminal study on a North American autopsy population (739 males and 273 females), Brooks and Suchey established the six-phase system with statistical age ranges and standard deviations. They found strong correlation between phase and chronological age, particularly in younger individuals, with a wide age range and overlap in phases 5 and 6.Our study aligns with their finding that phases 5 and 6 are less reliable, indicating that age estimation beyond 30 years needs cautious interpretation.[2] Another study by Kim et al. (2007, South Korea)Kim and colleagues tested the method on 186 Korean cadavers and concluded that while the method could be used, it tended to overestimate age in younger phases and underestimate in older phases.[3] When the results were compared our study did not show overestimation in younger age groups but did show underestimation and broad overlap in older phases, consistent with their findings.[3]

In a study by Kanchan et al. (2011, Mangalore, India) on 103 male cadavers from South India, Kanchan et al. found that the Suchey–Brooks method was reasonably accurate up to 40 years. They emphasized the variability in older age groups and the need for region-specific standards.Their findings closely mirror ours—reliable in young adults, but variable in older males, necessitating caution.[4]In a study by Saxena & Jeyaseelan (2007, Chennai, India) in a southern Indian population, the study revealed that the Suchey–Brooks method was less accurate beyond 40 years of age and recommended the development of Indian-specific standards. Our study supports this by suggesting cautious use in phases 5 and 6, while still finding acceptable accuracy in individuals ≤30 years.[5]

An international study by Rissech et al. (2006, Spain)in this population-specific analysis of the Suchey–Brooks method, Rissech et al. found that morphological changes varied in timing, and some features were less pronounced in the Spanish population, resulting in age estimation errors. This is similar to our findings, this study underscores the impact of genetic and environmental factors on phase transitions, particularly in later stages.[6] Another study by Sahni et al. (2012, Punjab, India) evaluated 120 pubic symphyses from male cadavers, the authors found that although the Suchey–Brooks method was applicable, the mean age of phase transition was often lower in the Indian sample

compared to the original American standards. Our study also observed earlier onset of degenerative changes, especially among females, reinforcing the need to adjust age ranges when applying the method in the Indian context.[7] The discussion of our study is summarized below in Table 8.

Across all six comparative studies considered for discussion, a consistent pattern emerges that the Suchey–Brooks method remains a valuable tool for age estimation, especially in young to middle-aged adults. However, most studies—domestic and international—underline the reduced accuracy in later phases due to greater inter-individual variability and overlapping age ranges.[8] Our findings from North Karnataka align well with these observations, reinforcing both the utility and limitations of the method. The study strengthens the case for region-specific validation and the integration of multiple skeletal markers for enhanced age estimation accuracy in forensic anthropology.

Conclusion

The Suchey–Brooks method employed in our study is applicable to the population of North Karnataka and provides reliable age estimation up to 30 years of age. It can be employed for both male and female individuals. However, caution must be exercised in applying this method to older individuals (phases 5 and 6), where morphological features are less distinct and show greater inter-individual variability. For improved accuracy, the method should be supplemented with additional skeletal indicators in higher age groups.

Limitations of the study: This study was executed as a single-center prospective analysis in North Karnataka, and the results may not be entirely applicable to other regions of India with distinct genetic, nutritional, and lifestyle contexts. The sample size, while sufficient for initial assessment, may not accurately reflect the complete demographic diversity of Karnataka or South India. Differences in occupational patterns, exercise, and bone health may have affected the shape of the pubic symphysis. Furthermore, interobserver variability and possible subjectivity in phase assessment, intrinsic to morphologic methods, may have influenced the accuracy of age estimation.

Future research implications: Future research should focus on multi-center studies with larger, demographically varied samples to authenticate and, if required, recalibrate the Suchey–Brooks standards for the Indian population. Utilizing advanced imaging techniques and 3D morphometric analyses could augment objectivity and enhance the applicability of age estimation methods in forensic anthropology and medico-legal practice.

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Identification of an Unknown Poison in Pharmaceutical Formulations – A Forensic Study of Culpable Homicide Case

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Abstract

Homicide, the intentional killing of one person by another occur for a variety of reasons, might be because of personal conflicts, domestic violence, revenge, financial gain, gang-related disputes. Most commonly encountered ways are using one's body parts as weapon, firearms in gun-prevalent societies, sharp objects like knives or machetes, blunt force trauma using bats or hammers, strangulation, poisoning, drowning, vehicular homicide, arson, and even explosives in cases of targeted killings or terrorism. Mixing of poison in food or water is an age old technique followed from ancient times to kill enemies for acquiring kingdom or personal gains. In recent times, mixing of poison in medicines is also practiced to commit homicide. A case was registered in Srikakulam District of Andhra Pradesh State, where the perpetrator followed the same technique by mixing unknown poison in pharmaceutical formulations (syrup and eye drops) in disguise of free distribution of covid medicines. A high performance thin layer chromatography (HPTLC) and UV-Visible spectrophotometer (UV-Vis) was used for the separation and identification of poison present in the pharmaceutical formulations seized in the crime scene. It was confirmed that Paraquat a herbicide poison was found in the seized material objects. The described HPTLC method can be used routinely in the forensic science laboratories for identification of Paraquat not only in pharmaceutical formulations but also in the viscera samples.

Key words: Homicide, Pharmaceutical formulations, Spot Tests, UV-Visible spectrophotometer High performance thin layer chromatography and Paraquat.

Introduction

Homicide, the intentional killing of one person by another, is among the oldest documented crimes in human history and based on intent mainly classified into criminal homicide, justifiable homicide and excusable homicide[1]. The reasons and societal reactions have behind the act has been changed over time, shaped by cultural, religious, and legal influences. Homicides occur for a variety of reasons, might be because of personal conflicts, domestic violence, revenge, financial gain, gang-related

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disputes. Even the political or ideological intension, such as assassinations or acts of terrorism comes under this category.

Criminal homicide comprises of the murder an intentional killing, manslaughter an unintentional killing or killing without having any plan and felony murder an unintended killing while committing robbery or arson. Criminal homicides are illegal, not accepted and punishable by law. Justifiable homicides are legally justified and occur under the circumstances of self-defence or in the defence of others. Excusable homicide is not criminal but, caused by negligence or lack of intension includes unavoidable accidents.

Homicide rates differ widely across the world due to factors like socio-economic conditions, effectiveness of law enforcement and availability of crime objects. Areas suffering with poverty, organized crime, and weak judicial systems often face higher homicide rates. The investigative methods and careful examination of causes behind homicide, plays a crucial role in arresting the crime rate and ensuring justice.

There are many ways in homicidal deaths depending on the situations, motives and methods. But commonly encountered ways are using one's body parts as weapon, firearms in gun-prevalent societies, sharp objects like knives or machetes, blunt force trauma using bats or hammers, strangulation, poisoning, drowning, vehicular homicide, arson, and even explosives in cases of targeted killings or terrorism and also mixing of poison in food or water[2]. Asit Kumar Sikary reviewed homicide poisoning in India and stated that homicidal poisoning prevalence varies from 0.3% to 3.7% from different parts of India. Organophosphates, paraquat, phosphide and arsenic are the commonly used poisons to commit homicide[3]. The objective of the present study is to find out the presence of paraquat in pharmaceutical samples of a homicide case.

Paraquat (N, N′-dimethyl-4,4′-bipyridinium dichloride), also known as methyl viologen, is a bipyridilium herbicide with a molecular formula($C_{12}H_{14}Cl_2N_2$). It is a non-selective contact herbicide used to control weeds and grasses. Due to its redox activity, it generates free radicals, making it highly toxic to humans and animals. World Health Organization (WHO) has classified paraquat as class II or moderately hazardous chemical, where as the International Pesticide Action Network has classified it as highly hazardous herbicide[4]. Despite its toxicity, fast-acting, easily accessible and inexpensive characteristics, it is widely used globally and particularly in many developing countries like India.

Due to high toxicity, even a small sip of paraquat can be fatal with no known antidote. There are some important evidences to diagnose the patient with paraquat poisoning which includes any history of ingestion either accidental or intentional, secondly based on any availability of containers, suicide note, thirdly based on immediate clinical symptoms like, burning effect in gastrointestinal tract starting from mouth/tongue/throat/ to stomach and intestine, copious vomiting, ulceration of mucous membranes[5].

The severity of poison is classified into three categories; mild (20 mg/Kg) (5ml), moderate to severe (< 40 mg/Kg) (5-10 ml), and fulminant (>40 mg/Kg (10 ml). Mild poisoning is characterized by minor gastrointestinal tract disorders with mild symptoms like, nausea, vomiting, fever, drooling, runny nose, burns on lips, redness of throat, tearing or dyspnea with bilateral crackles, crepitation, and pupil mydriasis. Moderate-to-severe poisoning develops severe symptoms like lung fibrosis, metabolic acidosis, kidney and liver damage often leads to acute renal failure, acute hepatitis, acute lung injury and progressive pulmonary fibrosis. Fulminant poisoning results in multiple organ failure and finally causes death within a few days.

A case report was published by Sriram etal[6] where paraquat was used as poison to kill a 40-year-old man who was poisoned by family members with paraquat-laced alcohol. The authors discussed clinical aspects, controlling of manufacture and sale of paraquat, by stringent rules of the regulatory authorities in India and also need to develop a suitable antidote.

Fan Chen et.al [7] reported a rare fatal case of homicidal paraquat poisoning of a 58 year old man by consuming paraquat-mixed medicine. For detection and quantification of paraquat in post-mortem samples, the Ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) was used.

The complexity of homicide cases makes them a major focus for law enforcement and forensic investigations. Advancements in forensic science have played a crucial role in solving homicide cases more accurately. Identification of unknown poison for committing homicide by mixing them in food or water is a challenging task to Forensic Scientist. Especially, in homicide cases the detection of unknown substance in a complex matrix is still difficult job. The matrix will interfere with the target substance by masking it and leading to errors in the process of identification. Most of the perpetrators select such a chemical which will not give any bad odour or taste or strong colour and easily miscible with the matrix selected to administer the victim. It is well known that cyanide[8, 9] is very poisonous substance and kills almost instantaneously when it is consumed in lethal dose. Procuring cyanide in rural and semi urban areas is not easy due to stringent rules of the Government. Hence, perpetrators look for alternatives to cyanide and easily available chemicals in their locality which should be fatal.

Brief history of the case: During the Covid time Government was distributing medicines (tablets, syrups, nutritional supplements etc) door to door, through Health workers as a precautionary measure to prevent spreading of the Covid Cases. In a village of Srikakulam District, a Lady Health worker wearing official uniform covering her head with scarf and mouth by mask, introduced herself to a farmer's wife, coming from Govt Hospital for distributing free Covid medicines. The Lady Health worker administered through mouth a syrup to the farmer's wife and instructed her to take the remaining syrup daily three times and also given eye drops and left the place. After some time the farmer's wife became unconscious and fell down. The inmates of the house were observed and immediately admitted her into hospital. After one week of treatment the patient was discharged without experiencing any organ dysfunction. The reason for survival may be quantity of the paraquat taken, early diagnosis and good supportive treatment[10].

The farmer was a given a complaint to the police and requested to file a case. During the course of investigation, it was found that the farmer has an illegal intimacy with a lady in that village. That lady wants to kill the wife of the farmer so that she can marry and live with him. In this connection, the lady perpetrator came to house of the farmer in the disguise as a Health worker from Govt. Hospital and administered the syrup which contains poison, to kill the farmer's wife.

The investigating officer seized the material objects, two syrup bottles and eye/ear drops bottle which were administered/given to the farmer's wife in the crime scene. The three samples were sent to the forensic science laboratory to find out any poisonous substance in them. The objective of this work is to identify any unknown poison present in the pharmaceutical formulation (syrups and eye/ ear drops) used to commit the homicide by the perpetrator.

Experimental

All the chemicals and reagents used were of analytical reagent grade. Sodium dithionite, methanol, ammonia solution, chloroform, sodium hydroxide pellets are obtained from SD Fine Chemicals Limited, Mumbai, India. Paraquat dichloride was purchased in a local fertilizers shop at Visakhapatnam, India. Ciprofloxacin eye/ ear drops (Sample-1) and Two syrup bottles labelled as Appetite-Fungal diastase pepsin syrup (Sample-2), Feronia-XT suspension-Ferrous ascorbate (Sample-3) and were received from the investigating officer.

Extraction and Analytical Procedure

Five ml of syrup and two ml of eye/ear drops were taken in a separating funnel separately, added a mixture of chloroform:methanol:ammonia (7 : 2.5 : 0.5 v/v/v) by shaking vigorously for two to three

minutes, thereby releasing pressure intermittently and allowed to separate the layers. The organic layer was taken in an evaporating dish. The extraction procedure was repeated twice and collected all the combined extracts into the dish. The combined extract was evaporated to dryness and leached into methanol for further analysis.

Chromatography separations were carried out with 1µl of each sample by spotting on 10 x10 cm silica gel 60F 254 (Merck, Germany) plate using applicator Linomat 5. The spotted plate was developed using mobile phase of Methanol : Ammonia mixture (100:1.5 v/v).

Spot Test

1 ml of extracted sample was taken on spot plate and added 0.5 ml of 1% sodium dithionite in 1N sodium hydroxide. An intense blue colour indicates the presence of bypridines and oligopyridines class of compounds.

Instrumentation

HPTLC

A TLC Scanner 4 and applicator Linomat 5, (CAMAG, Muttenz, Switzerland) were used for densitometric scanning in the absorbance mode and controlled by visionCATS V3.1 software. Deuterium and tungsten lamps were used as a radiation source. Spectrum scan speed was kept at 20 nm/s. HPTLC aluminium plates pre coated with silica gel 60 F254 (Merck, Germany) were used for spotting and scanned with slit dimensions of 4x0.45 mm at wave length 254 nm with data resolution 1 nm.

UV-Visible Spectrophotometer

A double beam UV-Visible spectrophotometer, Model UV-3200 (Lab India Analytical instruments Pvt. Ltd, Hyderabad) with a pair of 10mm matched quartz cells were used in the range 190-1100 nm with wavelength accuracy ±0.1nm and silicon photodiode as detector. All the samples and standard were analysed using methanol as solvent.

Results and Discussion

Physical examination was conducted on the seized pharmaceutical samples (two syrups and one eye/ear drops) for odour and colour. No characteristic odour was observed for all the samples. One of the syrup bottle is having syrup which is dark brown in colour but after careful examination a blue tinge was noticed here and there, the other syrup bottle was empty and the eye/ear drops were green in colour. Same brand syrup and eye/ear drops were purchased from the local market for colour comparison. The purchased two syrup samples were brown in colour with no blue tinge, whereas the colour of the eye/ear drops was a colourless clear liquid. All the three samples were tested for qualitative analysis of volatile poisons viz., alcohols, cyanide, chloroform etc and metallic poisons viz., arsenic, mercury, copper etc and found negative for all the above mentioned poisons. Based on the blue tinge of the syrup and green colour of the eye/ear drops of the seized samples it was suspected that a blue/green colour compound may be mixed in them. The pesticides like quinalphos, paraquat and cartap and also copper compounds which are in blue/green colour may be possible to mix in the crime samples as these pesticides/compounds were easily available in the local market.

After visual examination of blue/green colour of the crime samples, presumptive or screening tests (spot/colour tests) were conducted. These tests are rapid, low cost, simple and reliable to proceed for further confirmation analysis. Spot tests are colour based presumptive tests reliant on visible reaction of drug and diluents with specific functional groups[11]. A series of color tests like scott test, marquis test, mecke test and duquenois levine test were performed on small quantity of crime samples and found negative. Later, sodium dithionate spot test was performed for identification of bypridines and oligopyridene class of compounds. It was found that the sodium dithionate spot test was positive for eye/ear drops (Sample 1) and a syrup (Sample 3) which indicates the presence of bypridines and oligopyridines class of compounds. As paraquat belongs to the same class it may be present in the suspected samples 1 and 3.

Based on the visual examination and spot test of dithionite it was presumed that paraquat may be present in the suspected samples. To confirm the presence of paraquat in these samples, further,

instrumental methods of analysis like UV-Visible spectrophotometry and high performance thin layer chromatography were used.

UV-Vis Spectroscopy (Ultraviolet-Visible Spectroscopy) is an analytical technique used to measure the absorption of light in the ultraviolet and visible spectrum, typically within the wavelength range of 190 nm to 800 nm. The UV region spans from 190 to 400 nm, while the visible region ranges from 400 to 800 nm. It requires minimal sample preparation and can analyze both organic and inorganic substances in various states—solid, liquid, or gas. This technique is simple and cost-effective, making it widely used for both qualitative and quantitative analysis in fields such as forensic science, chemistry, pharmaceuticals, environmental science etc[12].

The extracted samples were scanned using UV-Visible spectrophotometer in the range of 200 to 400 nm and it was observed that at 257 nm wavelength maxima (λ_{max}) exhibited by them. The standard paraquat solution was also scanned in the above range and compared the pattern of the spectrum. Fig. 1 shows the combined spectrums of all the three samples and standard paraquat solution. Sample 1, sample 3 and standard paraquat exhibited wavelength maxima (λ_{max}) at 257 nm with similar pattern of the UV spectrums indicating the possibility of paraquat presence in both of them.

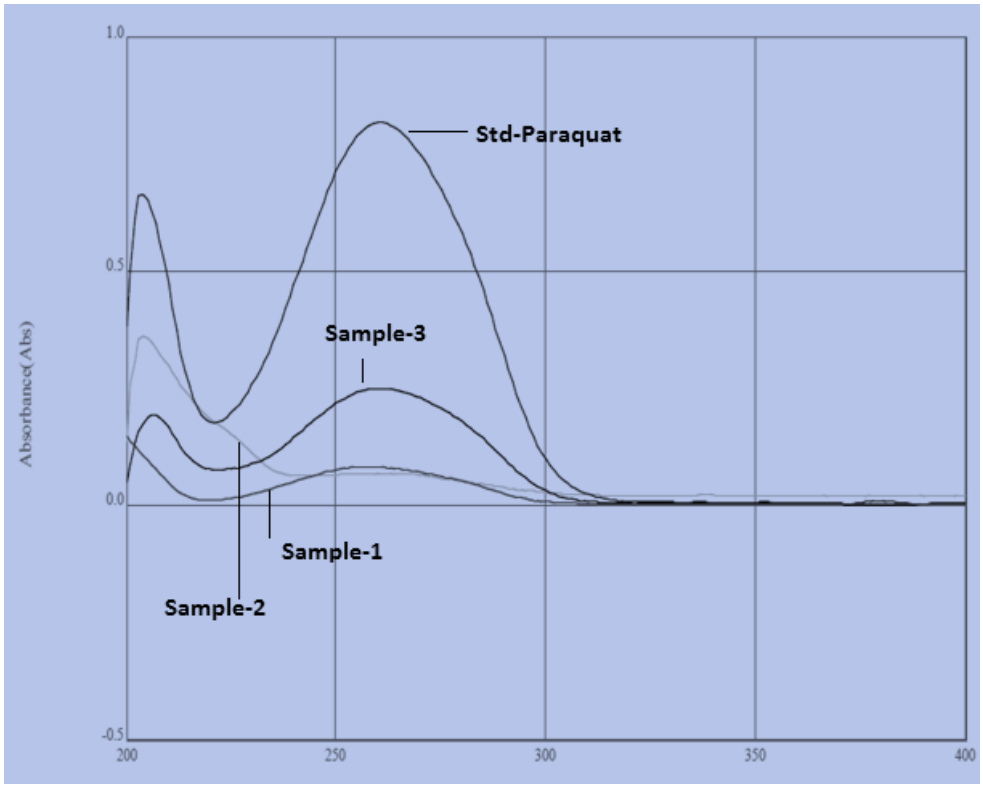


Figure 1: The combined UV spectrums of all the three samples and standard paraquat obtained from UV Visible Spectrophotometer.

For further confirmation of paraquat presence in the suspected samples, Thin layer chromatography (TLC) was used. It is one of the most widely used technique in almost all the forensic science laboratories in India for the separation and identification of unknown drugs, pesticides, chemicals in pure form and in biological matrices viz., viscera and body fluids[13]. TLC is primarily used because, the apparatus is low cost, simple, reliable, and selective and a number of samples can be run simultaneously over other techniques. Since it has lower sensitivity and resolution when compared

with High Performance Thin Layer Chromatography (HPTLC), in the present investigation the HPTLC was used for final confirmation of paraquat presence in the suspected samples. HPTLC is more sensitive with high resolution that can separate closely related compounds of multiple samples in a single run, with reproducible and accurate results for both qualitative and quantitative analysis[14].

As mentioned in the e*xtraction and analytical procedure*, the samples were extracted and reconstituted in methanol. The standard paraquat and samples were applied on a HPTLC plate using Linomat 5 applicator. The plate was developed in a TLC trough chamber having 10 ml of mobile phase Methanol: Ammonia mixture (100:1.5 v/v) which was previously saturated for 15 min. A distance of 70 mm from the starting point of the plate was developed and air dried for 20 min. Densitometric scanning was performed using TLC Scanner 4 on the dried plate at a wavelength of 254 nm. The obtained peaks in all the tracks were analysed and their Rf values were compared with standard and the presence of specific peak for paraquat at Rf *0.017 ± 0.010* is considered as the positive result of paraquat in the seized samples. The Rf values and wavelength maxima (λ_{max}) were given in Table 1. The three dimensional picture of all the spotted tracks of samples and standard were shown in Fig.2.

Table 1. The Retention factor (Rf) values of suspected samples with standard Paraquat.

S. No.	Name of the sample	Rf value (0.017 ± 0.010)	Wavelength(λ_{max})
1	Eye/ear drops	0.026	257nm
2	Syrup sample-1	0.026	257nm
3	Syrup sample-2	0.018	257nm
4	Standard Paraquat	0.010	257nm

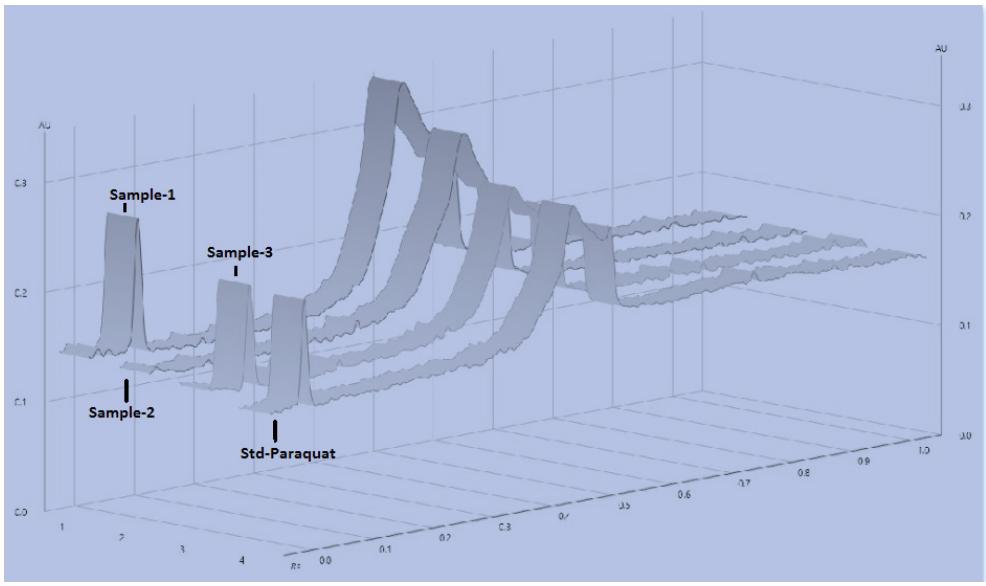


Figure 2: The three dimensional pictures of all the spotted tracks of samples and standard paraquat obtained from HPTLC instrument.

The pattern of the peaks shown in the three dimensional picture look similar indicates the presence of paraquat in sample 1 and 3 except sample 2.

For further confirmation UV based scanning was performed on HPTLC scanner for the peaks of standard paraquat, sample 1 and sample 3. The overlapped UV spectrum obtained from HPTLC

scanner was shown in Fig. 3. The pattern of the UV spectrum of sample 1and 3 were exactly matching with standard paraquat which indicates the presence of paraquat in them.

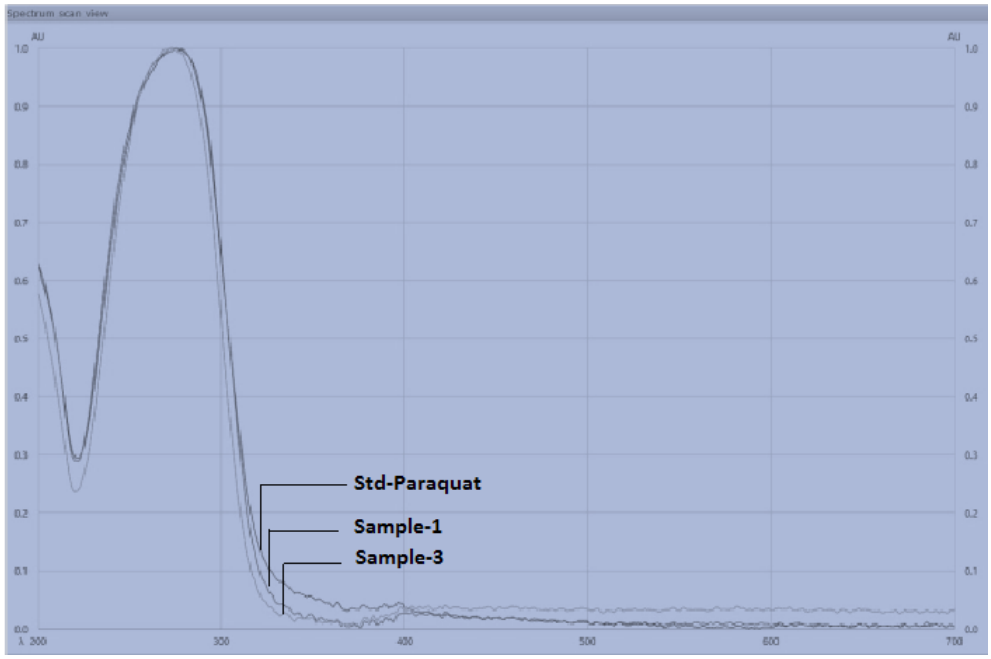


Figure 3: The overlapped UV spectrum obtained from HPTLC instrument.

Based on the above findings from visual examination, spot test, UV-visible spectrophotometer and HPTLC analysis it was confirmed that paraquat is present in the sample 1(Eye/ear drops) and sample 3 (Syrup).

A couple of spectrophotometric methods have been reported for the determination of Paraquat in food and biological samples[15, 16]. Both the methods were used a reducing agent in alkaline medium to develop colour and measured absorbance at 600 nm. Hence, in the present UV-Visible spectrophotometric method, the standard paraquat and samples, neither adding any reducing agent nor any alkaline medium, were measured at 257 nm exhibited good reproducible spectrums resulting the method is simple and reliable. Asif etal[17] reported a TLC method by using stannous chloride as spray reagent for identification of Paraquat and regular white chalk as sensor for the field detection of viologens. This method is suitable for identification of field samples only. In the present investigation, HPTLC method has been used, which is more sensitive and high resolution when compared with TLC as mentioned previously. A number of HPLC[18], UHPLC-MS/MS[19], LC-MS/MS[20] and GC-IT/MS[21] methods were developed for the identification of paraquat in biological/post-mortem samples. These instruments are costly and may not be available in all laboratories. Hence a combination of UV-Visible spectrophotometer and TLC/HPTLC method has been developed for identification of paraquat, which is simple, low-cost and available in almost all laboratories.

In recent times, a large number of paraquat poisoning cases were registered at RFSL Visakhapatnam. The above extraction procedure, spot test and instrumental methods viz., UV spectrophotometer and HPTLC methods were successfully applied for identification of paraquat in viscera samples.

Conclusion

A UV-Visible spectrophotometer and high performance thin layer chromatography were successfully used for the separation and identification of paraquat present in the pharmaceutical

formulations seized in the above homicide case. Confirmation of mixing of paraquat pesticide poison in the seized samples helped the investigation officer to proceed for further in the case. The described method was successfully applied not only for the pharmaceutical samples but also for viscera samples of our forensic science laboratory.

Conflict of Interest: None to declare

Source of Support: Nil

Ethical Clearance: Nil

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