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“Sanitizer a Lockdown Hooch! – A Case Series”

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Abstract

What began as a mysterious infectious outbreak in Wuhan in Dec 2019 soon engulfed the entire globe. The whole of India was placed under a complete lockdown (25 March to 14 April 2020) to prevent the further spread of coronavirus disease. In the absence of any definitive drug or vaccine for the disease, people seem to be trying novel methods to contain the virus which is proving fatal. Hand hygiene products are heavily used in community and healthcare settings in everyday life to maintain hand hygiene. During the lockdown period, many alcoholics suffered acute withdrawal and delirium episodes. WHO and FDA recommended alcohol-based hand sanitizers for frequent hand hygiene which contain mainly ethanol, isopropyl alcohol, and hydrogen peroxide in varying proportions¹. Methanol must never be used in such a product because oral, pulmonary, and skin exposure can result in severe local and systemic toxicity and even death². More than 4% methanol must be labelled as poison³. In a recent study by the Mumbai-based Consumer Guidance Society of India, 122 hand sanitizer tested, 37% were of poor quality and 4% contained the toxic chemical methanol⁴. Instead of liquor, these addicts resorted to consuming hand sanitizers. We have encountered six cases of sanitizer poisoning during the lockdown period from 24th March to 3rd May 2020 in KIMS Hubballi, Karnataka. Here we discuss a case series of poisoning due to the consumption of the sanitizer – a novel *Hooch* in the lockdown period.

Keywords: Sanitizer, Hooch, Lockdown, Poisoning.

Introduction

Methanol (CH₃OH), which has long been used in mummification in ancient Egypt, was obtained

from the distillation of wood, which in Greek roots was called methylene or wood wine⁹. It is a toxic alcohol used as a solvent or in denatured industrial alcohol. Methanol production reached industrial

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scale in 1923 and has found wide applications in various consumer industries, such as model cars, airplane fuel, perfumery, copy machine fluid, gas line antifreeze ("dry gas"), etc.¹⁰ Following the coronavirus disease 2019 (COVID-19) pandemic in Iran (February 19, 2020 to April 27, 2020), there has been a significant increase in methanol-induced morbidity and mortality. This was the greatest prevalence of methanol mass poisoning in the country in recent periods. Because methanol is less expensive and more readily available than ethanol, some fraudsters in Iran use it instead of ethanol in home-made alcohol. Therefore, it is important to increase public knowledge about the deadly consequences of consuming fake alcohol sold on the black market^{11,12}. The most common cause of methanol poisoning in India is adulteration of alcoholic drinks. These alcoholic drinks are illicit liquor produced by unauthorized persons. Methanol claims to give early kick when mixed with alcohol. Hence, adulteration is done. Secondly, it is cheaper than ethanol, which makes it suitable for mixing. Methanol ingestion is an uncommon form of poisoning that can cause severe metabolic disturbances, blindness, permanent neurologic dysfunction and death. While methanol itself may be harmless, it is converted in vivo to the highly toxic formic acid¹³. We have encountered six cases of sanitizer poisoning during the lockdown period from 24th March to 3rd May 2020 in KIMS Hubballi, Karnataka.

Case series

A 45-year-old male patient presented with complaints of loss of consciousness and vomiting with a history of consumption of hand sanitizer. On examination, the patient was unconscious, with poor respiratory effort and gasping. His vitals were not stable. The patient was intubated immediately and he died within one hour. Postmortem examination findings are mentioned in Table 1. Blood and viscera are preserved and sent to Regional Forensic Science Laboratory (RFSL), reports mentioned in Table 3.

A 42-year-old male patient presented with complaints of loss of consciousness with a history of consumption of hand sanitizer. On examination, the deceased was unconscious, with poor respiratory effort and gasping. At the time of admission, his pulse rate was feeble, saturation was 50% in room air and blood pressure was low. The patient was intubated immediately, and despite all efforts,

the patient died within one hour. Postmortem examination findings are mentioned in Table 1. Viscera are preserved and sent for histopathological examination, reports mentioned in Table 2. Blood and viscera are preserved and sent to RFSL, reports mentioned in Table 3.



Figure 1: Petechial haemorrhage in the left ventricle



Figure 2: Stomach contains green colour fluid



Figure 3: Mucosa of stomach - Congested

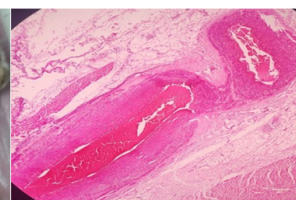


Figure 4: Section from coronaries showing occlusion

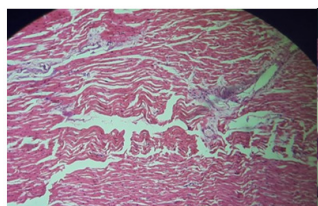


Figure 5: Section from myocardium showing waviness of fibres

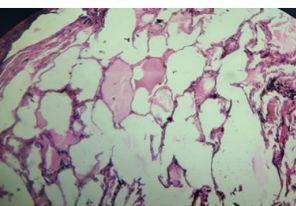


Figure 6: Section from lungs showing pulmonary oedema

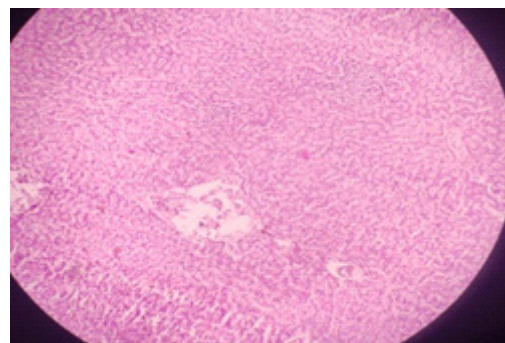


Figure 7: Section from liver showing fatty liver with portal hepatitis.

A 47-year-old female patient presented with complaints of loss of consciousness and convulsion with a history of consumption of hand sanitizer. On examination, the patient was unconscious and gasping. His vitals were not stable. The patient was intubated immediately, and despite all efforts, the patient died within four hours. Postmortem examination findings are mentioned in Table 1. Viscera are preserved and sent for histopathological examination, reports mentioned in table-2. Blood and viscera are preserved and sent to RFSL, reports mentioned in Table 3.



Figure 1: Hand Sanitizer.

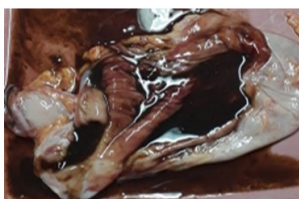


Figure 2: Stomach contains reddish colour fluid.



Figure 3: Mucosa of Haemorrhagic.

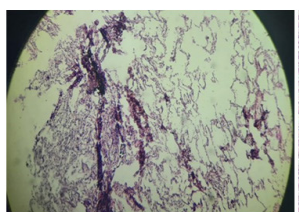


Figure 4: Section from lungs shows Interstitial Pneumonia.

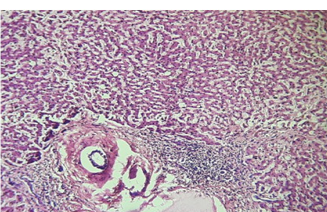


Figure 5: Section from liver shows Acute hepatic injury.

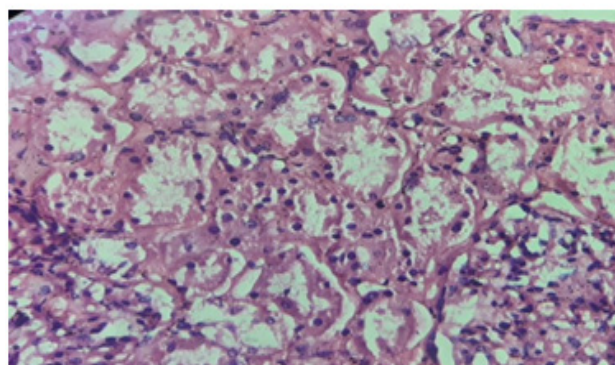


Figure 6: Section from kidney shows Acute tubular necrosis.

A 45-year-old male presented with complaints of loss of consciousness, convulsion and syncope with a history of consumption of hand sanitizer. On examination, he was unconscious and had poor respiratory effort. His vitals were not stable. The patient was intubated immediately, and despite all efforts, the patient died after 1 day. Postmortem examination findings are mentioned in Table 1. Blood and viscera are preserved and sent to RFSL, reports mentioned in Table 3.

A 50-year-old male presented with complaints of convulsion, gasping and syncope with a history of consumption of hand sanitizer. On examination, he was unconscious, with a feeble pulse, saturation not recorded and poor respiratory effort. The patient was intubated immediately, and despite all efforts, the patient died within two and a half hours. Postmortem examination findings are mentioned in Table 1. Blood and viscera are preserved and sent to RFSL, reports mentioned in Table 3.

A 29-year-old male patient was admitted with a history of consumption of hand sanitizer. On examination, the patient was conscious and stable. He was admitted for 3 days in our hospital and went against medical advice. Then he was admitted to a rehabilitation centre for withdrawal symptoms and recovered spontaneously.

Table 1: Postmortem findings.

	Case 1	Case 2	Case 3	Case 4	Case 5
Larynx & Trachea	Froth present.	Congested	Congested	Congested	Yellowish discoloration of epiglottis.
Lungs C/S	Voluminous & congested. Blood stained frothy fluid.	Voluminous & congested. Blood stained frothy fluid.	Voluminous & congested. Blood stained frothy fluid.	Voluminous & congested. Minimal blood stained fluid.	Congested. Blood stained frothy fluid.
Heart	Congested	Congested	Surface shows petechial spots.	Enlarged.	Congested
Stomach Smell Mucosa	150ml of watery fluid. Present Congested	100ml of brown colour fluid Present Haemorrhagic	100 ml of green colour fluid Present Haemorrhagic	250 ml of watery fluid. Absent Congested	50 ml mucoid dark fluid Present Thinned out at greater curvature.
Liver	Fatty changes	Congested	Congested	Congested	Fatty changes

Table 2: Histopathological findings.

	CASE 2	CASE 3
Heart	Myocardial infarction of left ventricle. Pericardium- Dense areas of haemorrhage. Coronary arteries- Occluded (Figure- 4)	Unremarkable.
Lungs	Pulmonary Oedema with early changes of acute lung injury. (Figure- 6)	Interstitial pneumonia. (Figure- 4)
Liver	Fatty liver with portal hepatitis (Figure- 7)	Acute hepatic injury (Figure- 5)
Kidney	Acute tubular necrosis	Acute tubular necrosis (Figure- 6)

Table 3: Methyl alcohol in blood.

	CASE 1	CASE 2	CASE 3	CASE 4	CASE 5
RFSL REPORT	Methyl alcohol- 82.26mg/100ml blood. Ethyl alcohol- 12.53mg/100ml blood(8 & 1/2hrs after death)	Methyl alcohol- 53.95mg/ml blood.(18hrs after death)	Methyl alcohol- 71.75mg/100ml blood.(10hrs after death)	Negative (11hrs after death)	Phenol (12hrs after death)

Discussion

Alcohol-based hand rubs should not contain methanol for obvious reasons. The unexpected presence of methanol poses a series threat to public health. During the lockdown period, many alcoholic dependents suffered acute withdrawals

and delirium episodes. Some of them struggled hard to fight their addictions to alcohol as all liquor shops have been closed down¹⁴. Instead of liquor, these people had resorted to hand sanitizers as novel hooch. Methanol has a weak ability to kill viruses but can be toxic to humans. When a person is exposed to methanol, it is absorbed through the

skin, stomach, or lungs. Methanol itself is not highly toxic, but it is metabolised to toxic metabolites. 10 – 30ml of methanol can lead to death for an adult. It is fatal due to CNS depressant properties and effects of metabolites. Formic acid inhibits mitochondrial cytochrome C oxidase causing the symptoms of hypoxia at the cellular event and also causing metabolic acidosis. After ingestion, CNS depression, headache, dizziness, nausea, lack of coordination and confusion begin. Once initial symptoms passed second set of symptoms arose 10 to 30 hours after initial exposure to methanol including blurring of vision, loss of vision and acidosis. These symptoms result in the accumulation of toxic levels of formic acid in the blood and may progress to death by respiratory failure. Health authorities and manufacturers repeatedly remind consumers that alcohol-based hand rub is for external use only. The consumption of surrogate alcohol is a major health problem. When taken by mouth, methanol, if ever as a substitute for isopropyl alcohol or ethanol, in the hand rub will cause much greater mortality and morbidity¹⁵. We have encountered six cases of sanitizer poisoning during the lockdown period. In case number 1, 2, and 3, a high amount of methyl alcohol was found in the blood. But in case number 4 it was negative. In case number 5, though a history of hand sanitizer poisoning, however, phenol was identified in the blood. In Canada, Two Ontario residents had died after ingesting a hand sanitizer⁵. In USA, a 42 years old man died after ingesting a hand sanitizer⁶. In China, a hospital mistakenly purchased industrial alcohol for surgeons to disinfect the hands before surgery. It was used 3-5 sessions per week. There was soon skin dryness and desquamation of the areas exposed to the hand rub. Six months later, 5 surgeons in the same unit developed erythema and rash in the affected areas. Four surgeons stopped using this product and recovered spontaneously. The fifth surgeon had further exposure and he developed mild visual impairment which gradually improved after stopping this product. The sixth surgeon continued to use the hand rub until blurred vision occurred⁷. During the COVID 19 pandemic, Iranian media reported that nearly 300 people had died and over a 1000 fallen ill after consuming methanol amid false rumours that it can help cure the disease caused

by coronavirus⁸. In our cases, specific therapy was not initiated because the physician did not think of methanol poisoning in a hand sanitizer.

Conclusion

The government should have made alternate arrangements to handle withdrawal cases. Encourage people to use soap instead of hand sanitizer. Strictly regulate and monitor commercially available hand sanitizer. Avoid locally available hand sanitizer. Awareness among healthcare professionals will facilitate the early detection, management, and prevention of poisoning incidents of public health significance. Appropriate public education is mandatory to fight the misinformation that is being spread through social media.

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Vertical and Horizontal Autosomal Sharing of Alleles in First Degree Relationship: A Case Study

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Abstract

This case study details the identification of a deceased individual through paternity and sibship analysis using allele sharing among alleged relatives. Bone and blood samples from the deceased, as well as reference blood samples from an alleged son and brother, were provided. DNA was extracted, quantified, amplified, and analysed using an 8-capillary array DNA analyzer. Remarkably, each autosomal locus in the DNA profiles of the alleged son and brother shared at least one allele with the corresponding loci in the deceased's DNA profile. Y-STR analysis confirmed their immediate patrilineal relationship. To assess the paternity and sibling relationships, respective indices were calculated, confirming the relationships to the deceased. This is the first case observed where both the son and brother shared autosomal DNA fragments at all loci without the mother's reference. The study underscores the importance of DNA statistics in establishing relationship strength solely based on STR results, enabling reliable conclusions without additional testing.

Keywords: Allele sharing, Autosomal DNA, Kinship analysis, Paternity index, Sibship index.

Introduction

Kinship analysis for first-degree relationships (FDR) using Short Tandem Repeat (STR) technology is a powerful method in genetics for determining familial connections. STRs are simple sequence repeats of DNA, where a short sequence of base pairs (1-6 bp) is repeated, constituting about 3% of the human genome¹. The number of repeats varies significantly among individuals (~5-50), with 4 bp repeat STRs being particularly useful for genetic profiling, kinship analysis, and crime investigation^{2,3}.

Kinship is established through degrees of relationship, which refer to the closeness of familial connections, determined by genetic analysis methods such as STR analysis, SNP analysis and sibship analysis either manually, or through software⁴⁻⁷. FDR include parent-child and full siblings, who share about 50% of their DNA segments in contrast to half-siblings who share only one common parent and therefore share only 25% of their DNA. During meiosis, numerous double-strand breaks are generated per chromosome, with some resulting

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in crossing-over events⁸. This leads to the random segregation of each parent's half genome into their offspring, causing full siblings to receive a more or less similar half of their genome from each parent⁹. Second-degree relationships, such as grandparent-grandchild and half siblings, share about 25% of their DNA. Third-degree relationships, like first cousins and great-grandparent to great-grandchild, share about 12.5% of their DNA, and so on.

In sibling cases, the STR alleles (the alternative forms of the DNA segments, usually two in case of diploid autosomal DNA) play a crucial role in determining the likelihood of a sibling relationship¹⁰. Among full siblings, the chance of sharing alleles at a given STR locus is distributed as follows: there is a 25% chance that they will share both alleles, a 50% chance that they will share one allele, and a 25% chance that they will share no alleles¹¹. These probabilities stem from the fact that full siblings inherit their genetic material from the same set of parents, leading to a predictable pattern of allele sharing. By analysing multiple STR loci, geneticists can use these probabilities to accurately determine and confirm sibling relationships, which is crucial in forensic and genealogical investigations. In this particular case, while analysing 21 autosomal STR loci, we observed only the first and second scenarios of allele sharing, making this a unique instance of allele inheritance among two siblings.

In kinship analysis, DNA profiles are created by analyzing multiple STR loci and comparing them statistically to calculate the likelihood of various relationships. A primary statistic in kinship analysis is the paternity index (PI), which evaluates the probability of a man being the biological father of a child. The child's DNA profile is compared to the alleged father's profile at each STR locus. For each locus, a likelihood ratio is determined by comparing the probability of the child's allele given the alleged father's alleles against that of a random individual from the population. The product of these ratios across all loci results in the combined paternity index (CPI). A CPI above 1 supports paternity, while values exceeding 100 are generally considered conclusive evidence. The sibship index is another critical metric, assessing the likelihood of two individuals being biological siblings. It compares the genetic similarity

observed between them against expected values for siblings versus unrelated individuals. Likelihood ratios for each STR locus are calculated based on allele frequencies and combined into a combined sibship index (CSI). A higher CSI indicates a greater probability of a sibling relationship. This approach is particularly valuable in scenarios where paternity or maternity data is absent, such as inheritance cases or identifying unknown relatives.

In this case study, we used DNA statistics of amplified STR markers for kinship analysis to determine the likelihood of biological relationships (paternity and brotherhood) between individuals, utilizing allele frequency data from the population of Madhya Pradesh, India¹². Blood and bone samples from the deceased person, along with reference blood samples from two putative relatives (the son and the brother), were provided for comparison, but no reference sample from the deceased's wife was available. Autosomal and Y-STR profiling were performed on all three individuals, revealing a unique pattern of allele DNA inheritance among the deceased person and his alleged brother, similar to paternal DNA inheritance observed in vertical transmission of alleles to his son.

Materials and Methods

DNA Extraction

In the laboratory, blood sample as well as a sternum bone piece of deceased individual were received, accompanied by reference blood sample from the alleged son and a brother, for DNA profiling and comparison. The sternum bone was thoroughly cleaned using chemical-grade water followed by 70% ethanol and allowed to dry at room temperature. Bone powder was prepared from the surface of the sternum using a hacksaw blade, and approximately 2 grams of the powder was placed in a 15 ml propylene tube. The sample was incubated with 0.5M EDTA (3-5 volumes) on a rotary mixer at 30 rpm at room temperature (RT), with EDTA renewed twice daily for three days, resulting in a paste-like consistency due to significant demineralization. Lysis of the bone powder and reference blood samples was performed in 1.5 ml microcentrifuge tubes at 56°C using a lysis buffer containing 10 mM Tris-Cl (pH 8.0), 200 mM NaCl, 1% SDS, 10 mM EDTA, 1 mM DTT, and

20 mg/ml proteinase K¹³. Following lysis, samples were centrifuged, and the supernatant was mixed with equal volume of Phenol: Chloroform: Isoamyl alcohol (25:24:1), pH 8.0. After gentle mixing and centrifugation, the aqueous phase was collected, and DNA was precipitated with absolute ethanol at -20°C for at least 30 minutes and then centrifuged at 14000 rpm. The DNA pellet was washed with 70% ethanol, centrifuged at 14000 rpm at RT, pellet was dried, and resuspended in TE buffer for subsequent analysis. The extracted DNA was quantified using real-time polymerase chain reaction (RT-PCR) with the Quantifiler® Trio DNA Quantification kit (Applied Biosystems)¹⁴.

STR amplifications and Fragment Analysis

For the amplification of autosomal STRs and Y-STRs, we used the Investigator® 24 Plex kit and the Investigator® Argus Y-28 QS kit (Qiagen Inc.) for multiplex analysis^{15,16}. The autosomal STR kit allowed the simultaneous amplification of 21 autosomal STR loci, one Y-STR locus, a gender marker (Amelogenin), and two quality sensor (QS) markers. The 25 µl PCR mixture included 7.5 µl of Fast Reaction Mix, 2.5 µl of Primer Mix, and 15 µl of DNA/water, ensuring a final DNA template concentration of 1 ng per reaction. Positive and negative controls were included but are not detailed here. The amplification protocol involved an initial denaturation at 98°C for 30 seconds, annealing at 64°C for 55 seconds, and extension at 72°C for 3 seconds for 3 cycles. This was followed by

27 cycles at 96°C for 10 seconds, 61°C for 55 seconds, and 72°C for 5 seconds, with a final extension at 68°C for 5 minutes and 60°C for 5 minutes. PCR products (1.0 µl) or ladder were mixed with 10 µl of Hi-Di™ formamide and 0.4 µl BTO Size Standard dye in a 96-well plate, denatured at 95°C for 5 minutes, snap chilled, and loaded onto the Genetic Analyzer 3500 (Applied Biosystems). The Investigator® Argus Y-28 QS Kit was used for Y-STR profiling, co-amplifying 27 Y-STR markers and a QS marker as per the kit protocol.

Calculation for Paternity and Sibling Index

Data analysis for calculating the duo paternity index and sibship index was performed on a comparative table (Supplementary Material -1). The paternity index (PI) was determined by the ratio of A to B, where A represents the likelihood ratio of the alleged father being the biological father (null hypothesis), and B represents the population allele frequency of a random man (alternative hypothesis). The value of B (autosomal STR frequency) was derived from the STR frequency data of Madhya Pradesh State⁷. The combined paternity index (CPI) was calculated by multiplying all the individual paternity indices (PI) for each locus (refer to Table 1). The CPI indicates the strength of genetic evidence supporting the hypothesis that the alleged man is the child's father, rather than any other man from the population.

Table 1: Paternity index and sibship index calculation of deceased person with alleged son and the alleged brother respectively.

S. No	STR LOCUS	Sample Source & Alleles Called			Paternity Index	Full Sibship Index
		Deceased Person	Alleged Son	Alleged Brother		
1	Amelogenin	X, Y	X, Y	X, Y	-	-
2	TH01	8, 9	6, 8	9, 9.3	1.969	0.590
3	D3S1358	16, 16	14, 16	16, 16	2.024	6.373
4	vWA	14, 16	16, 19	16, 17	1.196	0.848
5	D21S11	28, 30	29, 30	28, 30	1.232	6.732
6	TPOX	8, 11	11, 11	8, 11	1.222	1.736
7	DYS391	11	11	11	--	-
8	D1S1656	13, 16.3	13, 16	13, 16.3	1.786	55.429
9	D12S391	23, 23	20, 23	21, 23	6.757	3.628

Continue....

10	SE33	20, 20	20, 29.2	20, 30.2	5.556	3.028
11	D10S1248	13, 14	14, 14	12, 14	1.779	0.695
12	D22S1045	11, 15	11, 15	11, 15	1.430	1.984
13	D19S433	13, 14	14, 15	13, 14.2	1.111	0.606
14	D8S1179	10, 16	10, 10	10, 13	2.778	0.944
15	D2S1338	18, 20	20, 26	18, 20	2.119	8.565
16	D2S441	10, 11	10, 11	9, 10	1.328	0.582
17	D18S51	16, 16	12, 16	16, 16	3.906	19.273
18	FGA	24, 24	24, 25	23, 24	2.994	1.747
19	D16S539	12, 13	11, 12	12, 13	1.389	7.257
20	CSF1PO	10, 11	11, 11	10, 11	1.773	3.704
21	D13S317	11, 12	11, 12	11, 12	2.173	3.681
22	D5S818	12, 12	11, 12	11, 12	1.572	1.031
23	D7S820	11, 12	11, 12	11, 12	2.709	4.930
					(CPI)= 2.83×10^6	(CSI)= 1.52×10^9

NB: The Combined Paternity Index (CPI) and the Combined Sibship Index (CSI) are here the likelihoods that the deceased person is the biological father or biological full sibling of the alleged individuals. This indicates that the alleged father has 2.83×10^6 times more possibility to be the father of the alleged son compared to an arbitrary person or 1.52×10^9 times more to be the full sibling of the alleged brother compared to an arbitrary person.

2. Formula for calculating Sibship Index				
S. No.	Sharing of Alleles	Full sibling (F)	Half sibling (H)	Unrelated (U)
1	Share two alleles (both are pq)		$p+q+4pq$	$8pq$
2	Share two alleles doubly (both are pp)	$(1+p)^2$	$2p(1+p)$	$2p^2$
3	Share one allele double (both are pp or pq)	$1+p$	$1+2p$	$4p$
4	Share one allele (both are heterozygous, pq and pr)	$1+2p$	$1+4p$	$8p$
5	Share no alleles (pq or pp vs rs or rr)	1	2	4

Supplementary Material - 1. Formula used in the case study for calculation of duo paternity index and sibship index (Courtesy: <https://dna-view.com/>).

1. Formula for calculating Duo Paternity Index			
S. No.	Father	Alleged Child	Duo Paternity Index
1	q	q	$1/q$
2	q	pq	$1/2q$
3	qr	q	$1/2q$
4	pq	pq	$p+q/4pq$
5	qr	pq	$1/4q$
6	r	q	0

Foreestablishment of sibling relationship, sibship index was calculated using the formula available at DNA view website (<https://dna-view.com/>) which were derived from the symbolic iKinship program¹⁷(Supplementary Material -1). The proportion $F: U$ was used for estimating the likelihoods of the full

sibling versus unrelated person using amplified STR types assuming full-sibship and unrelated respectively. From these proportions likelihood ratios of full to half-sibship (F/H) was calculated for each set of alleles for each amplified locus. The combined sibship index (CSI) was calculated by multiplying

the individual values obtained for each locus studied (refer to Table 1). Paternity and sibship indices for each locus were computed based on the population data of Madhya Pradesh¹⁸.

Discussion

The DNA profiles of the deceased person, his alleged son, and his alleged brother were generated using validated Qiagen Kits (Investigator® 24 Plex Kit and the Investigator® Argus Y-28 QS Kit) to establish their biological relationships. Y-STR profiling confirmed their shared patrilineal ancestry. The alleged son shared at least one allele at all the amplified loci with the deceased person. The Duo paternity index was calculated, indicating that the deceased person is 2.83×10^6 times more probability to be the father compared to an arbitrary man from the same population.

The alleged brother's autosomal profile was also compared, revealing that he shared at least one allele at all the autosomal amplified loci with the deceased

person. This is a rare occurrence, as in a randomly mating population, the segregation of alleles is independent, and the chances of sharing of all 21 forensic STR loci among siblings are extremely low. The probability distribution for allele sharing among full siblings at a given STR locus is: 25% chance of sharing both alleles, 50% chance of sharing one allele, and 25% chance of sharing no alleles. In this particular case, while analysing 21 autosomal STR loci, we observed only the first scenario (9 events) and the second scenario (12 events) of allele sharing, making this a unique instance of allele inheritance among two siblings. The Combined Sibship Index (CSI) indicates that the alleged brother is 1.52×10^9 times more probability to be a full sibling compared to an arbitrary man from the same population. Additionally, Y-STR profiling of the deceased person, the alleged son, and the alleged brother revealed an identical profile across 27 loci (see Table 2), confirming their common immediate patrilineal ancestor.

Table 2: Y-STR profiling of deceased, alleged son and alleged brother confirming their same patrilineage.

S. No.	STR LOCUS	Sample Source & Alleles Called		
		Deceased Person	Alleged Son	Alleged Brother
1	DYS389I	13	13	13
2	DYS391	11	11	11
3	DYS389II	29	29	29
4	DYS533	12	12	12
5	DYS390	22	22	22
6	DYS627	18	18	18
7	DYS458	19	19	19
8	DYS393	12	12	12
9	DYS19	16	16	16
10	DYS437	14	14	14
11	DYS449	34	34	34
12	DYS460	10	10	10
13	DYS576	17	17	17
14	YGATAH4	13	13	13
15	DYS481	24	24	24
16	DYS448	19	19	19
17	DYS518	36	36	36
18	DYS439	11	11	11
19	DYS549	12	12	12

Continue....

20	DYS438	9	9	9
21	DYS456	16	16	16
22	DYS643	9	9	9
23	DYS570	17	17	17
24	DYS635	20	20	20
25	DYS385	15, 16	15, 16	15, 16
26	DYS392	11	11	11

Conclusion

In this forensic case study, the autosomal STR profile of an alleged brother showed an unusual resemblance to a paternal relationship. At each of the 21 loci amplified from the deceased's bone sample and the alleged brother's sample, they shared at least one allele, a rare occurrence attributed to identity by descent from a common ancestor. In the absence of the child's mother's reference blood sample, the deceased shared at least one allele at all loci with the alleged son, while the son did not share alleles at two loci with his uncle, ruling out a first-degree relationship with the uncle.

Further analysis revealed a higher paternity index for the alleged brother when considered as the son, and a significantly lower sibship index when the alleged son was considered as the brother, reflecting their paternally bonded relationship. The half-sibship index between the deceased and the alleged brother was less than one, excluding such a relationship. This case, lacking the mother's reference sample and mitochondrial DNA analysis, highlights a unique challenge for forensic analysts. The results suggest that based on the shared alleles, the alleged son and brother cannot be excluded as the true son and brother of the deceased. This study highlights the forensic importance of STR statistics in kinship analysis and highlights the potential for integrating genealogical studies in the future to resolve complex patterns of STR inheritance and establish relationships more accurately.

Ethics Approval and consent to participate

This article does not include any studies involving human participants or animals conducted by any of the authors. The authorization for examining the samples (bone sample and reference blood samples) was granted by the Superintendent of Police, Hyderabad, Telangana.

Competing interests: All the authors have declared that there is no competing interest in publication of this case report.

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Authors' contributions

DB extracted DNA and carried out capillary electrophoresis. PCK carried out electrophoresis and statistical calculations. AKR conceptualized the article, analysed the results, and wrote the paper.

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Unveiling the Health Consequences of a 55-Year-Old with Nubain, Alcohol, and Smoking Abuse

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Abstract

The case study describes a high-risk substance user adult patient with Nubain, smoking, and alcohol abuse. This research study tries to find out the effect of substance abuse on the patient's health and social functioning. Initially, the patient started with marijuana for fun with friends, which further led to Nubain injections, alcohol intake, and smoking. Due to prolonged consumption, the patient's body eventually developed an intolerance to these substances, resulting in severe health complications.

The patient was admitted to a nearby hospital in emergency with sudden severe abdominal pain. The patient was diagnosed with CBC, ultrasound, and x-ray to identify his condition. The complications were so serious that the patient needed the attention of a specialized doctor, including very low hemoglobin levels and melena.

Despite all these admissions and other medical interventions, there was no improvement in the patient's condition. The patient ultimately died due to substance use. This study identifies early intervention and comprehensive care as important in improving health and social outcomes. It points to the dangerous and lethal effects of substance abuse, hence the importance of prevention and timely medical interventions.

Keywords: Nubain injection, alcohol, smoking, health risks, substance abuse

Introduction

Substance misuse represents one of the key public health concerns. Misuse of drugs, consumption of alcohol, and tobacco misusing all include a category of serious health disorders such as problems of the heart, liver, and respiratory organs, among others. 2019 research showed that a combination of alcohol with opioids or cocaine raised health risks whenever

dangerous conditions occurred in the process, such as issues related to heart conditions and liver diseases.⁽⁷⁾ 2021 research outlines the risk of smoking, heavy drinking, and drug use for heart disease and serious organ damage.⁽¹¹⁾

Nalbuphine: This is also known as Nubain, a semi-synthetic opioid. Although very effective in the treatment of pain described from moderate to severe,

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there's quite a high potential for abuse. In the cases where there is an abuse of Nubain, most especially with other depressants of the central nervous system, there are serious complications that can arise, and they include respiratory depression and impairment of various organs. These risks delineate how much the study of long-term effects is justified in substance abuse, particularly with the use of multiple substances. ⁽⁶⁾

It therefore discusses a 55-year-old male patient who was exposed to severe multi-organ damage following long-term Nubain use in combination with alcohol and tobacco. It is a case, thus, to raise awareness of the danger of drug use. Early intervention and timely treatment need to be emphasized. By sharing this study, we would like to contribute to the discussions of substance abuse, show its long-term impacts, and press for better prevention and rehabilitation strategies.

Case Presentation

The patient had been taking Nubain for 20 years. Although rehabilitation was attempted, the patient continued using Nubain. Over time, he developed high blood pressure, which he attributed to medication. Consequently, he decided to stop Nubain. However, despite discontinuing the medication, the patient continued to consume alcohol and cigarettes.

In the middle of the night, he was taken to the hospital as the patient had acute stomach ache. The doctors wanted to perform an endoscopy on him, but his red blood cell count was too low. The patient needed a hematologist before the operation, but such specialist was not available. So he was transferred to another hospital where Internists & Hematologists were available.

One of his throat veins was swollen when the doctors performed the endoscopy. They decided to attend to the swollen vein first, as that might lead to bleeding during the endoscopy since the patient was suffering from pancytopenia and needed to be handled with care. While the patient was recovering from the throat procedure, he started to develop blood in his stool. During this time, the doctors couldn't do anything more, which included the endoscopy and the operation, because the patient needed a

blood transfusion for the surgery. While recovering from the procedure, the patient's abdomen starting swelling up with fluid accumulation.

The doctor removed some of the fluids to relieve the patient's pain. The patient continued these regular consultations for proper medication until the doctor estimated that the patient had about seven months to live.

The patient was admitted to the hospital with low hemoglobin levels and melena. All possible laboratory tests were conducted on him. The health condition of the patient did not improve even after medical intervention, and he died. This is a very serious consequence of substance abuse, which indicates the importance of early intervention and comprehensive care to reduce the burden of disease and social problems.

Methods

The laboratory tests and procedures performed to diagnose the disease of the patients include: A CT scan was done to view internal organs, bones, and soft tissues and give comprehensive information about the condition of the patient and any abnormalities and injuries sustained. Chest X-ray was used to treat and diagnose pathologies in the lungs, heart, and chest wall and helped in diagnosing infections and diseases of the lung and heart conditions. A CBC measures several components of blood, including red and white blood cells, hemoglobin, and platelets, to detect infections, anemia, and other disorders.

Blood chemistry tests included FBS, which was utilized for the diagnosing and monitoring of diabetes; HBA1C, for reflecting average blood glucose level over the past 2-3 months; and SGOT and SGPT for assessing liver functions and in detecting liver damage. BUN and creatinine were used to assess kidney functions.

Additional tests included electrolyte tests (sodium and potassium) to assess fluid balance, kidney functions, and heart health. Alkaline phosphatase testing was done to diagnose liver and bone diseases, while total protein testing was carried out to assess overall health and diagnose various conditions. Coagulation tests evaluate the blood's ability to clot, diagnosing bleeding disorders and monitoring anticoagulant therapy.

The medications administered to the patient included sofosbuvir, omeprazole, spironolactone, and propranolol.

Results

Diagnostic Findings: The CT scan report shows non-obstructing nephrolithiasis on the right, indicating kidney stones in the right kidney that were not causing any blockage. There was a renal cortical cyst on the left, which is a cyst in the cortex of the left kidney. The patient had hepatosplenomegaly, meaning there was an enlargement of both the liver and spleen. Additionally, there was an atheromatous abdominal aorta, indicating the presence of atherosclerosis (plaque buildup) in the abdominal aorta. The most important finding is the suggestion of diffuse liver parenchymal disease with signs of cirrhosis, including hepatic foci that may indicate hepatocellular carcinoma.

The fecal analysis result showed many bacteria and moderate yeast cells, which could indicate an infection. The blood chemistry report shows a fasting blood sugar (FBS) result of 5.82 mmol/L, which is slightly elevated compared to the normal values for adults (3.89 to 5.8 mmol/L). The coagulation tests show a prothrombin time (Protime) result of 13.0 seconds, within the normal range of 11.5-15.5 seconds, but another value of 16.4 seconds is slightly above the normal range. The activity result is 66.5%, which is below the normal range of 78-106%.

The chest PA X-ray report showed new horizontally oriented linear opacities in the left middle lung field, suggesting subsegmental atelectasis (partial collapse of a small area of the lung) or non-specific parenchymal fibrosis (scarring of lung tissue) in the left lung. The blood chemistry test showed a creatinine result of 5.47, which is significantly higher than the normal range for both males and females, indicating impaired kidney function. The total bilirubin result was 19.55 $\mu\text{mol/L}$, which is slightly elevated compared to the normal range of 0-17.1 $\mu\text{mol/L}$.

The hematology report shows a hemoglobin (HGB) result of 78 gm/L, which is significantly lower than the normal range for both males (130-180 gm/L) and females (120-160 gm/L), indicating severe

anemia. The urinalysis results indicate the presence of blood and pus cells in the urine, which could suggest a urinary tract infection (UTI) or other underlying conditions. The presence of many bacteria further supports the possibility of a UTI.

The last CT scan reported a diffuse liver parenchymal disease with signs of cirrhosis, including ill-defined, hypodense hepatic foci in segments IVB and VI, which may indicate hepatocellular carcinoma (LIRADS V). Additionally, round, rim-enhancing, hypodense hepatic foci in segments VII and VIII could be part of the same neoplastic process. There are several worrisome findings for metastasis if a primary malignancy is confirmed, such as thrombus formation within the extrahepatic portal vein and possible thrombus formation within the left port venous branches, a pleural-based nodule in the left lung, retroperitoneal and periportal lymphadenopathy, ascites, dilated portal and splenic veins, and collateral vessel formation. Suggesting portal venous hypertension. Portal vein thrombosis and arteriportal shunting are also noted.

Other findings include hepatic cysts in segments VI and IVB, calculous cholecystitis, a renal cyst in the left kidney (Bosniak I), an umbilical hernia, bilateral minimal pleural effusion (slightly progressing, more on the left), cardiomegaly, a normal-sized prostate with concretions, subcutaneous edema, non-specific extra-testicular calcifications in the left testicle, degenerative spondyloarthropathy with L1-L2 disc disease, and an atherosclerotic aorta, which eventually lead to the death of the patient.



Figure 1: Enlarged and prominent lymph nodes (encircled) are noted in the paraaortic region.

Discussion

The results of this study point to the serious health consequences of the combined use of Nubain, alcohol, and smoking. The Child-Pugh score is determined by scoring five clinical measures of liver disease and the possibility of eventual liver failure. Class A (5-6 points) least severe liver disease, one to five-year survival rate: 95%. Class B (7-9 points) moderately severe liver disease, one to five-year survival rate: 75%. Class C (10-15 points) is the most severe liver disease, with a one- to five-year survival rate of 50%.⁽¹²⁾ Based on the medical certificate, the patient has chronic liver disease CP B (7) due to alcoholic liver disease.

Model for end-stage liver disease (MELD) scores range from 6 to 40, depending on the severity of liver disease. A MELD score greater than or equal to 10 may be referred to a hepatologist or liver specialist. MELD scores are meant to assess the risk of death within three months, which may support a decision to recommend a liver transplant to avoid this high risk.⁽¹³⁾ Based on the medical certificate, the patient has chronic hepatitis C, with MELD Na 10.

The patient also has portal hypertension due to decreased platelets, splenomegaly, and recent bleeding esophageal varices, which further underlines how critical the state of the patient was. Among the findings were an atheromatous abdominal aorta consistent with atherosclerosis, a known complication of drug misuse.

These facts are supported by the study in 2019, which further stated that combining alcohol with opioids and cocaine, among other drugs, might result in hazardous health effects characterized by cardiovascular disorders and liver damage. It can be

analyzed from the case study that the abuse of Nubain, combined with alcohol and smoking, is responsible for severe cardiovascular and hepatic complications. Similarly, a study in 2021 said that smoking, heavy alcohol drinking, and drug abuse result in major health problems, including cardiovascular diseases (CVD), which tally with the symptoms presented by the patient.⁽⁷⁾

Nubain, or nalbuphine hydrochloride, is a narcotic medication used to treat moderate to severe pain, such as post-surgery or childbirth. The usual dose is 10 mg for an average adult weighing about 70 kg, administered subcutaneously, intramuscularly, or intravenously, and repeated every 3 to 6 hours as needed.⁽¹⁾ One innovation enhancing pain control is the nasal spray form of Nubain called Apain, a noninvasive and effective alternative to intramuscular albuphine.⁽²⁾ Some recovery rooms use a device to administer 5 mg of morphine as additional analgesia.⁽³⁾

Nalbuphine, a synthetic opioid in clinical practice for over 40 years, interacts with both the μ -opioid and the κ -opioid receptors of the receptor. The Thai FDA has recognized its effectiveness in treating moderate to severe pain.⁽⁴⁾ Though its mixed agonist/antagonist properties were believed to reduce its abuse potential, dependence has been reported among bodybuilders misusing it to manage pain and enhance their training.⁽⁵⁾

Despite clinical benefits, nalbuphine is dangerous, especially when combined with other brain-acting drugs, and is prescribed cautiously due to the risk of severe respiratory depression, particularly when starts or when doses increase.⁽⁶⁾

Kappa receptor (KOR) function in neurocircuits of addiction. This simplified scheme represents a sagittal section of a rodent brain illustrating brain regions involved in drug abuse (Circles). Receptor density is indicated for each region and black lines show the opioid-receptor-regulated pathways identified in the studies discussed in this review. KORs in excitatory amygdala (AMG) neurons projecting to the bed nucleus of the stria terminalis promote stress and anxiety. KORs in the nucleus accumbens input negatively regulates motivational processes, modify the hedonic value of nociceptive events, and shape motivational behaviors in response to painful experiences. KORs in AMG are related to inducing the affective state of addiction

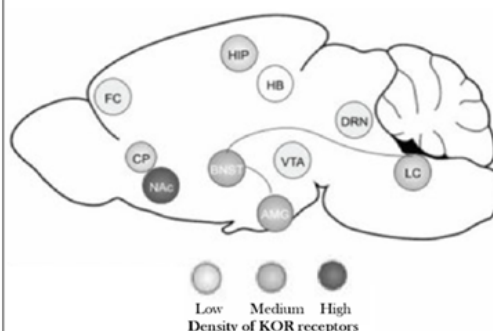


Figure 2: Kappa receptor (KOR)

Recent research reconsiders nalbuphine's merits amid the opioid addiction epidemic, investigating its potential to reduce stronger opioid use. KORs located in specific brain regions modulate motivation and nociceptive stimuli. For instance, KORs in the nucleus accumbens assist in motivation and pain, while in the amygdala they relate to addiction centers. ⁽¹⁰⁾

A 2022 study found that children and adolescents who frequently smoked and drank alcohol were happier with peer relations, likely due to social experiences from substance use. This aligns with reports of increased peer time among Nubain, smoking, and alcohol users.

Studies from 2021 identified opioid addiction disorder as a public health concern, with risk factors including genetic changes, bipolar disorders, and substance-related behavioral disorders. ⁽¹¹⁾

Health problems arise from combining Nubain with opioids or cocaine, as explained in a 2019 study. The pattern of Nubain use with tobacco and smoking is linked to liver impairment, cardiovascular disorders, and even extreme drug abuse. ⁽⁷⁾

Studies quantified the negative effects of heavy alcohol consumption and smoking while taking drugs, raising cardiovascular disease (CVD) risks. Abdominal aortic atherosclerosis is diagnosable from a CT scan due to aorta hardening from drug misuse. Research from 2021 indicates drug users without CAD are at a higher risk of ischemic heart disease, often caused by coronary heart disease. ⁽⁸⁾

Historically, alcohol mixed with opioids, morphine, or cocaine treated many diseases, leading to circulation problems and liver trouble. Addiction quickly manifested withdrawal symptoms as the drugs started to wear off. The interaction between alcohol and drugs remains a mystery. ⁽⁷⁾

The patient had alcoholic liver disease and chronic hepatitis C, advancing to chronic liver disease CPB with a 10 MELD Na score. The liver is diffusely affected with cirrhosis and suspected hepatocellular carcinoma. The situation worsens with intrahepatic biliary ducts, together with thickened walls, reducing clearance. The patient also has potential bleeding esophageal varices, white blood cells, and a loss in platelet size, indicating portal hypertension.

Implications of the Findings:

The outcomes of the study warrant these important considerations. In particular, the association of Nubain or polydrug use involving alcohol and smoking with debilitating maladies, particularly cardiovascular diseases and liver insults, leads to the suggestion that these patients should be evaluated for multiple substance use, which enhances the quality of health care provided. It is important to comprehend these relationships since they determine the effectiveness of prevention and treatment approaches. ⁽⁸⁾

The Government of India's Ministry of Social Justice and Empowerment, in its 2019 report under the National Drug Dependence Treatment Centre, observed that there is a significant use of substances. Substance abuse could be best approached by adopting evidence-based preventive strategies, which were suggested to the policymakers. ⁽⁷⁾

Policies to promote public health should aim at specific preventive measures against using multiple drugs at a given time. This study expands the current body of knowledge by bringing forth new dimensions to these relationships to direct further investigations and policy formulation processes.

Limitation of the Study:

Even though much valuable information is obtained from this study, it has several limitations. The single sample size may not allow the generalization of findings to a wider population. Further, data are self-reported, introducing possible inaccuracies in reporting. No control group is included in this study design; such a design would better isolate the effects of Nubain, alcohol, and smoking abuse. These limitations need to be overcome in future research, which should involve larger and more diverse samples, coupled with more robust study designs. The findings could also be confounded by some variables that were not fully controlled, such as the presence of other underlying health conditions.

Conclusion

This study highlights the significant health risks associated with the combined use of opioids, alcohol, and smoking—a synergistic “triple threat”

to health. Professionals in forensic and related fields should recognize that individuals often engage in polydrug use, which can complicate case analyses. Acknowledging this overlap is crucial for accurately assessing and addressing such situations.

Healthcare providers must proactively engage with patients about the concurrent use of multiple substances, enabling them to better understand patients' behaviors and offer appropriate interventions. Additionally, preventive measures are essential. Public health practitioners play a pivotal role in raising awareness about the dangers of polydrug use through targeted campaigns and educational initiatives.

However, the study's impact could have been enhanced with a more diverse participant pool, encompassing individuals from varied backgrounds. Future research should prioritize inclusivity to provide a comprehensive understanding of the risks associated with the simultaneous use of these substances, ultimately informing more effective interventions and policies.

Implications for forensic professionals:

This study will give valuable help to forensic professionals, especially in forensic medicine, by stressing the severe complications and combining Nubain, alcohol, and smoking. This study will emphasize the importance of thorough assessments in evaluating individuals suspected of substance abuse.

Forensic experts involved in substance abuse can use these findings to better understand the physical and psychological effects of polysubstance use, especially in cases of addiction, overdose, or criminal behavior. Forensic practitioners should recognize the risk of multiple substances, which could influence decision-making in cases such as determining the cause of death. Forensic professionals can make more informed, accurate assessments and legal evaluations by considering the complex interactions between substances.

Funding Sources:

I declare that I am the sole funding source for this case study, and all financial support has been provided by me.

Ethical Clearance: There is no ethical clearance required for this study, as it does not involve any procedures or data that necessitate approval from an ethics committee.

Declaration of conflicts of interest statement: I declare that I have no conflicts of interest related to this case study. All information and findings presented are based solely on the data, and the documents are our personal family copies intended solely for our use.

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A Case report of Unknown Identity: Investigating a Death on Railway Tracks

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Abstract

Unidentified bodies pose substantial challenges in forensic and medico-legal investigations, requiring a thorough and multidisciplinary approach. This case report discusses the forensic investigation of an unidentified male, approximately 40 years old, discovered deceased on the railway tracks. In this case, severe traumatic injuries were observed, particularly to the head and face, suggesting a high-impact collision, likely with a moving train. However, the absence of personal identification documents complicated the process of establishing the individual's identity.

The forensic examination revealed multiple traumatic injuries, including a crushed head, fractures in the upper limbs, and significant abdominal lacerations. Internal findings such as congested organs, ruptured spleen, and lacerated liver supported the high-impact trauma hypothesis. The presence of post-mortem staining and rigor mortis provided further insight into the timing of death. Notably, a tattoo in Urdu and circumcision suggested the deceased's religious background, aiding in preliminary identification efforts.

This case highlights the complexities involved in distinguishing between pre- and post-mortem injuries, and emphasizes the importance of forensic techniques such as DNA profiling and dental record comparisons in resolving cases involving unknown victims. The investigation underscores the need for a comprehensive, multidisciplinary approach to railway fatalities, combining scene analysis, autopsy, and ancillary testing to determine the cause of death and support identification efforts. Such cases necessitate coordinated efforts among forensic experts, law enforcement, and medical examiners.

Keywords: Unidentified Male, Railway Tracks, Forensic Investigation, Blunt Force Trauma, Autopsy Findings, Medico-Legal Examination

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Introduction

The discovery of unidentified bodies poses significant challenges in forensic and medico-legal investigations, demanding a meticulous and multidisciplinary approach to determine the cause of death and establish the identity of the deceased^[1]. This case report details the investigation of an unknown male, approximately 40 years old, found dead on the railway tracks in a capital city of a state. Railway-related fatalities can result from various scenarios, including accidental deaths, suicides, and homicides, each requiring distinct investigative strategies^[2]. On the morning, the lifeless body of the unidentified male was discovered on the railway tracks, triggering a comprehensive forensic examination. The initial assessment suggested severe traumatic injuries consistent with a high-impact event, likely involving a moving train. However, the lack of identification documents or personal belongings at the scene necessitated a thorough investigation to rule out foul play and uncover the victim's identity.

This report aims to provide an in-depth analysis of the forensic procedures undertaken, from the scene investigation to the autopsy findings and subsequent ancillary tests. It highlights the complexities involved in distinguishing between pre- and post-mortem injuries and emphasizes the role of toxicological analysis in understanding the victim's state at the time of death.

The identification process, including the use of advanced forensic techniques such as DNA profiling and dental records, is also discussed. Through this case report, we aim to shed light on the investigative challenges and underscore the importance of a coordinated effort among forensic experts, law enforcement, and medical examiners in resolving cases involving unidentified victims on railway tracks.

Case Description

An unidentified male, approximately 40 years old, was found deceased on the railway tracks. The following observations and findings were noted during the forensic examination:

Physical Characteristics

- **Build:** Average build, weighing approximately 80 kg and measuring 180 cm in height.
- **Rigor Mortis:** Rigor mortis had passed from both the upper and lower limbs.
- **Post-Mortem Staining:** Present on the back.
- **Identifying Marks:** A tattoo mark on the dorsum of the hand with an Urdu word (Figure: 1) and a circumcised penis, suggesting the deceased was of Muslim religion.



Figure: 1



Figure: 2

Injuries

- **Head and Face:** The head and face were crushed from front to back. All structures above the mandible were destroyed, and the brain was absent from the body bag. (Figure:2)
- **Right Shoulder:** Swelling was noted. Upon examination, a fracture of the right humerus shaft was found, approximately 5 cm distal to the shoulder joint.
- **Left Forearm:** Swelling was observed. Upon examination, a fracture of the ulna was found, approximately 5 cm distal to the elbow joint.
- **Abdomen:** A laceration measuring 12 x 4 cm was present on the left side of the abdomen, muscle deep, located 1 cm above the iliac crest and 18 cm from the umbilicus. (Figure: 3)
- Multiple contusions of sizes varying from 2x3 cm to 8x4 cm were found on the lateral aspect of the left thigh, along with grease staining. (Figure: 4)
- Multiple graze abrasions are present on the entire surface of the back of the right arm.
- Additionally, multiple abrasions of various sizes and shapes are present on the front of the right knee.



Figure: 3



Figure: 4



Figure: 5

Internal examination:

All internal organs were found to be congested. The lungs showed black pigment deposition and congestion. The spleen was found ruptured, with approximately 500 ml of clotted blood present in the abdominal cavity. Multiple lacerations and ruptures were observed on the anterior surface of the liver. (Figure: 5) The heart was congested, with the right ventricle filled with blood and the left ventricle empty

Negative findings: There were no signs of respiratory failure, asphyxia, or poisoning, including cyanosis, ligature marks on the wrists and ankles, and hemorrhagic spots in the gastric mucosa.

Discussion

The severe traumatic injuries to the head and face in this case indicate a high-impact collision, most likely involving a moving train. Research by Rupani Raja et al. highlights that facial injuries are frequently seen in railway track accidents (29.72%), second only to road traffic accidents (56.75%)^[3,4]. The presence of rigor mortis suggests that the body had been deceased for a considerable time before being discovered. Additionally, the post-mortem staining on the back implies that the body was lying in a supine position for a period after death. A study by Kumar S. found

that out of 20,877 autopsy cases, 3,169 (15.17%) were unclaimed bodies, with railway and road traffic accidents accounting for 516 (68.25%) accidental deaths in males and 301 (88.01%) in females^[5]. The presence of a tattoo in Urdu and circumcision offers potential clues regarding the deceased's religious background, which may assist in identification^[6]. In such cases, it is crucial that the legal system effectively collects and evaluates forensic evidence found at the scene of the incident to ensure justice^[7]. This forensic analysis plays a vital role in bridging the gap between medico-legal investigations and criminal justice, aiding both identification and accountability in trauma-related deaths^[8].

Opinion:

The time since death is likely 24 to 36 hours, as rigor mortis has passed, indicating a timeframe consistent with decomposition. The cause of death appears to be high-impact trauma from a moving train, evidenced by severe head injuries, multiple fractures, and internal organ damage, particularly to the spleen and liver. Identification may be aided by the Urdu tattoo and circumcision, suggesting a Muslim background, but further investigation is needed. Recommended actions include DNA profiling, toxicology analysis, and fingerprint matching, along with a detailed scene investigation to establish circumstances surrounding the death and confirm identity.

Conflict of interest: There is no conflict of interest among the authors.

Source of funding: There is no funding agency to support the study

Ethical clearance: This case report does not require ethical clearance as it is based on a single case analysis.

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From Anatomy to Analysis: Current Trends and Future Directions in Bite Mark Forensics

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Abstract

Bite marks play a crucial role in forensic science. This review talks about their anatomy, characteristics, and the history of development within the discipline. It covers methods used in the analysis of bite marks, highlighting advancements in imaging and comparative analysis. It further talks about current challenges faced by the forensic experts and the effects of new technologies. It focuses on future directions for bite mark forensic science, emphasizing the necessity of standardized processes and inter-professional collaboration to improve precision and reliability.

Key Words: Bite marks, Forensic Odontology, Anatomy, Dental Structure, and Imaging Techniques.

Introduction

Bite marks are impressions which human or animal teeth leave on any surface, mainly skin. Generally, they are associated with crimes related to violence, sexual assault or rape, or child abuse. As in cases of violence, the bites can help characterize the size, shape of the teeth, and how deep they imprint on any surface.¹ this may prove useful in identifying suspects or the victims.²

Bite marks can be used in age and sex identification, diet analysis, and dental modification detection. They usually appear on victims, suspects,

and objects like food or tools, especially in sexual offenses and child abuse cases. Forensic odontologists use them to link the crime scene, suspect, and victim.³

The bites help in crime detection since the ante-mortem dental profile is compared with the post-mortem dental profile to try and identify victims in mass disasters. Fresh marks suggest a fresh offense, and their appearance can hint at the perpetrator's motive and psychological state.

History

The application of bite marks has been in existence for a long time, but it was standardized only during

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the 20th century. Forensic odontology got academic recognition in the middle 1900s and evolved from the generalized dental traits to a more precise and systematic identification method.

Preliminary Use of Bite marks:

1. Powai Rape Case (2015) - Mumbai, Maharashtra

Forensic odontologists assisted Mumbai police by analyzing bite marks found on a 28-year-old victim, which contributed significantly to securing a conviction.⁴

2. Surat Child Rape and Murder Case (2017) - Surat, Gujarat

In this tragic case of a seven-year-old girl, bite mark evidence played a key role in identifying and convicting the suspect. The bite patterns on the victim's body were matched with the suspect's dental impression.⁵

Evolution of Techniques and Methodologies:

Bite mark analysis began as an intuitive process, based upon comparisons of photographs to casts of the dentition. It was, however, in violent crimes like child abuse, sexual assault, and murder that it would one day prove important. For example, serial killer Ted Bundy was convicted in 1978 based on dental evidence of a bite left on the body of a victim named Lisa Levy.⁶

Challenges and Controversies in Early Use:

Bite marks help in crime detection by comparing the ante-mortem and post-mortem dental profiles, thereby helping in victim identification in mass disasters. Fresh marks indicate recent offenses and may reveal the motive and mental state of the perpetrator.^{7, 8}

Anatomy and Characteristics of Bite marks

Understanding the dental characteristics of Bite marks, structure of teeth always helps in solving

such cases and identifies the victim or suspect. Sometimes unique characteristics of the person's tooth differentiate him from other people and helps in solving crime.

Dental Structure:

There are four types-teeth are found in the mandible and maxilla. There are incisors, premolars, molars, and canines. People have two cycles of teeth: 20 primary or deciduous teeth and 32 permanent teeth.

A tooth is divided into two parts: the crown and the root. The crown is covered by enamel, which is the hardest substance in the body, while the dentin beneath forms most of the tooth's structure. The pulp is at the center, and the root is covered by cementum.⁹⁻¹²

The shape and size of teeth determine the origin of a bite mark and make it different from other types of injuries. Maxillary incisors produce rectangular marks, while central incisors are the widest. The individual variations in the size, shape, and wear of teeth can connect a bite mark to a specific person.¹³⁻¹⁵

Affecting Factors:

Bite mark interpretation is influenced by pressure, skin type, movement, and surface texture. Deep marks are made by high-pressure bites, while low-pressure ones fade quickly. Marks appear clearer on smooth surfaces. They change in color, texture, and size as they heal. Decomposition further alters marks.^{16, 17}

Types of Bite marks:

Nonhuman bites are different from human bites [figure 1&2]. Animal bites are deeper than human bites. They can be differentiated by tooth morphology and their tooth alignment, shape, structure etc.

There are 7 different types of bite marks.¹⁸

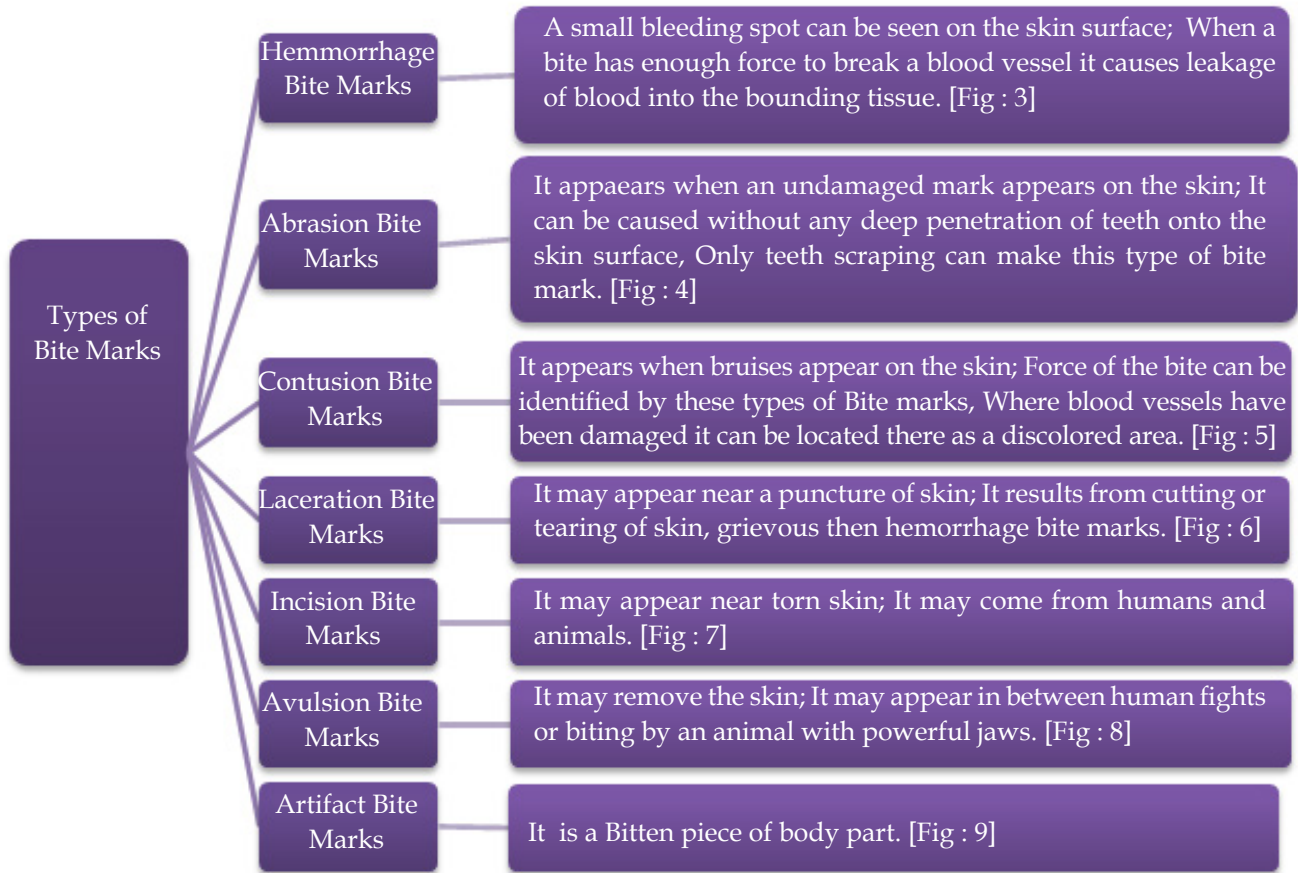


Fig 1: Types of Bite Marks



Fig 2, 3 Human & Animal Bite mark



Fig: 6, 7 Contusion & Laceration bite mark

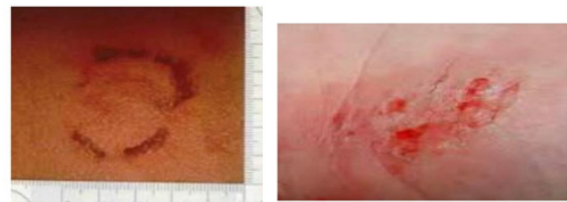


Fig: 8, 9 Incision & Avulsion bite mark



Fig 4, 5 Hemorrhage & Abrasion bite mark



Fig: 10 Artifact bite mark

Methodologies in Bite marks

Bite marks are evidence that disappears with time, so its collection and preservation are the most important steps that forensic odontologists have to take. Both Collection and preservation must be done with care.

Collection and Preservation of Bite marks:

It is vital to include inoculation of a description of the bite mark, location, size, shape, color, kind of injury, and other details that may help gather dental evidence. Among those evidence that are derived from victim and suspect include history, photographs, tissue samples, imprints and saliva samples. Here some guidelines are accepted by the American Board of Forensic Odontology (ABFO).^{19,20}

PHOTOGRAPHY:

Orientation and close-up photos should be taken with and without the placement of a scale next to the Bite marks. Scale placement should be in the same plane as the Bite marks, showing both linear and circular references to help eliminate distortion, good film resolution, along with proper color balance, are needed

SALIVARY SWABBING:

When there is no variation in the bite site from the time the bite was inflicted salivary samples must be retrieved.

IMPRESSIONS:

Impressions of the bite site region should be taken when the surface detail is of an adequate amount to provide useful information. The material used should meet the American Dental Association requirements and sufficient must be provided to ensure that the body site contour is adequately supported and repeatable.^{21, 22}

Comparison of Bite marks:

After collection and preservation of Bite marks it is necessary to compare it with suspect and victims Bite marks to give an opinion about the event that had happened. Comparison of Bite marks also helps in age estimation and sex determination of deceased.

AGE ESTIMATION FROM BITEMARKS:

Bite marks help in the estimation of a person's age by different appearance and characteristics.

The following are the possible assistance by Harvey on dental age estimation.^{23, 24}

1. Dental bacteria appearance,
2. The earliest manifestation of mineralization,
3. The completeness of the interrupted tooth,
4. Development velocity of enamel and neonatal line,
5. Clinical outburst,
6. Length of the roots of teeth,
7. The extent to which the deciduous are resorted,
8. Attrition of the crown,
9. Formation of cementum.

SEX DETERMINATION FROM BITEMARKS:

Teeth are used to determine sex in addition to age. Forensic dentists examine teeth and cranium features, particularly in partial remains, to aid in gender determination. Traits such as tooth shape, crown size, root length, and skull patterns characterize male and female features. PCR amplification ensures accurate sex identification from remains.^{25, 26}

3D TECHNIQUE:

3D scanners and computer-assisted analysis improve the accuracy in forensic odontology applicable both for skin and objects. It reduces exposure to pathogens, simplifies the handling of evidence, improves communication between members, and allows remote testimony from experts.²⁷

Contact 3D scanner [Fig: 11] have the risk of damaging evidence and are not suitable for concave surfaces, so non-contact methods such as laser scanning are preferred. Although 3D laser scanners [Fig: 12] are accurate, they may distort sharp incise edges and therefore affect tooth morphology. Accuracy margins must be considered in forensic applications.²⁸

In 2003, Thali et al introduced photogrammetric for the presentation of 3D skin bite marks. This technique creates a 3D replica from overlapping 2D

images and is cost-effective, using free software with low technological requirements. Its accuracy matches laser scanners and is widely used in recording 3D skin injuries in forensics.²⁹⁻³¹

Bite marks can only be identified using identifiable dental features such as nicks or chips. Absence of these, especially in orthodontic patients or in skin bites, makes identification of criminals impossible. 3D scanners and computer methods show promise but have issues with small sample sizes and invalidated software.^{32, 33}



Fig 11: 3D Contact Scanner & Fig 12: 3D Laser Scanner

Current Trends and Future Directions

As we all know, Forensic is still developing in India. It will take some time to develop in many forensic fields, Forensic Odontology is also one such field. There is still a lot of research going on in this field, with time something new will come here in India.

Advances in Technology and Methodology:

Post-mortem dental examination is essential for the identification of a person, usually by matching dental radiographs with ante-mortem records. Digital technology has also revolutionized forensic odontology to enhance diagnostic visualization and treatment processes to the advantage of forensic dentistry and medicine.³⁴

DENTAL IMAGING EXAMINATION:

Some research used digital imaging and/or data processing software for the evaluation of pulp area, size, tooth length, and root anatomical form in permanent and primary teeth. Research studies concluded that the outcome was informative for age estimation and individualization purposes.³⁵⁻³⁸

Researchers observed the appearance of the Dental Radiography images of teeth with a single root canal and teeth that were previously endodontically treated to conclude that it possessed very specific morphological traits. Images were recorded in a digital data bank system.³⁹

However, the manuscript has highlighted that mental foramen position, ramous height and width, and mandible angle as established by Lateral Cephalography and Digital Panoramic Radiography formed the keystones for gender determination and age prediction of a given person.^{40, 41}

Researchers determined that postmortem full-body Computed Tomography (CT) [Fig: 13] imaging could be a useful tool in individual dental identification processes after confirming the viability of dental identification using this type of imaging.⁴²

The "Fusion Function of Imaging Analysis Software" enables the analysis of tooth root apices in postmortem CT scans or dental radiographs across different imaging modalities. Forensic dentistry has advanced with the use of dental cone-beam CT, where algorithms assist in image processing and matching tooth morphology for improved dental radiograph analysis.⁴³

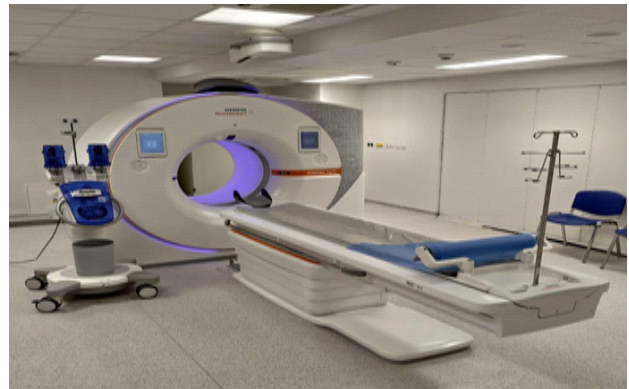


Fig 13: Computed Tomography

AI in FORENSIC ODONTOLOGY:

AI-based models have advantages in identifying individuals from dental casts, radiographs, and records. The limitation of human error is obviated. A proof-of-concept study related to the identification of bite mark using artificial neural networks appears promising but requires further developments and training to enhance accuracy.⁴⁴

Interdisciplinary Approaches in Forensic Odontology:

Forensic odontology is divided into three main categories: (1) identification of individuals in criminal cases or mass disasters, (2) examination of injuries to jaws, teeth, and oral tissues, and (3) evaluation of bite marks in cases of abuse, assault, or self-defense.⁴⁵

Identification by dental means gains more importance because the dental tissues are often preserved even if the deceased person is skeletonized, decomposed, burnt, or dismembered. Dental tissues are often used to determine the age, sex, and ethnicity of a person.⁴⁶

TEETH - A SOURCE OF DNA:

Teeth are resistant and their morphology is well preserved, with enamel protecting the pulp from external damage. The hydroxyapatite crystals in enamel help stabilize and bind DNA, making it possible to easily recover DNA from teeth. However, the crushing of teeth destroys the morphology, though DNA may still be recovered.⁴⁷

IDENTIFICATION METHODS OF DENTURE:

Identification prostheses that don't deform by extreme temperatures are more efficient. Registration of Denton-prostheses with adequate markings including metallic bands, micro-engraving, barcodes or electronic microchip is encouraged.⁴⁸

Legal Implications in Bite Marks

The question was the subject of several high-profile cases in recent years where a number of dental professionals on both sides presented valid arguments for or against the contention that the markings inoculated the defendant. These glaring discrepancies in interpretation have sometimes made some people doubt the validity and scientific objectivity of the bite marks.⁴⁹

LEGAL STATUS OF BITE MARKS:

1. **ADMISSIBILITY:** Since judges and juries are not necessarily equipped with scientific knowledge, they need expert testimony to determine admissibility. The Frye test in 1923 sets down three requirements for admissibility: 1) the discovery must be verified by a principle, 2) it must follow well established methods, and 3) it must be

accepted by experts in the relevant scientific field.⁵⁰

2. **IMPARTIALITY:** Criminal defense attorneys question the objectivity of bite mark investigators, as most forensic dentists work for law enforcement, which could create bias towards prosecution, though not all show this bias.⁵⁰

Conclusion

Bite marks are an important part of forensic science, and their contribution to the criminal case is unique. The technological advancements in analysis still face problems like subjectivity and variations in human dentition. To overcome such issues, future developments must focus on standardizing procedures, improving education for forensic professionals, and fostering multilateral cooperation. These measures would enhance the probative value and admissibility of bite mark evidence in court, thus improving criminal justice outcomes.

Conflict of Interest: Nil.

Source of Funding: No external funding was received for this study.

Ethical Clearance: Not applicable.

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A Study of Pattern of Fatal Thoraco-Abdominal Injuries in RTA Cases: An Autopsy Based Cross Sectional Study

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Abstract

Road Traffic Accident (RTA) is any vehicular accident occurring on the roadway i.e. originating on, terminating on, or involving a vehicle partially on the roadway. Road traffic accident ranks among the top causes of death in the world; after ischemic heart disease, it is projected to become the second leading cause in 2024. In developing countries, around 85 percent of all deaths caused by road accidents occur, and nearly half of these accidents happen in the Asia-Pacific region. India is responsible for around 10 percent of all road accident deaths in the whole world. Road traffic accidents in India is emerging as a major cause of death and injury with subsequent disability and burden on economy of a nation. The cost of burden due to RTA is estimated to be around 1-2% of a country's gross national product (GNP) specifically in lower-income countries. In this study we have analyzed the 109 cases of road traffic accidents brought for autopsy examination by the police to the Department of Forensic Medicine & Toxicology, JGMCH, Jalpaiguri, West Bengal from the January 2024 to June 2024. All data were analyzed by appropriate statistical tool (SPSS software). In this study we found that the peak incidence of RTA was observed in the age group 21-40 years comprising 41.28% of the cases. Males (88.07%) outnumbered females (11.93%) and most of the accidents occurred between night times from 8pm to 6am (48.62%). In 47.78% cases there was immediate death and cases are seen mainly in two wheeler occupants (42.20%). In two wheelers accidents, drivers (84.78%) were more commonly affected and in four wheelers accidents, front seaters were affected (55.55%). It has been observed the lungs injuries are common 32.11% cases compared to heart injuries (18.34%) cases in thoracic involvement and liver with 14.67 % cases is more commonly affected abdominal organ compared to spleen (2.75%) in abdominal injuries.

Key words: Road Traffic Accident, RTA, Thoraco-abdominal injuries, Lung injuries, Heart injuries, Liver injuries, Spleen injuries.

Introduction

The World Health Organization (WHO) describes an accident as "an unpremeditated event resulting in

recognizable damage." Road Traffic Accident (RTA) is any vehicular accident occurring on the roadway i.e. originating on, terminating on, or involving a vehicle partially on the roadway.^[1] Most automobile

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accidents are accidental, few are suicidal and still fewer are homicidal in manner. RTA is a public health issue that greatly affects individuals, families, communities, and nations. Pattern of injuries and circumstances of death often indicate the manner.^[2] Road traffic accident ranks among the top causes of death in the world; after ischemic heart disease, it is projected to become leading cause of death in developing countries, around 85 percent of all deaths caused by road accidents occur, and nearly half of these accidents happen in the Asia-Pacific region.[the second leading cause in 2024.^[3] India is responsible for around 10 percent of all road accident deaths in the whole world. Road traffic accidents in India is emerging as a major cause of death and injury with subsequent disability and burden on economy of a nation.^[4] RTA is a serious public health issue that greatly affects individuals, families, communities, and nations. The cost of burden due to RTA is estimated to be around 1-2% of a country's gross national product (GNP) specifically in lower-income countries.^[5]^[6] As per the data released by the Ministry Of Road Transport And Highways, during the calendar year 2022, road crashes in India claimed about 1.68 lakh lives and caused injuries to more than 4.4 lakh people and number of fatal accidents and numbers of persons killed in road accident increasing since 2005. Decadal trend reveals increasing trend in number of accidents, person killed and injuries.^[18] In Southeast Asian countries RTA are among the top five causes of fatality. In 2011, India witnessed 4.97 lakhs reported road traffic accidents, 142485 reported deaths, an accident every one minute and death every 3 minutes.^[19] It has been reported that involvement of the abdominal organs like the liver, spleen, bladder, and kidney is in descending order of frequency in road traffic crashes.^[7] Amongst accidental occurrence, road traffic accidents are the commonest cause for thoraco-abdominal injuries and 45.08 % of victims in both fatal and non-fatal categories had injuries to thoracic region(23.21%), followed by injuries to thoraco-abdominal region (23.21%) while the injuries to abdominal region comprised only 17.20% cases.^[8] After the strict implementation to use the helmet in two wheelers, the number of head injuries due to road traffic accidents has subsequently decreased, but injuries fatal thoracic and abdominal regions are still on the rise. Due to the anatomical position,

thoracic and abdominal viscera are commonly injured in road traffic accidents. The objectives of the present study is to describe the distribution of injuries and demographic profile and also to study the pattern of thoraco-abdominal injuries sustained as a result of road traffic accidents as most of the RTA studies are focused to prevent injuries to brain but injuries to the thoracic and abdominal viscera are also causing significant mortality during RTA so we conducted the study of autopsied cases of RTA at the mortuary of Department of FMT, JGMCH from January 2024 to June 2024, the data of study can be used for the development of a strategy for the prevention of thoraco-abdominal fatalities due to RTA and creating public awareness on road safety.

Objective: The objectives of the present study are to describe the distribution of injuries and demographic profile and also to study the pattern of thoraco-abdominal injuries sustained as a result of road traffic accidents and autopsied at the mortuary of Department of FMT, JGMCH from January 2024 to June 2024, which can be used for the development of a strategy for the prevention of mortality due to RTA and public education on road safety.

Materials and Methods

It is a prospective cross sectional study conducted to analyze the pattern of Fatal Thoraco-abdominal injuries cases in road traffic accidents autopsied in the mortuary of Department of FMT, JGMCH, Jalpaiguri from January 2024 to June 2024. Total 109 cases of fatal thoraco- abdominal injuries cases were included in this study. Detailed information on the cases was based one valuation of post-mortem findings. Data was analyzed with age, sex, nature of the collision, time of the collision, survival period, distribution of injury, internal findings, and cause of death, which were acquired from police inquest, history obtained from medical records and relatives. We have analysed all data with the help of latest version of SPSS software.

Inclusion criteria: 109 cases of road traffic accidents autopsied at the mortuary of Dept. of FMT, JGMCH, Jalpaiguri.

Exclusion criteria: Decomposed cases and unknown brought dead RTA cases were excluded from the study.

Observation

Table 1: Epidemiological profile of deceased

AGE DISTRIBUTION:		
Age	Frequency	Percentage of age
Up to 20 years	7	6.42
21 to 40 years	45	41.28
41 to 60 years	38	34.86
>60 years	19	17.43
Total	109	100
SEX DISTRIBUTION		
SEX	No. of cases	Percentage
Male	96	88.07
Female	13	11.93
Total	109	100
DRUG HISTORY		
Alcohol & Other Drugs	No. of cases	Percentage
Present	18	16.51
Absent	91	83.49
Total	109	100

The age of the victims varied from 1 years to more than 60 years. The peak incidence was observed in the age group 21-40 years comprising 41.28% of the cases. It was also observed that 34.86% belonged to the age group 41-60 years. Individuals in the age group below 20 years were the least affected (17.42%). Males comprised a majority and constituted 96 (88.07%) compared to females who were only 13 (11.93%). The male to female ratio in the study was 7.38:1 (Male=96 Female=13). It is seen from the above table most of the victims were not under the influence of alcohol and other drugs with 91 cases (83.49%) and only in 18 cases (16.51%) evidence of alcohol and others drugs were present.

Table 2: Profile of road users

TIME OF INCIDENT		
Time of incidence	No. of victims	Percentage
6 AM to 12 Noon	19	17.43
12 Noon to 8 PM	37	33.95
8 PM to 6 AM	53	48.62
Total	109	100

Continue.....

TIME OF DEATH		
Time of Death	No.of cases	Percentages
Instant Death	43	39.46
Immediate Death	52	47.78
Delayed Death	14	12.86
Total	109	100
ROAD USER		
Road User	No. of victims	Percentage
Pedestrian	38	34.86
Two Wheeler Occupant	46	42.20
Four Wheeler Occupant	18	16.51
Heavy Vehicles Occupant	7	6.42
Total	109	100

Most of the incidents irrespective of the cause, occurred between 8PM to 6AM comprising 53 cases (48.62%) of total cases followed by at the time 12 Noon to 8 PM 37 cases comprising 33.95 % and the least number of incidents occurred between 6AM to 12 Noon comprising 17.43% of the total cases. It is seen from above table that most of the death occurred of my study population is immediate death with 52 cases (47.78%), followed by instant death with 43 cases (39.46%) and delayed death occurred in only 14 cases comprising with 12.86%. It is seen from the above table that most of the victims in the study were two wheeler occupants with 46 cases (42.2%) and followed by pedestrian 38 cases (34.86%) and four wheeler occupants were 18 cases comprising 16.51%, and Heavy Vehicles Occupant in only 7 cases (6.42%).

Table 3: Profile of two wheeler users

TWO WHEELER USER		
Two wheeler user	No. of cases	Percentage
Driver	39	84.78
Back Sitter	7	15.21
Total	46	100
HELMET WEAR		
Helmet	No. of cases	Percentage
Present	19	41.30
Absent	27	58.70
Total	46	100

Among the all deaths in this study due to two wheeler accidents, most of deceased were driver comprising 39 cases (84.78%) followed by back sitters 7 cases (15.21%). It is seen from the above table that most of the two wheeler user not wear the helmet during driving with 27 cases (58.70%), followed by 19 cases (41.30%) used the helmet.

Table 4: Profile of four wheeler users

FOUR WHEELER USER		
Four wheeler users	No. of cases	Percentage
Driver	5	27.78
Front sitter	10	55.55
Back sitter	3	16.67
Total	18	100
SEAT BELT USERS		
Seat Belt	No. of cases	Percentage
Use	6	33.34
Not use	12	66.66
Total	18	100

It is seen from the above table that most of the four wheeler user was dead who were sitting in front of the car with 10 cases(55.55%), followed by Four wheeler driver 5 cases (27.78%), then back sitter with only 3 cases comprising with 16.67%. It is seen from the above table that most of the four wheeler user not used the seat belt during driving with 12 cases (66.66%), followed by 6 cases (33.34%) who had used the seat belt.

Table 5: Thoracic injuries

Chest Injuries		
Types of injuries	No. of cases	Percentages
Contusion	23	21.10
Laceration	11	10.09
Abrasion	17	15.59
Fracture clavicle	3	2.75
Ribs fracture(Lt.)	4	3.66
Ribs fracture(Rt.)	7	6.42
Both side Rib Fracture	18	16.51
Sternum fracture	2	1.83
Absent	61	55.96
Lungs		
Lungs injury	No. of cases	Percentages
Laceration	12	11.00

Continue.....

Contusion	23	21.11
absent	74	67.89
	109	100
Heart		
Heart injury	No. of cases	Percentages
Contusion	16	14.67
Laceration	4	3.67
Absent	89	81.66
	109	100

It is seen from the above table of the chest wall injuries that contusion were present in 23 (21.10%) cases, both side rib fracture was present in 18 (16.51%) cases, followed by abrasion injury were present with 17 (15.59%) cases, right sided ribs fracture was present with 7 (6.42%) cases, followed by left sided ribs fracture was presented with 4 (3.36%) cases, fracture clavicle was present with 3 (2.75%) cases and 2 (1.83%) cases with fracture sternum. It is seen from the above table that lungs injuries were absent in 67.89% cases and heart injuries were absent in 81.66% cases. Contusion of the lung was present in 12 (11.0%) cases & contusion of the heart was present in 4 (3.67%) cases.

Table 6: Abdominal Injuries

Abdominal wall injuries		
Types of injuries	No. of cases	Percentages
Contusion	9	8.25
Laceration	6	5.50
Abrasion	22	20.18
Absent	72	66.05
	109	100
Liver		
Liver injury	No. of cases	Percentages
Contusion	5	4.58
Laceration	11	10.09
Absent	93	85.33
	109	100
Spleen		
Splenic Laceration	No. of cases	Percentages
Present	3	2.75
Absent	106	97.25
Total	109	100

Continue.....

Pelvic region		
Pelvic Injury	No. of cases	Percentages
Fracture pubic bone	6	5.51
Dislocation of symphysis pubis	8	7.34
Absent	95	87.15
Total	109	100

It is seen from the above table that abrasion on abdominal wall was present in 22 (20.18%) cases, followed by contusion of abdominal wall (8.25%) cases and then followed by fatal laceration of the abdominal wall in only 6 (5.57%) cases and in most of the 72 cases (66.05%) there was no abdominal wall injury. It has been observed that liver with 14.67% cases is more commonly affected abdominal organ compared to spleen (2.75%) in abdominal injuries. In 87.15% cases there were no injuries to the pelvic bones and in 8 cases (7.34%) there was dislocation of pubis and in 6 cases (5.51%) there was fracture of pubic bones. In 10.09% cases there were liver laceration that proved to be fatal and only in 2.75% cases fatal laceration of spleen were found.

Discussion

In the present study the age of the victims varied from 1 to above 60 years. The peak incidence was observed in the age group 21-40 years comprising 41.28% of the cases followed by age group of 41 to 60 years of age (34.86%). This finding is in accordance with studies done by Tyagi, Sinha and Sengupta, Banerjee, Salgado.^[9] ^[10]^[11]^[12] In the present study males comprised a majority and constituted 96 (88.07%) compared to females who were only 13 (11.93%). The male to female ratio in the study was 7.38:1. Sinha and Sengupta reported 80% male and 20% female incidence ^[10]. A male preponderance is in consistence with the study reported by Cardoso, and Seow^[13] ^[14]. Gole, Soni and Gonade has also observed that out of 105 cases of fatal head injury during RTA, 89% male and 11% female were observed male and female ratio is 7.75:1^[20] It is seen from the above table that most of the victims in the study were two wheeler occupants with 46 cases (42.2%) and followed by pedestrian 38 cases (34.86%) and this finding is not in accordance with the findings observed by Dikshit who found that most common victims were pedestrians constituting 50% of total cases followed by motorcyclists constituting 18.28% of total cases as

their study was conducted in between 1966 - 1976 and at that time two and four wheelers were not prevalent so pedestrians were more exposed to RTA.^[15] It has been observed the lungs injuries was common (32.11% cases) compared to heart injuries (18.34% cases) in thoracic involvement and liver with 14.67% cases is more commonly affected abdominal organ compared to spleen (2.75%) in abdominal injuries. Dr. Anindya kumar Goswami (2009-2012) a study of pattern and distribution of injuries in fatal road traffic accident cases for last three years in the jurisdiction of Kolkata police morgue, Kolkata. This study shows that, among the 42 cases the liver injury, contusion was present in 36 cases and laceration was present in 6 cases. The findings in our study are not similar to the findings of the above mention author ^[16].

In the present study shows that, most of the two wheeler user not wear the Helmet during driving with 27 cases (58.70%), followed by 19 cases (41.30%) used the Helmet and this finding is similar to the findings of Michael Johnson at Ohio in which only 77 (23%) of those studied wore helmet, whereas 254(77%) were not helmeted^[17].

Conclusion

This study attempts to contribute to the body of knowledge on road safety. It is hoped that it will inspire and facilitate increased cooperation, innovation and commitment to prevent road traffic accident ultimately preventing the fatal thoraco-abdominal injuries. Incorporation of safety measures in the two and four wheelers to protect the thoracic and abdominal region and most important is creating awareness among general public which will help in reducing morbidity & mortality due to fatal road traffic accidents.

Conflict of Interest: No conflict of interest.

Source of Funding: Self.

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Relationship Between Arm Span and Human Stature

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Abstract

Background: Incomplete human remains may be recovered from crime scenes in some cases. Stature estimation from this remains may be very important in the personal identification of the corpse or living. If the stature could be estimated it can definitely narrow down the search in missing individual data base. This study was intended to find out correlation between arm span and human stature and to derive a regression model to find out human stature from arm span.

Materials and methods: This cross sectional study was conducted on 100 subjects, 50 males and 50 females aged between 21 and 60 years in the department of Forensic Medicine in Govt. Medical College, Thrissur.

Results: In females the average arm span is 159.91 ± 5.61 cm, and mean height is 157.44 ± 4.93 cm, correlation coefficient (r) is 0.8388. In males the average arm span is 173.83 ± 6.02 cm and mean height is 170.85 ± 5.2 cm, correlation coefficient (r) is 0.8934. Thus stature is strongly correlated with arm span. The knowledge of correlation between arm span and stature is important for identification of unknown bodies when a part of body with intact upper extremities on the axial skeleton is available and from that the height of the person can be calculated in situations like mass disasters- landslide, aeroplane crash, railway occurrence, animal attack, homicides with dismembered body etc and a comparison can be made with the available missing persons data. It can also be used for identifying stature in cases like amputation of the lower limb done due to various diseases and spinal deformities.

Key Words: Stature, arm span, upper extremities, forensic anthropometry, identity

Introduction

Stature estimation from various skeletal elements has been an area of vital interest among researchers.¹ Many varieties of mathematical techniques were developed to derive stature from both living, dead and

skeletal remains. Trotter and Gleser method is among the mostly used techniques.²⁻⁴ Identification and identity establishment makes an autopsy complete.

The need of stature identification becomes necessary in mutilated fragments, shattered remains,

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amputated and decomposed bodies collected from mass disasters like tsunamis, bomb blast, earth quakes, road traffic or aeroplane crash, war, landslide, flood etc. The importance and significance of cephalometry, craniometry, somatometry and osteometry for the identification of human remains have been described and later on a new term 'Forensic Anthropometry' was coined.⁵

The foundation from which anthropology has evolved includes the big 4s Age, stature, sex and race.⁶ The key methods used for stature estimation are Mathematical and Anatomical methods. In the mathematical method it takes advantage of higher linear correlation between stature and long bones. One can use a regression equation with long bone as the independent or dependent variable that reflects the relationship between an individual's stature and the chosen bone. In anatomical method, which is commonly known as "Folly's method", stature is reconstructed by summing the measurements of skeletal elements which contribute the height and a correction factor is added for the soft tissues.⁷

Stature estimation from bones is a time consuming process which involves the preparation, cleaning and washing of bones. Because of this reason Forensic anthropologists are using percutaneous measurements instead of bone measurement. The measurement of arm span for identifying human stature can be added to the arsenal and the correlation between arm span and stature was found to be most reliable.⁸

Arm span is the maximum distance between the tips of the longest fingers of both hands when the person extends both arms at the level of the shoulders. Stature is the distance measured from the point where the heel touches the floor to the highest point of head when the person is in erect position.⁹ This study was done to find out the relationship between arm span and human stature. Another purpose of the study was to derive a regression equation to find out stature from arm span. Studies had been conducted by Agnihotri AK et al, Sen J et al and Saco-Ledo G et al to know the correlation between stature and foot length, stature and foot breadth, stature and tibial length.¹⁰⁻¹² Investigators like Gardasevic J et al, Arifi F et al and Supare MS et al had conducted studies to find out relationship between arm span and stature.¹³⁻¹⁵ Study by Mohan Kumar S and Kamate V among farm workers of North

Karnataka region shows that there were differences in body dimensions from the anthropometric data of other part of India.¹⁶ We have conducted this study among students and staff at Govt. Medical college, Thrissur, Kerala to find out correlation between arm span and stature and to find out a regression formula to detect stature from arm span.

Objectives:

1. To find out relationship between arm span and stature of an individual.
2. To formulate a regression model to predict stature of an individual from arm span.

Materials and Methods

This is a cross sectional study on 100 subjects, 50 males and 50 females aged between 21 and 60 years in the department of Forensic Medicine in Govt. Medical College, Thrissur. Sampling technique used was simple random sampling. Subjects were taken in the age group of 21-60 years as to include the persons whose ossification centres are completely fused.

Inclusion criteria:

All Staffs and post graduate students over age 21.

Exclusion criteria:

1. Subjects with apparent symptomatic diseases, deformities, fracture, amputation or history of any surgical procedures of upper limbs.
2. Who are not willing to participate in the study.

Measurement of stature:

The subjects were asked to stand erect with their heel together and backs straight as possible so that the heels, buttocks, shoulders and the head touch the rod of stadiometer (in Frankfurt plane). The arms were hung freely by the sides. Reading was taken from the stadiometer scale at the vertex point (highest point on their head) with sensitivity 0.5 cm. The reading was taken three times and their average value was taken.

Measurement of arm span.:

It was measured using a measuring tape up to 5mm accuracy. Arm span was measured as the distance between tip of middle fingers on the stretched upper limbs.

Results

Table 1. Total study population

Individuals analysed	
No. of males	50
No. of females	50
Total	100

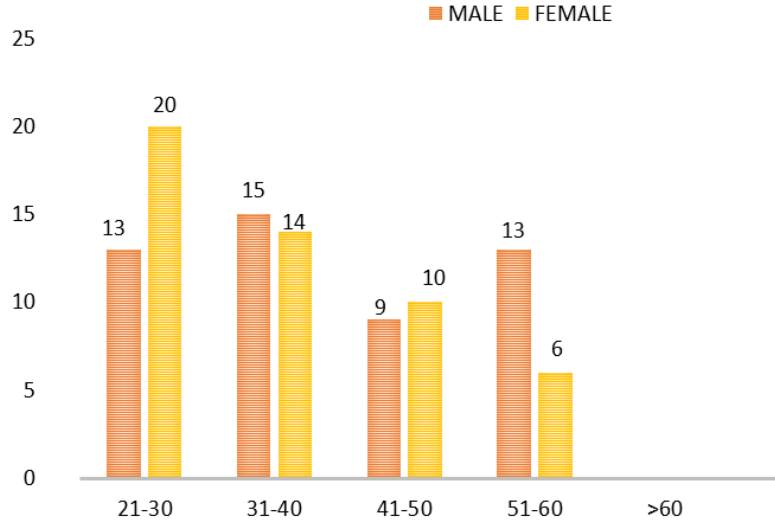


Figure 1: Age wise distribution of study population.

The age group of the female subjects ranged from 21 years to 60 years with the mean age of 35.54 ± 8.49 SD. The age group of male subjects ranged from 21 years to 60 years with the mean age of 37.39 ± 9.58 SD. Majority of the subjects belongs to the age group 21-30 years. Even though there are more women in the 21-30 age group and less women in the 51-60 age group this would not have impact on the finding because of the fact that in both these cases ossification centres are fused and height and arm span remain constant.

Arm span and height were calculated by standard procedure.

The height of subjects varies from 144 cm to 183cm with mean height of 164.15 ± 8.42 SD

Arm span varies from 146 cm to 185 cm with mean of 166.87 ± 9.08 SD.

Correlation between arm span and stature

In males the mean arm span is 173.83 ± 6.02 cm. Correlation coefficient (r) is 0.8934

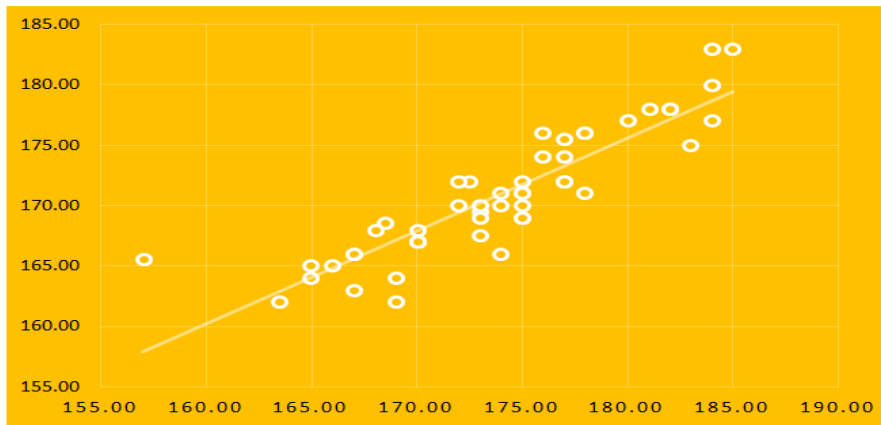


Figure 2. Correlation between arm span and stature in males

In females the mean arm span is 159.91 ± 5.61 cm.

Correlation coefficient (r) is 0.8388.

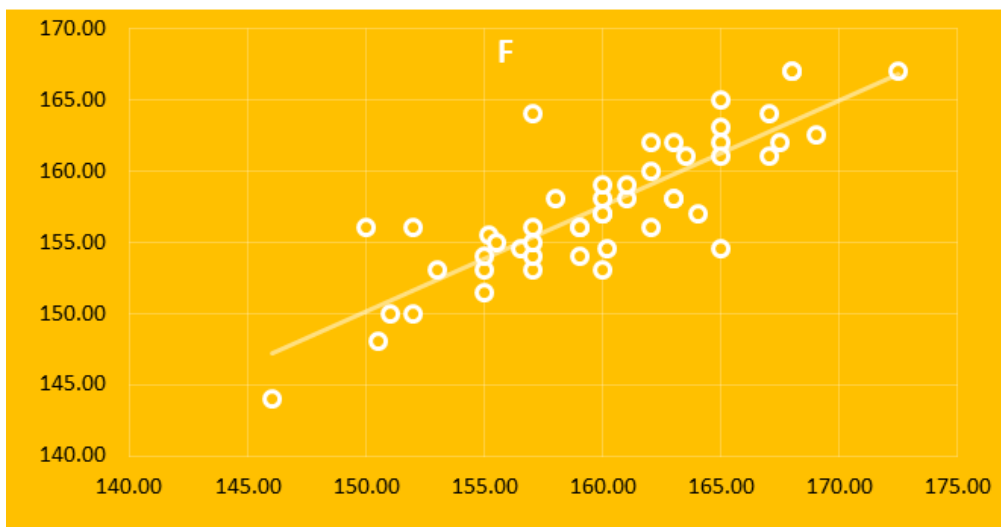


Figure 3. Correlation between arm span and stature in females

In males and females combined, the mean arm span is 166.87±9.08 SD

Correlation coefficient is (r) is 0.9483

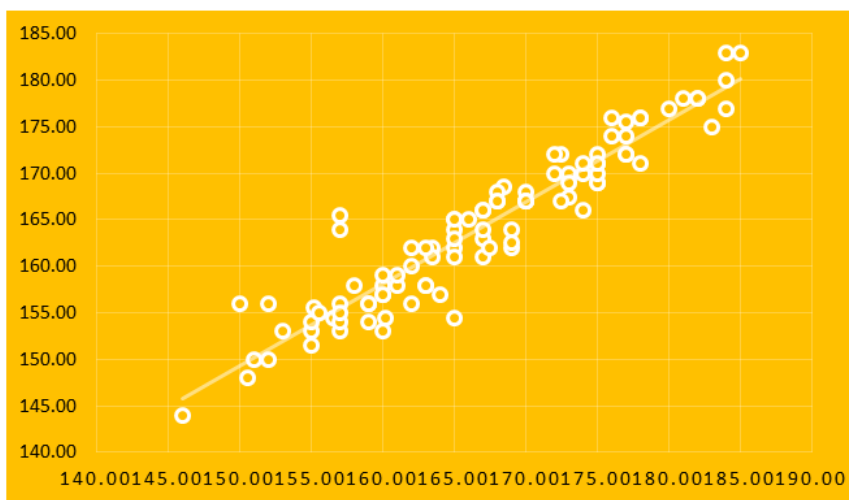


Figure 4. Correlation between arm span and stature in males and females combined

Table 2: Correlation coefficient of males and females

Correlation coefficient (r)	
Correlation coefficient (r) in males	0.8934
Correlation coefficient (r) in females	0.8388

REGRESSION EQUATION

- The Regression equation

Height of individual (Y) =intercept (a) + slope (b) x X,

Where, X = Arm span

Y= Height of individual

- The regression equation for individuals(including both males and females) was found to be:

$$\text{Height of individual} = 17.475 + 0.87895 \times X$$

P values, P < 0.001, SE for intercept (a) = 4.966, SE for slope (b) =0.03

Regression equation for males

$$Y = 36.788 + 0.771 X$$

Subject Regression coefficient Standard error-p-value

Male 0.771 0.056 < 0.0001

Constant = 36.778, SE=9.735, P < 0.0001

Regression equation for females

$$Y = 39.393 + 0.738 X$$

Subject Regression coefficient Standard error p-value

Female 0.738 0.069 < 0.0001

Constant = 39.393 SE = 11.064, P < 0.0001

Discussion

This study was designed to show the relationship between human stature and arm span and also to formulate a regression model to estimate stature from arm span. It was conducted among 100 subjects between 21 and 60 years of age in Govt. Medical College, Thrissur.

50 males and 50 females (total of 100 subjects) were analysed during the study period of one year. The mean age of females in the present study was 35.54 ± 8.49 SD and majority belongs to the age group 21-30 years. The mean age of males in the present study was 39.24 ± 10.30 SD and majority belongs to the age group 21-30 years.

Stature of males varies from 162 cm to 183 cm; the mean stature was 170.85 ± 5.20 SD.

The stature of females varies from 144 cm to 167 cm; the mean stature was 157.44 ± 4.93 SD.

The stature of males and females taken together varies from 144 cm to 183 cm and the mean stature of the same was $164.15 \text{ cm} \pm 8.42 \text{ cm}$.

The arm span ranges from 146 cm to 185 cm with mean of $166.87 \text{ cm} \pm 9.08 \text{ SD}$. The arm span in females ranges from 146 cm to 172.50 cm with mean of $159.91 \pm 5.61 \text{ SD}$.

The arm span when the males and females taken together ranges from 146 cm to 185 cm with mean of $166.87 \text{ cm} \pm 9.08 \text{ SD}$.

The present study showed that there is statistically significant correlation between stature and arm span in both sexes. The correlation coefficient (r) in males is 0.8934. The correlation coefficient in females were 0.8388. The correlation coefficient when males and females taken together is 0.9483.

The presence of sexual dimorphism of anthropometric parameters were considered in the study and the results in this study showed that there was statistically significant difference in mean value of the measurement. Different regression equations are necessary for estimation of stature from arm span in both sex groups. Sexual dimorphism in height and arm span measurements can be due to environmental, hereditary or other hormonal impacts. Estrogen and progesterone play a vital role in closure of growth plates in long bones. They also play a part in the secretion of growth hormones.

The findings of the present studies were in agreement with numerous previous studies. Study by Gardasevic J et al concluded by saying that relation between arm span and stature is high and significant in their study sample, and the arm span is apparently a reliable indirect anthropometric measurement used for estimating stature in males from the Federation of Bosnia and Herzegovina.¹³ Study by Arifi et al reached conclusion that arm span reliably predicts stature and has a high value of correlation with validity of 95% in male as well as female, which have given very high value (0.776 and 0.688) of correlations between them.¹⁴ Study by Supare MS et al among medical students of Maharashtra found correlation coefficient (R) of 0.89 in male and 0.90 in female and concluded that there was a strong correlation between stature and arm span.¹⁵

Conclusions

There was statistically significant correlation between arm span and human stature. The regression equation formulated from arm span is a best indicator of stature. There were no statistically significant difference between estimated and actual stature in female and male subjects in the study.

In conclusion, stature can be estimated when the measurement of arm span is possible. The regression equation derived can be used in cadavers with fairly

accurate results. This can be helpful in medico-legal cases as well as in anthropology studies. This study demonstrate that the stature and arm span is significantly higher in males when compared to females.

A direct relationship was observed between these measurements in both males and females. The regression equations for estimation of stature from arm span is $Y = 36.788 + 0.771 X$ for males and $Y = 39.393 + 0.738 X$ for females, where X is the arm span. The regression equation and slope obtained correlate with previous studies. There was difference in slope and intercept of the equation which is suggestive of ethnic and racial variations of the individuals. Similar studies should be conducted in different geographical locations and among different population groups in view of previously described variations. The possible limitation of study was that only one geographical location was used that is Govt. Medical college, Thrissur. This may affect the generalizability of the test results when applied to other geographic locations. However in future this regression equation derived could be tested on different populations to validate their accuracy across ethnic groups.

Ethical clearance: Institutional Ethics committee, Order No:B6-155/2019/MCTCR(28), Date:20/12/2019, Govt. Medical college, Thrissur

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Moot Court for Teaching – Learning of Court Procedures in MBBS Students in Central Region of India - An Educational Interventional Study

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Abstract

Background: As a doctor we all need to attend the court of law either as a first treating doctor or treating doctor of a speciality or as subject expert as an expert witness.

There are many incidents reported and also we experience as a part of society and medical institutes, where a doctor tries to avoid attending a case having legal implication either poisoning or some injury by any means so that he/she can avoid attending the court.

Usually it's not because the doctor feels incompetent in treatment but most of the time it is the apprehension to attend the court.

It was also observed that when a doctor goes to court for a few times they will become more confident and comfortable, but first experience remains more or less same. It gave us an idea that if Indian medical graduates, can get some practical experience during MBBS (even in simulation situation) e.g. "moot court1" with the theory class on legal procedure, this apprehension can be removed or minimised and in future they will not be hesitant to attend cases with legal implication.

Primary Objective:

Students will get more practical knowledge about court procedures.

Secondary Objectives:

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1. Future IMG will become more confident in court
2. Future IMG will not hesitate to attend medico-legal cases in future during their professional life.
3. Introducing moot court to the students as simulation technique for the court procedure.
4. To assess the effectiveness of moot court in comparison of didactic lecture on court procedure by comparing pre and post-test about the topic.

Methodology: It is an Educational interventional study.

Convenient sample technique was used for sampling method, in which 130 students of Phase III part 1 MBBS, of MIMS & R, Bhopal (MP), participated. Informed consent was obtained. Data collected by pre and post-test and paired t-test was used to analyse them.

Results: On observing in pre and post-test of the sample size 99 and using paired samples t-test (with 95% Confidence Interval of the Difference) mean value in post-test increased from 13.63 to 17.34, showed the improvement in the knowledge of the students about topic. In the study Std. Deviation decreased from 3.14 to 2.47. Std. Error Mean decreased from 0.32 to 0.25. In the study t-value is 12.1770 and P value is <0.0001.

Conclusion: Overall this study shows that "Implementation of the Moot court as a simulation technique for teaching - learning of court procedures" is beneficial for improving the knowledge of the students about the court procedures.

Keywords: Court Procedure, Medicolegal cases, Moot Court

Introduction

As a practicing physician it is also a moral responsibility of all the medical practitioners to respond to the summons/warrants etc. whenever required.

But it is very common to see that Doctors tries to avoid any case having legal implication. Even when forensic PG attend first court evidence they also feel a little bit anxious, even though as forensic PG students, they have guidance from the senior teachers and prepared for the case under their guidance.

So it is very much understood how all newly graduates must have felt, for attending the court as an expert witness, without any practical expert guidance for only "How to Act in Court".

In "Court room exposure to medical students: a practical approach to legal procedures in Indian scenario" by Gupta Sanjay² et al also concluded that the average knowledge level improved significantly.

After discussing this type of scenario with forensic medicine and other subject experts, in formal as well as informal meetings, it became evident that practitioners have this dilemma because during MBBS, as a student they attend the theory classes on the legal procedures in the subject of forensic medicine

but practical knowledge or exposure to real court procedures are not part of the curriculum till now. Hence, implementation of moot court as a simulation technique can be helpful for the students to get more practical knowledge about court procedures.

At first as a Pilot Project, we prepared a moot court¹ as simulation technique for better learning of the court procedure with student of the II MBBS (batch 2017 PCMS, Bhopal), then presented this "moot court" in a Dental college in a fellowship programme³ on forensic odontology to understand the impact of it on both, performers as well as audience and it really gave a better understanding to the both audience as well as performers. It was an experiment at that time but it gave an encouragement to go further on it. Both performers as well as audience gave positive response verbally, but no questionnaire or pre and post-test were used during that time. So it encouraged us to start this project as research work with following the proper guidelines.

Before going further here is a brief description of the moot court and discuss the literature about it.

According to Catherine Elise Barrett⁴, simulation means an imitation or an act that is only mock. As a teaching method, simulation can be interpreted as an activity that describes the real situation. The point is that students (with teacher's guidance) perform a

role in mock simulations to try to describe the actual events. In the simulation activity, participants or role holders perform a mock environment of the actual event.

In the current project, addition to acting as lawyers or judges, students were also assigned to serve as "law clerks, reporters, or amicus brief writers and also expert witness.

To get a real evaluation of the "Moot court for teaching - learning of court procedures in medical education" this research project was started. Here, students argue a mock appellate case before judges to understand "their overall act in court".

Material and Method

As court procedure taught in MBBS as theory lecture, in this project we tried to give practical experience to the students of court so they can be more comfortable in courts in future and also used this project to analyse and evaluate the impact of this practical experience, to improve student's knowledge about court procedure.

Study Design: Educational interventional study - A Quantitative Study.

Data collection method:

For getting the data and preparing the project, a meeting was held with various faculties from different departments including Community medicine as experts in this work, to discuss the difficulties they faced in the court proceedings. They also shared their experiences and what can be added in this project and what they think the challenges can be faced during this project and how to overcome them.

A meeting was also held with the 13 coordinator students, who participated in "moot court" during previous basic course of medical education workshop in the institute.

Pre and Post-test prepared in the google form that covered both theory as well as practical aspect of the legal procedure for the medical professionals. Questions in pre and post-test were same.

A theory class as interactive learning, was taken during second MBBS of the same students, as a part of curriculum and then this project was started with

a pre-test than project of moot court and in the end post-test was taken, to get to know the improvement of the knowledge (if any) of the students about the legal procedures.

The students of the Phase III Part 1 MBBS were divided in 10 groups and among them 13 coordinators were appointed for these 10 groups. They helped in the sessions to train the participants for this project.

As these coordinators were already exposed to the moot-court (during previous basic course of medical education workshop) they were excluded from the pre and post-test.

Before starting the project, students of the phase III part 1 were sensitized about the project and written informed consent was obtained from the willing students.

The students who were willing to participate in the project were asked to take a pre-test with the help of google form on the topic "legal procedures" (students were asked to prepare for pre-test, as it was not a surprise test).

Different real life medicolegal circumstances were provided to the different groups and were asked to prepare for moot court competition.

To get the guidance for procedure of moot court competition, reference from the Amity International Moot Court Competition⁵, 2021 amity law school, Noida Amity University, Uttar Pradesh rules, procedure and guidelines of the competition and Sixteenth Nelson Mandela World Human Rights Moot Court Competition⁶, University of Pretoria, South Africa, were used. For this project group dynamics and SDL were also used.

Process of validation:

For the validation of the project, a meeting was held with 2 senior subject expert from L N Medical College Bhopal and RKDF Medical College Bhopal, along with faculties of the department of the forensic medicine and toxicology of MIMS & R, Bhopal, discussed about various aspects of the Pre and Post-test, rectified what needed as per suggestions and then validated the Pre and Post-tests and complete method.

Project was put in front of the IEC and got the ethical clearance.

Status of Ethical permission: Got the ethical clearance from the IEC (REG. No. ECR / 1190 / INST / MP / 2019 / RR-22) of the LNMC & RC & JK HOSPITAL, BHOPAL with ref. no. LMNC&RC/Dean/2024/Ethics/046.

Sampling Method: Convenient sampling technique (As the study was an Educational interventional study and it will be directly visible if there are any impact in either way positive/ negative/ neutral on the students, as they are currently studying in the department of forensic medicine)

Participants: The students from the Phase III part 1 MBBS, MIMS & R, and medical Faculties of various subjects from MIMS & R and other Medical colleges of Bhopal, MP.

Sample size: Students - 130 (Phase 3 Part 1, MIMS& R)

Inclusion criteria:

Students - 130 (Phase III Part 1 MBBS, MIMS & R)

Exclusion criteria:

- Who were not willing to participate and didn't give written consent.
- Who were not present during the session both in the practice session and competition.
- 13 coordinator students (who participated in MOOT COURT during previous BCME workshop) did not participate in pre and post-test.
- Students whom responses of either pre or post-test were not collected due to some technical error were excluded.

Informed consent obtained (Annexure- 1 INFORMED CONSENT FORM STUDENT)

Moot Court Competition and data collection

The moot court competition was held in which 35 faculties attended the competition as Judges and guests. All 10 groups performed "moot court".

Three senior faculties, having vast court procedure experience as medical professionals were invited to become Judges in this competition and willing faculties from various departments of MIMS & R and other medical colleges of Bhopal, were invited as guests.

After the competition, students (other than coordinators) took Post-test in the google form.

Statistical analysis:

Responses of the Pre & Post test were recorded. Only 99 student's both Pre and Post-test data were collected, other's either Pre or Post-test were missing. So only 99 student's data was used for the study, in MS Excel and used Paired t-test for the comparison. Statistical analysis was done using SPSS-20 software. The data was expressed as means and percentages.

Results/Observations

On observing in pre and post-test of the sample size 99 and using paired sample t-test (with 95% Confidence Interval of the Difference), mean value in post-test increased from 13.63 to 17.34.

Std. Deviation decreased from 3.14 to 2.47. Std. Error Mean decreased from 0.32 to 0.25. Here t-value is 12.1770 and P value is <0.0001.

So the current project shows by comparing pre and post-test and using paired samples t-test (Table no-1), the mean value of the post-test increased and concluded that the level of knowledge increased by using this moot court as simulation technique in medical education too.

Table No 1: Comparison Of Pre And Post-Test

	Mean	Number of observation	Std. Deviation	Std. Error Mean	t value	P value
Total score pre-test	13.63	99	3.14	0.32	12.1770	<0.0001 (significant)
Total score post-test	17.34	99	2.47	0.25		

Discussion

Sakhalkar Ujjwala⁷ in her book "Developing skills through moot court and mock trials from preparation to performance" write all about the fundamental premise that Mooting and mock trials can be used as an effective educative tool, that helped a lot to prepare this project as "Moot Court" as teaching learning method in M.B.B.S. students. As it is still at a very early phase in medical education.

As mentioned above, Ringel⁸, Lewis. (2004) wrote, their same article, about how a moot court is an extremely fluid pedagogical tool which can be used for more than learning about the law or the judicial process. It has been used in a variety of disciplines including political science, media, history, journalism, sociology, art, economics, business, and the life sciences to educate students about a variety of subjects such as history, journalistic rights, antitrust laws, or professional ethics.

In this study more emphasis was given on the behaviour of a "Doctor in the courtroom as an expert witness", so that conclusion can be made for whether it can be helpful for the MBBS students in their professional life in the future or not too.

Hamzeh Abu Issa⁹ et al also in their study shows that the Moot Courts should be an integrated part of the study curricula by allocating a subject for it, to grant all students the opportunity to participate in it, in order to enhance and develop their skills. They in their research paper "The Impact of Moot Courts on the Quality of Legal Education: Students of the Faculty of Law at the Applied Science Private University as a Model", concluded that Moot Court is a norm for Law students and definitely have positive impact on their knowledge that is why this is a part of their studies for a very long time.

This current project also concluded by using paired samples t-test, mean value of the post-test increased that level of knowledge increased by using this moot court as simulation technique in medical education too.

In "Court room exposure to medical students: a practical approach to legal procedures in Indian scenario" by Gupta Sanjay² et al also concluded that the average knowledge level improved

significantly by the courtroom exposure when compared to didactic lecture, which also shows the same results of this study.

Edward F. Kammerer¹⁰, Jr et al wrote that "students are motivated to participate in moot court for academic reasons, they believe their critical thinking and public speaking skills improve, and that, despite its heavy work load and difficulty, moot court is also fun". Same response in present study shows in the comments of the students and faculties too.

On searching among peers of the subject Forensic Medicine and toxicology in India, it came to know that "Moot Court" is used as teaching learning method in some of the medical institutes e.g. GMC, Bhopal where acts done by PGs and Faculties, also in SIAMS, Indore and D Y Patil medical college, Pune by the under graduate students of the MBBS.

Studies of the effectiveness of Moot Courts in medical education, are not many found till time but it is very common as student centered simulation technique as mentioned by Carlson¹¹, J. & Skaggs, and Neil. (2000) in "Learning by Trial and Error" or Tindwani Manik¹² in "Legal research for a moot court problem" and Bose Sohini¹³ in "Tips on How to Be a Good moot court Researcher" and many more, in many other courses e.g. Law and Politics successfully.

Although we are discussing about moot court in real life situation, there is also concept of virtual moot court too that was discussed as pilot study by Ireland et al¹⁴ as a second life mooting project in 2010 at university of Western University (UWS) in Australia. We can also use this in India if needed.

This study on the effect of the "Implementation of the Moot court as a simulation technique for teaching - learning of court procedures" in medical students shows that it is a very effective way to teach the students about the legal procedure.

Conclusion

- To gain practical knowledge, practical ways of study in any way, either in "real or simulation" are more effective than only theory lectures.

- Active participation of the students is beneficial not only for that particular topic, but it also gives them and faculties encouragement to become better in every way.
- “Implementation of the Moot court as a simulation technique for teaching – learning of court procedures” in medical students shows that it is a very effective way to teach the students about the legal procedure.
- As this study involved mainly one institute, more studies in different areas of the country are recommended.
- It can also be performed as role play with the volunteers every year, if all the student’s participation is not possible, as it will be less time consuming but yes results will not be at par.
- It is recommended, that at least 1 court attendance during internship with senior (PG or Faculties from Forensic medicine) should be mandatory.

Ethical Clearance: From IEC of the LNMC & RC & JK HOSPITAL, BHOPAL

Source of Funding: Administration of the MIMS & R, Bhopal (MP)

Conflict of Interest: Nil

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Clinical and Epidemiological Profile of Cases of Poisoning Brought to a Tertiary Care Centre Located in a Rural Border District of South Kerala

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Abstract

Background: Poisoning remains a critical public health issue in India, with varying epidemiological and clinical characteristics across regions. Identifying demographic trends/types of poisoning/clinical presentations is crucial for improving prevention and treatment strategies. This study aims to analyze the general characteristics, clinical manifestations and trend patterns of poisoning cases in a hospital setting.

Methods: A retrospective study of 247 patients with a history of poisoning during the last 10 years was conducted. Data on patient demographics, type and manner of poisoning, clinical features and factors precipitating consumption of poisonous substances were collected from medical records. Descriptive statistical analysis was used to assess patterns and trends of poisoning cases.

Conclusion: The study highlights that poisoning incidents are predominantly reported among males and individuals aged 25–50 years, with a significant proportion being linked to suicidal intent. Drug-related poisoning was the most common and alcoholism was a prevalent comorbidity. These findings underline the need for targeted interventions, improved mental health support and effective management of poisoning cases to reduce the burden of toxicity in the population.

Keywords: Poisoning, clinical profile, suicidal poisoning, organophosphates, toxicology, retrospective analysis

Introduction

Poisoning is a significant global health issue, with its incidence and impact varying greatly, depending on geographic, social and economic factors. In developing countries like India, poisoning is still one of the major causes of morbidity and mortality, particularly in rural areas. The patterns

in consumption of poison are largely influenced by the availability of the toxic substances, local practices and societal issues such as poverty, mental health crises and inadequate regulatory oversight in procurement/sale of toxic agents.¹

In India, poisoning cases often involve consumption of agrochemicals/pharmaceutical

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agents/household chemicals as well as bites/stings of venomous creatures. The increasing trend of misuse of pharmaceutical products and self-poisoning have become a matter of public health concern.^{2,3}

Understanding the epidemiological and clinical features of poisoning is essential for designing targeted prevention strategies and improving clinical outcomes. This retrospective study aims to explore the demographic profile, clinical presentations and factors precipitating poison consumption in cases admitted to a tertiary care hospital. The trends in poisoning in this part of state are not seen studied in the recent years which may provide insight into the current underlying causes and may help propose measures for prevention and better management of poisoning cases in a rural area. Hence it was felt that the study of this kind would serve the above purpose.

Materials and Methods

Study Design and Setting: This was a retrospective observational study, after perusal of case records, conducted at a tertiary care hospital, in a rural setting of a border district of South Kerala, focusing on those patients admitted with a positive history of poisoning and treated in wards/ ICUs for more than 48 hours. The study period spanned from January 2013 to December 2023.

Study Population: The study included 247 patients who were admitted with a confirmed history of poisoning. Criteria for inclusion was a positive history of poisoning upon admission and in whom the clinical picture was similar to that of a toxidrome. Exclusion criteria included patients whose medical records were incomplete or in whom a clinical diagnosis of poisoning could not be confirmed.

Data Collection: Data was collected from the hospital's medical records, including patient demographics/type of poison consumed/manner of poisoning/clinical features/comorbidities/precipitating factors/duration of hospital stay/outcome/complications developed.

Statistical Analysis: Data was coded and entered into MS Office Excel and analysis was done by using SPSS version 27. Descriptive statistical methods were used to analyse the data.

Ethical Considerations: The study was conducted following approval from the hospital's Institutional Ethics Committee, ensuring patient confidentiality and data security. No information revealing identity was used in the analysis or reporting of results.

Results

General characteristics of study subjects

247 patients with history of poisoning were enrolled in the current study. Their mean age was found to be 34.38 ± 18.19 years with the youngest victim being 1 year of age and oldest, 80 years. Nearly half (46.2%) of the poisoning cases affected age group of 25-50. More than half (57.1%) of the study subjects were males. Nearly one fourth (20.6%) of the study subjects gave history of addiction, 98% of them being alcoholic. About half of the study subjects had comorbidity; more than half suffering from psychiatric/psychological disorder (52.5%) followed by hypertension (26.2%) (Table No.1).

Table No.1: Distribution of study subjects based on general characteristics (n=247)

General Characteristics	Frequency(%)
Age(years)	
<25	82(33.2)
25-50	114(46.2)
>50	51(20.6)
Gender	
Male	141(57.1)
Female	106(42.9)
Addiction	
Present	51(20.6)
Absent	196(79.4)
Type of Addiction(n=51)	
Smoking	10(19.6)
Alcoholism	50(98.0)
Substance abuse	5(9.8)
Comorbidity	
Present	122(49.4)
Absent	125(50.6)

Type of comorbidity(n=122)	
Bronchial Asthma	11(9.0)
COPD	5(4.1)
Diabetes Mellitus	23(18.9)
Hypertension	32(26.2)
Dyslipidemia	7(5.7)
CVD	11(9.0)
CKD	1(0.8)
Seizure disorder	8(6.6)
Hypothyroidism	19(15.6)
Psychiatric/psychological disorder	64(52.5)

Trend of poisoning

The incidence of poisoning appeared to be higher during the months of April/May/October (10.9% each) followed by September/November/December (9.3% each). About 18.2% of the cases with history of poisoning reported with history and symptoms of poisoning on Sundays followed by Wednesday (15.8%). Suicidal cases constituting nearly one fourth (20%) of the total cases reported on Wednesday followed by Tuesday (16.7%). No cases of suspected/confirmed homicide were reported to this hospital during this period.

Pattern and manner of poisoning

All the cases were admitted with history of either ingestion of poison (70.8 %) or following bites by creatures (29.2%). Cases of poisoning by other routes were not observed in the present study.

Age Groups: The highest incidence of poisoning occurred in the 20-40 age group, among both genders.

Gender Distribution: A higher frequency of poisoning incidents among males was noted in certain categories, particularly among those with access to organophosphorus, rat poison and neurotoxic agents and among females in incidents related to drugs and cleaning agents like Harpic/Dettol.

Types of Poisons: Drug poisoning was prevalent among all age groups. Organophosphorus compounds and rat poison were more common in males (20-40 years). Ingestion of hydrocarbon, especially kerosene was more common among children (<20 years) in both genders. Accidental ingestion of sanitizer was observed in children especially male toddlers during the Covid period.

Manner of poisoning: Only accidental and suicidal poisoning cases were observed. There were substantial number of accidental poisonings, particularly in males (20-40 years) and females (40-60 years). Suicidal attempts were common in both genders (20-40 age range).

Table No.3: Distribution of study subjects based on pattern and manner of poisoning(n=247)

Age(years)	Male				Female			
	<20	20-40	40-60	>60	<20	20-40	40-60	>60
Type of poison								
Organophosphorus	0	6	5	1	0	2	1	0
Rat poison	0	13	1	0	3	5	0	0
Pyrethroid	0	0	1	4	2	1	0	0
Carbamate	3	4	7	1	2	1	1	0
Organochlorine	0	1	0	0	0	1	0	0
Paraquat	0	0	1	0	0	1	0	0
Hydrocarbon	4	2	1	1	3	0	0	0
Sanitizer	2	0	0	0	0	1	0	0
Enamel thinner	0	1	1	0	1	0	0	0
Mosquito repellent	2	0	0	0	0	0	0	0
Dettol	0	0	0	0	0	1	0	1
Harpic	0	0	0	0	1	2	0	0

Continue.....

Drugs	5	19	8	2	9	22	6	3
Formic acid	0	2	0	0	0	3	1	0
Neurotoxic snake	3	0	0	0	0	1	3	0
Hemotoxic	0	2	1	1	0	1	2	1
Non-venomous	0	4	4	2	1	1	2	1
Unknown bite	3	6	2	1	2	3	7	2
Stings	2	3	4	3	1	0	0	2
Naphthalene	0	1	0	1	0	0	0	0
KMnO4	0	0	0	0	1	0	0	0
H2O2	0	0	0	0	0	1	0	0
Manner								
Accidental	18	19	12	9	12	7	13	7
Suicidal	6	45	24	8	14	40	10	3

Overview of Drug Poisonings

Age and Gender Distribution

Males: The 20-40 age group showed significant misuse/abuse of analgesics (50%) and antipsychotics (75%). No incidents of poisoning with antiepileptics/antidepressants were noticed in the younger (<20) age groups. Occurrences of multidrug poisonings (20-40 age group) (63.6%) were also observed.

Females: Similar patterns of abuse/misuse with analgesics were also prominently found (66.7%) in the <20 age group. Abuse of antidepressants showed higher incidence in the 20-40 age group. Poisoning with eltroxin was more prevalent in the 20-40 age group (71.4%). Those in the younger age group (<20years) showed minimal exposure with cases reporting consequent to excessive intake of

analgesics in 4 cases and homeopathic medication in 1 case only.

Types of Drugs involved: Antiepileptics misuse was minimum. Significant over consumption of analgesics was noticed in both genders (20-40 age group). Eltroxin poisoning was more prevalent in the females (20-40 age group). Antidepressant misuse/Multi drug poisoning showed a worrying trend (20-40 age range) among both genders. Antipsychotic misuse was more prevalent (20-40 age group) in males. The combination of multidrugs involved mainly psychiatric medication along with analgesics/antibiotics/antihistamines/antihypertensives. Incidence of ingestion of Homeopathic medicines/oral hypoglycemic agents/bronchodilators though negligible was present.

Table no. 4: Incidence of drug poisonings among various age groups

Drugs	Male				Female			
	<20	20-40	40-60	>60	<20	20-40	40-60	>60
Antiepileptics	0	1	0	1	0	0	2	0
Analgesics	4	5	1	0	4	2	0	0
Antidepressants	0	3	1	1	0	5	1	0
Eltroxin	0	0	0	0	1	5	0	1
Antipsychotics	0	3	1	0	2	2	0	0
OHA	0	0	1	0	0	0	0	0
Multidrug	0	7	4	0	1	6	1	2
Homeopathic	1	0	0	0	0	0	0	0
Antihypertensive	0	0	0	0	0	1	2	0
Bronchodilator	0	0	0	0	1	0	0	0
Calamine lotion	0	0	0	0	0	1	0	0

Clinical features of poisoning

Symptoms of poisoning(n=247)

Almost three fourth 184(74.5%) of the study subjects presented with symptoms.

1. Common Symptoms Across Poison Types:

Nausea/vomiting were the most common symptoms across different poison types, particularly so with those who had ingested organophosphates/rat poison/pyrethroid/organochlorine and certain drugs. Headache/abdominal pain were also noticed to occur with moderate frequency, particularly in those who had taken organophosphates/rat poison/paraquat.

2. Specific Poison-Related Symptoms:

Organophosphorus poisoning presented predominantly with neurological/gastrointestinal symptoms. Symptoms like headache/nausea/vomiting/abdominal pain/difficulty breathing were found to be predominantly prevalent among those who partook carbamates and organochlorines. Those brought with history of consumption of rat poison revealed symptoms of tiredness/headache/nausea/vomiting/difficulty breathing. Pyrethroid poisoning was associated with headache and nausea but had a lower overall severity in symptoms compared to those who had ingested organophosphorus compounds. Patients who ingested Paraquat presented with severe respiratory distress along with burning sensation in the stomach/abdominal pain. Intake of Hydrocarbons mainly caused headache/cough/difficulty breathing demonstrating heightened potential for developing aspiration pneumonia. Tiredness/dizziness/burning sensation in throat were common in those brought with sanitizer intake. Patients admitted with Dettol/Harpic poisoning showed relatively lower severity in symptoms, though they often manifested nausea/vomiting/occasionally difficulty in breathing. Tiredness/headache,/nausea/vomiting were the common symptoms associated with various types of drug intake. Ingestion of Formic acid showed significant gastrointestinal involvement, particularly abdominal pain/vomiting and occasionally

hemoptysis. Neurotoxic and hemotoxic snake bites showed a distinct pattern with localized pain and swelling at the site of bite along with systemic manifestations like numbness/confusion/drowsiness/loss of consciousness in neurotoxic snake bites. Cases with history of bites or stings by unknown creatures presented with localized symptoms like itching/pain at the bite site/swelling/discoloration, along with occasional systemic effects like blurred vision/shivering/difficulty breathing and a few manifested with anaphylaxis showing periorbital edema/rashes over the body.

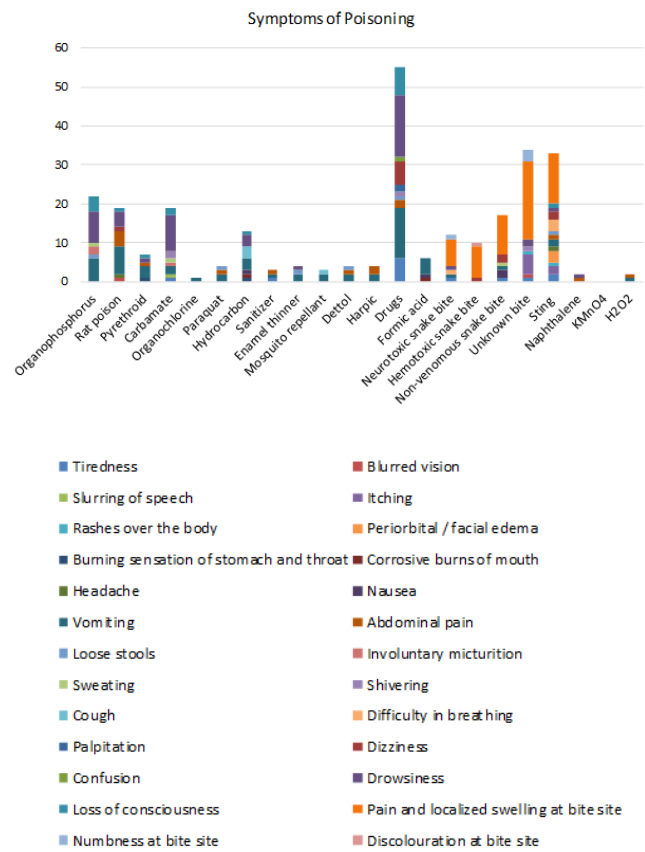


Figure No.1 Symptoms of poisoning(n=247)

Signs of poisoning

About 158(63.6%) of the study subjects presented with history, symptoms and signs of poisoning on the day of admission and among them, almost half of them presented with hypertension along with tachycardia(37.3%).

Specific poison related signs

Organophosphorus poisoning cases presented with altered sensorium/bradycardia/tachycardia/

tachypnoea/fasciculations/ptosis. The most common signs in rat poisoning were altered sensorium/tachycardia/ptosis. Patients with Pyrethroid poisoning showed altered sensorium/tachycardia/mild bradycardia. Those brought with history of Carbamate poisoning manifested with altered sensorium/tachycardia/fasciculations. Organochlorine poisoning caused tachycardia/ptosis/fasciculations. Tachycardia/tachypnoea and signs of lung involvement were prevalent in Paraquat Poisoning. Patients who presented with history of Hydrocarbon poisoning showed tachycardia/fasciculations. Sanitizer/Dettol/Harpic/Enamel Thinner poisoning cases presented with tachycardia. Mosquito repellent poisoning caused Tachycardia/fasciculations/ crepitations. Those with drug overdose presented with tachycardia frequently. Formic Acid poisoning cases also revealed tachycardia and hypotension. Those who suffered Neurotoxic snake bites presented with tachycardia/ptosis while those with Hemotoxic snake bites presented with tachycardia/hypertension/elevated BT/CT. Those who presented with Non-venomous/unknown bites showed tachycardia predominantly. Chemical exposures (KMnO4/H2O2/Naphthalene) showed limited systemic signs.

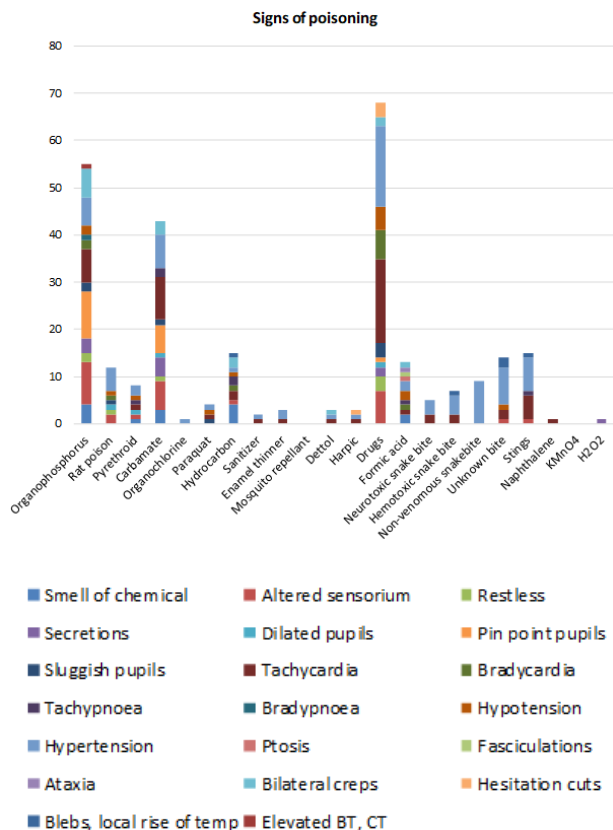


Figure No.2 Signs of poisoning(n=247)

Factors precipitating poisoning

On assessment of factors precipitating poisoning among suicidal cases, more than half (55.3%) of them were disinclined to reveal the specific triggering factor probably due to fear of the family or near ones being stigmatised. Family problems were reported as the precipitating factor by 16.7%.(Table No.5).

Table No.5: Factors precipitating poisoning(n=150)

Factors	Frequency(%)
Interpersonal stressors	7(4.7)
Problems in family	25(16.7)
Failure in love	6(4.0)
Disturbed marriage	23(15.3)
Financial crisis	6(4.0)
Not known	83(55.3)

Outcome of poisoning

A significant proportion of the patients (109 patients,44.1%) fully recovered from the effects of poisoning after treatment. However, a small but notable percentage of patients (8,3.2%) succumbed to the deleterious effects of the poison, particularly those treated following ingestion of organophosphates/carbamates/paraquat/formic acid/hydrocarbon.13 patients (5.3%) were discharged at request, before complete recovery as they wanted to continue treatment elsewhere.

Table No. 6: Outcome of poisoning

Outcome	Frequency(%)
Recovered	109(44.1)
Improved	117(47.4)
Death	8(3.2)
Discharged at request	13(5.3)

Duration of Hospital Stay and the need for ventilatory support

Mean Duration in Ward and ICU

Patients brought with history and clinical features of poisoning with Organophosphates/Carbamate/rat poison/pyrethroid/ hydrocarbon/cleaning agents/drug ingestion/formic acid required an average stay of more than 2 days in the hospital.

The highest mean duration of ICU stay was seen in Organophosphate poisoning followed by ingestion of formic acid and carbamate.

Ventilatory Support

Mean ventilatory support of more than 2 days were required for organophosphate and paraquat poisoning. Carbamate poisoning required mean ventilatory support for more than 1.5 days.

Table No. 7: Mean duration of hospital stay and the need for ventilatory support

Type of poison	Mean duration in ward	Mean duration in ICU	Ventilatory support (Days)
Organophosphorus	2.47±1.68	5.53±4.64	2.53±4.27
Rat poison	2.73±1.91	1.82±0.50	-
Pyrethroid	2.13±1.25	2.13±2.10	-
Carbamate	3.26±3.29	4.32±4.39	1.63±2.98
Organochlorine	1.50±0.71	0.50±0.71	-
Paraquat	1.00±1.41	3.50±2.12	2.50±3.54
Hydrocarbon	3.18±3.82	2.73±3.32	0.18±0.60
Sanitizer	0.67±0.58	1.33±0.58	-
Enamel thinner	1.33±0.58	1.33±0.58	-
Mosquito repellent	2.00±0.0	1.00±1.41	-
Dettol	2.50±2.12	0.50±0.71	-
Harpic	3.00±3.46	2.33±1.53	-
Drugs	2.38±2.06	1.47±1.10	0.14±0.83
Formic acid	2.17±1.33	4.67±2.66	-
Neurotoxic	1.57±1.33	1.57±1.51	0.71±1.89
Hemotoxic	1.25±1.03	1.63±0.52	-
Non-venomous bite	1.53±0.99	1.07±0.59	-
Unknown bite	1.92±0.79	1.27±1.34	-
Sting bite	2.67±1.72	1.53±1.88	-
Naphthylamine	1.00±0.0	1.00±0.0	-
KMnO ₄	1.00±0.0	1.00±0.0	-
H ₂ O ₂	1.00±0.0	2.00±0.0	-

Complications developed in various types of poison

Organophosphorus poisoning resulted in complications in 33.3% of patients, that included aspiration pneumonia and intermediate syndrome. In those who had consumed rat poisons, 13.6% of patients experienced liver dysfunction. Aspiration

pneumonia was the primary complication noted in both pyrethroid and carbamate poisoning. Paraquat poisoning led to complications such as alveolitis/liver dysfunction/multi-organ dysfunction syndrome. Kerosene ingestion/mosquito repellent exposure resulted in chemical pneumonitis, while turpentine

ingestion was associated with acute kidney injury. Formic acid ingestion caused complications in 50% of individuals, which included acute kidney injury/liver dysfunction/metabolic acidosis/hyperkalemia. Neurotoxic bites were complicated by necrotizing fasciitis/gangrene/acute kidney injury with hypoxic ischemic encephalopathy observed in one patient. Hemotoxic bites led to cellulitis, while wasp stings resulted in rhabdomyolysis. An insect bite reaction resulted in bronchiolitis in one individual.

Discussion

This study presents a comprehensive analysis of poisoning cases admitted to a tertiary care centre in a rural border district of South Kerala over the past decade.

Demographics and Patterns of Poisoning

The predominance of poisoning cases among males (57.1%) and individuals aged 25-50 years (46.2%) aligns with existing literature. The elevated incidence of suicidal poisoning attempts, accounting for 20% of cases, underscores a pressing mental health crisis prevailing in this demographic segment. This raises concern and suggests that societal factors, such as relationship stressors and economic hardships may contribute to high rates of suicidal behaviour.

The study also revealed a significant association between substance addiction and poisoning incidents. This emphasizes the interconnection between substance use disorders and the propensity for poisoning, often linked to impulsive behaviour in stressful situations.^{4,6}

Types of Poisoning

Drug-related poisoning was identified as the most common among all categories, particularly involving analgesics and antipsychotics. The higher incidence of drug poisoning among the 20-40 age group points to pharmaceutical agent misuse, especially as this demographic segment also reported a considerable number of multidrug poisonings. This signifies the importance of regulating pharmaceutical misuse/the necessity for the implementation of stricter regulations surrounding the prescription and availability of certain medications, alongside enhanced public education on safe usage and identification of factors triggering their misuse.

Organophosphates emerged as a notable contributor to poisoning incidents, particularly among males. The widespread use of these agrochemicals in rural regions underscores the need for better education on their safe handling and storage, with alongside efforts to reduce accessibility and misuse.⁵

Trends in Incidence

The analysis revealed seasonal peaks in poisoning cases during specific months, particularly April, May, October (pre-monsoon period). This suggests that environmental or societal factors, such as setbacks in agricultural activities/economic crises during festive periods, may influence the seasonal rise in poisoning incidents.⁷

Precipitating Factors

The assessment of precipitating factors for suicidal poisoning cases indicated that a significant proportion (55.3%) were disinclined to reveal the real triggering cause, possibly out of fear of stigmatization of family or loved ones, while interpersonal and family problems were noted in a smaller fraction of cases. This highlights the complexities involved in identifying the real cause of suicidal behaviour and the multifaceted nature of factors leading to poisoning incidents. Enhancing mental health support systems and community mental health outreach programs could help address underlying issues and reduce the incidence of suicidal behaviour.⁴

Clinical Presentation and Outcomes

Clinical symptoms varied depending on the poison type, with nausea/vomiting being the most prevalent across cases. Organophosphate poisoning frequently led to neurological sequelae, illustrating the serious implications of exposure to these toxic agents. Respiratory distress was notably high in cases involving paraquat and hydrocarbons, indicating the need for timely and effective management strategies in emergency settings.

In terms of outcomes, nearly half of the patients(44.1%) admitted fully recovered, while a small percentage (7.9%) succumbed to poisoning, particularly in cases involving highly toxic substances like organophosphates and paraquat. This underscores the critical nature of poisoning by these

substances and the need for specialized immediate medical interventions, along with the importance of early recognition of poisoning symptoms in improving patient outcomes.

The average duration of hospital stay for patients varied depending on the severity of poisoning, with mild cases typically requiring 1-3 days, while more severe cases, particularly those involving organophosphate and paraquat poisoning, necessitated prolonged hospitalization of 5-10 days.^{8,9}

Complications Developed

Complications related to poisoning were observed in several patients, with respiratory failure, acute renal injury and neurological deficits being the most common. These complications highlight the importance of follow-up care and rehabilitation for affected individuals and the necessity of implementing community mental health programs to mitigate long-term health issues.^{10,11,12}

Conclusion and Recommendations

This study highlights the multifactorial nature of poisoning cases in South Kerala, with distinct demographic patterns, prevalent substance use issues and significant clinical implications. It calls for comprehensive public health strategies, including enhanced mental health support/stricter regulations on toxic substances and targeted educational campaigns about safe medication use and the risks associated with careless handling of agricultural chemicals. By addressing these factors, it may be possible to reduce the burden of poisoning and improve overall community health outcomes.

Limitations of this study

The population represented is restricted to a rural area bordering Kerala and Tamil Nadu. Cases of poisoning of profound severity usually seek to get treatment from very high tech tertiary care centres or government medical colleges which is a peculiar treatment seeking behaviour to this part of the state. Even if fulminant cases report for treatment initially, they seek referral probably due to other social pressures. Financial constraints of the less privileged was also cited as a reason for seeking referral to other centres.

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Conflicts of interest statement: nil

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Study on Knowledge and Awareness on Medical Ethics and Infamous Conduct among Medical Students in a Medical College, Hyderabad

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Abstract

This cross-sectional study aims to assess the knowledge and awareness of medical ethics, as well as infamous conduct, among medical students at a medical college in Hyderabad, Telangana, in light of advancements in medicine and research. The study group consisted of 150 participants, including 35 undergraduate medical students, 66 internees, and 49 postgraduate medical students. After obtaining informed consent, participants were evaluated using a pre-structured questionnaire, with their responses graded accordingly. The data were analyzed using MS Excel.

The results revealed a significant lack of knowledge and awareness regarding infamous conduct among medical students, including internees and postgraduates. However, internees demonstrated a better understanding of the basic principles of medical ethics compared to postgraduate students, who exhibited notably poor knowledge levels. The study highlights the urgent need for stricter curriculum implementation, continued medical education programs, and workshops to enhance the standards of medical ethics education.

Key words: Medical ethics, Infamous conduct, Medical students.

Introduction:

The term "ethics" is derived from the Greek word "ethos," meaning custom or character. In 1803, Thomas Percival, a physician and author, developed the first modern code of medical ethics, introducing the terms "medical ethics" and "medical jurisprudence."

In 1847, the American Medical Association adopted its first code of ethics, which was primarily based on Percival's work.

Unethical practices were commonly observed in the early 20th century, often involving exposing individuals to risks for the broader benefit of

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society. The Nuremberg Code (1947) became the first international set of ethical principles for clinical research. Later, in 1964, the World Medical Association¹ established the Declaration of Helsinki to provide ethical guidelines for medical research. Similarly, in 1982, the Indian Council of Medical Research (ICMR) released its policy statement outlining the ethical considerations in human research. Tom Beauchamp and James Childress later defined four fundamental principles of medical ethics: respect for autonomy, non-maleficence, beneficence, and justice².

The principle of "autonomy" refers to an individual's ability to make rational, independent decisions, emphasizing self-determination and freedom. This principle emerged as a response to the paternalistic approach traditionally seen in healthcare. "Beneficence" involves actions that prioritize the well-being of others, ensuring that decisions serve the best interests of patients and their families. "Non-maleficence," which is encapsulated by the phrase "do no harm," advises healthcare providers to avoid causing harm, even if they are unable to provide a benefit. This principle is closely associated with the Hippocratic Oath taken by physicians. The principle of "justice" involves the fair and unbiased distribution of healthcare resources to all individuals.

Despite these ethical guidelines, there have been notable instances of clinical trials conducted without participants' knowledge or consent, such as the Tuskegee Syphilis Study and the Thalidomide tragedy. In response to evolving medical research, the ICMR updated its guidelines in 2006.

The National Medical Commission's Code of Medical Ethics^{3,4,5} Regulations, 2002, requires all doctors to adhere to its rules and regulations. Any violations of the ethical code are considered infamous conduct and are subject to penal action. Additionally, the ICMR⁶ has established 12 general principles for biomedical researchers working with human subjects, with ethics committees ensuring that research upholds the safety, dignity, and well-being of participants.

Integrating medical ethics and humanities into the curriculum for medical undergraduates will help shape future doctors who are not only skilled in their profession but also grounded in ethical principles.

The aim and objective of this study is to assess the knowledge and awareness of medical ethics and infamous conduct among medical students. The study objective is to evaluate the level of awareness in different groups, illustrate how inadequate knowledge can lead to unethical behavior, and compare the findings with other similar studies conducted both in India and abroad. Ultimately, this research will help guide medical students to practice medicine ethically, minimizing the risk of legal issues arising from unethical conduct.

Material and Methods

A cross-sectional, prospective study was conducted to assess the knowledge and awareness of medical ethics and infamous conduct among medical students at a medical college in Hyderabad, Telangana. The study included 150 participants, consisting of 35 final-year MBBS students, 66 internees, and 49 postgraduate students, regardless of gender. The study was conducted from September 1, 2022, to August 31, 2023. Informed consent was obtained from all participants prior to their inclusion in the study, with the anonymity of participants maintained throughout.

A questionnaire was developed based on the principles of medical ethics and infamous conduct as outlined in the National Medical Commission's *Professional Conduct, Etiquette, and Ethics Regulations, 2002*. The key components covered in the questionnaire were:

- Respect for autonomy, Non-maleficence, Beneficence and Justice.
- Professional secrecy, Informed consent
- Association with pharmaceutical and manufacturing companies
- Dichotomy, covering, and advertisement
- Addiction, alcohol use, and improper conduct with patients

Each component of the questionnaire was evaluated by grading participants' knowledge and awareness as poor, fair, good, or excellent. Data was collected for all responses and analyzed statistically using MS Excel 2019.

Results

Cross sectional prospective study on knowledge and awareness on medical ethics and infamous conduct among Medical students was conducted in a

Medical college, Hyderabad, Telangana. 150 medical students were participated the study among 35 undergraduates, 66 internees and 49 post graduates.

Table 1: Study Results of each variables in grading in total population.

S No	Variable	Poor	Fair	Good	Excellent
1	Respect for Autonomy	66.6%	19.3%	13.3%	0.66%
2	Non Maleficence	66.66%	23.33%	9.3%	0.66%
3	Beneficence	60.6%	23.33%	14%	1.33%
4	Justice	46%	32%	18%	4%
5	Professional Secrecy	24%	41.3%	22%	12.66%
6	Informed Consent	12.66%	50.66%	26.66%	10%
7	Association with manufacturing firms and pharmaceutical companies	35.33%	43.33%	9.33%	12%
8	Dichotomy	47.33%	34.66%	13.33%	4.66%
9	Covering	35.33%	41.33%	20%	3.33%
10	Advertisement	30%	46%	20%	4%
11	Addiction and Alcohol	16.665	54%	22.66%	6.66%
12	Awareness on improper conduct with patient	33.33%	49.33%	14%	3.33%

Autonomy

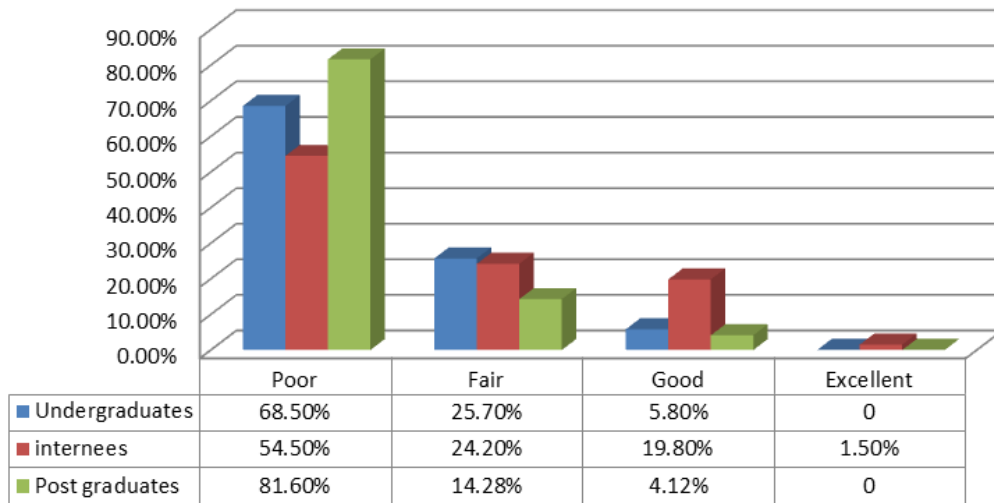


Fig 1: Showing the results of Autonomy among under graduates, Internees and post graduates.

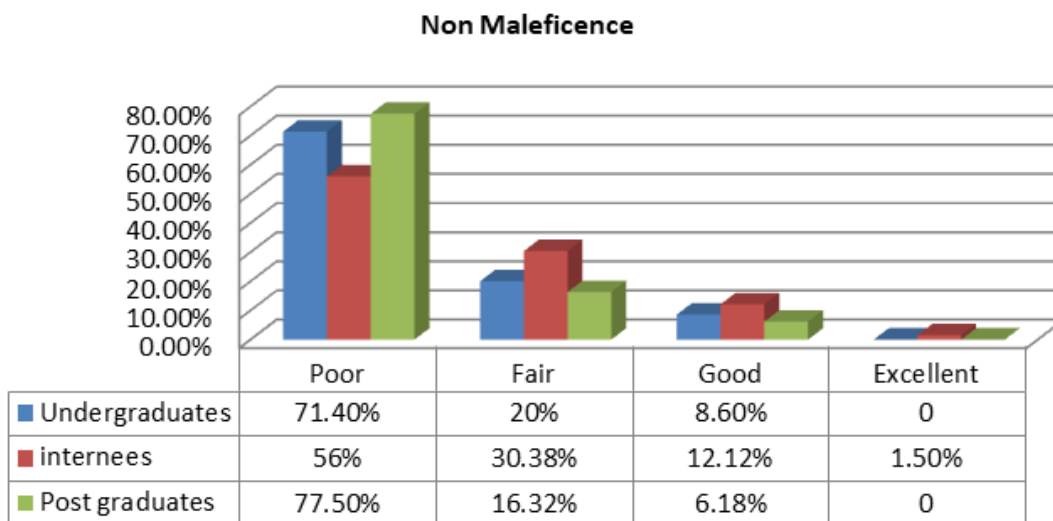


Fig 2: Showing the results of Non-maleficence among under graduates, Internees and post graduates.

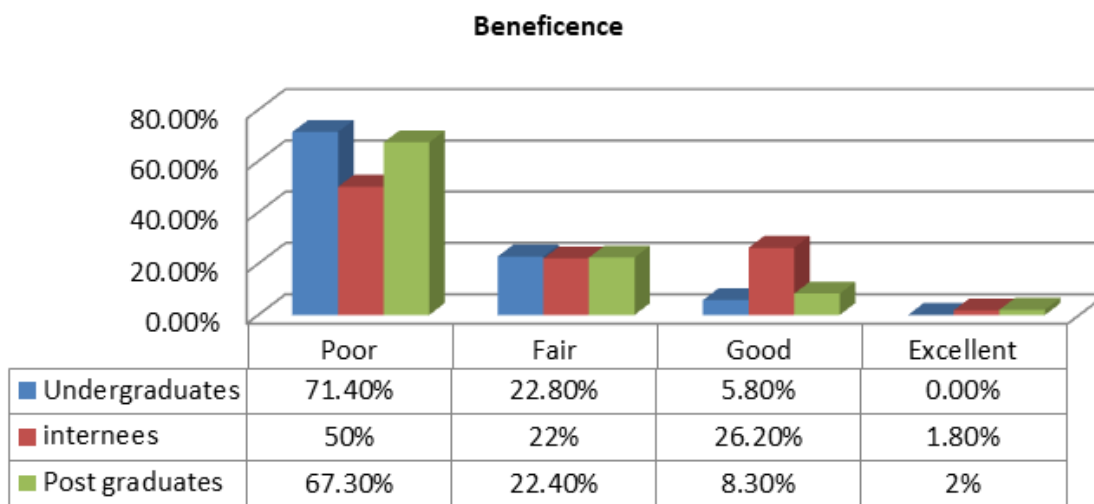


Fig 3: Showing the results of Beneficence among under graduates, Internees and post graduates.

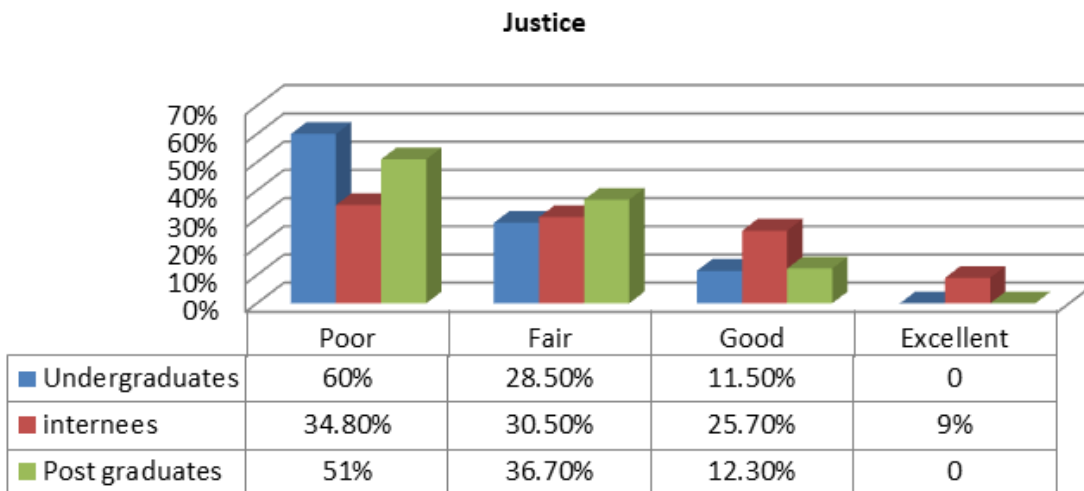


Fig 4: Showing the results of Justice among under graduates, Internees and post graduates.

Discussion

A cross-sectional study on medical ethics and infamous conduct was conducted among medical students (undergraduates, internees, and postgraduates) at a medical college in Hyderabad, Telangana. A total of 150 students, including both male and female participants, took part in the study. The study focused on four widely accepted principles of medical ethics: autonomy, non-maleficence, beneficence, and justice, as well as key components of infamous conduct.

Autonomy: The overall awareness of autonomy in the study population revealed that 66.6% had poor knowledge, 19.3% had fair knowledge, 20% demonstrated good knowledge, and only 1% exhibited excellent knowledge. Among undergraduates, 68.5% had poor knowledge, 25.7% had fair knowledge, 5.8% had good knowledge, and none were classified as excellent. In internees, 54.5% had poor knowledge, 24.2% had fair knowledge, 19.8% had good knowledge, and 1.5% had excellent knowledge. Among postgraduates, 81.6% were poor, 14.28% were fair, 4.12% were good, and none demonstrated excellent knowledge. Autonomy is a key principle in healthcare, underpinning informed consent, yet awareness was notably low, especially among postgraduates.

Non-maleficence: In terms of non-maleficence, 66.6% of the total study population demonstrated poor knowledge, 23.33% had fair knowledge, 9.3% had good knowledge, and only 0.66% had excellent knowledge. Among undergraduates, 71.4% had poor knowledge, 13.62% had good knowledge, and no students had excellent knowledge. In internees, 56% had poor knowledge, with 13.62% demonstrating good knowledge. Among postgraduates, 77.5% showed poor knowledge, 6.18% exhibited good knowledge, and no one had excellent knowledge. Postgraduates showed particularly poor awareness of non-maleficence.

Beneficence: Regarding beneficence, 60.6% of the study population had poor knowledge, 23.33% had fair knowledge, and 15.33% had good knowledge. Among undergraduates, 71.4% had poor knowledge, 5.8% demonstrated good knowledge, and none showed excellent knowledge. In internees, 50% had

poor knowledge, while 28% demonstrated good knowledge. Postgraduates again exhibited poor knowledge, with 67.3% having poor awareness, 10.3% demonstrating good knowledge, and no one showing excellent knowledge. Postgraduates appeared weakest in their understanding of beneficence.

Justice: Results related to justice revealed that 60% of undergraduates, 34.8% of internees, and 51% of postgraduates exhibited poor knowledge. In contrast, 34.7% of internees, 11.5% of undergraduates, and 12.3% of postgraduates demonstrated good knowledge. Internees showed relatively better knowledge in this area.

Overall, awareness of the basic principles of medical ethics was found to be poor among undergraduate and postgraduate medical students, with internees showing comparatively better knowledge.

Infamous Conduct: Knowledge and awareness of infamous conduct were also found to be lacking in the study population. Professional secrecy was well understood by 34.66%, association with pharmaceutical companies by 21.33%, dichotomy by 17.99%, covering by 23.33%, advertising by 24%, treating under the influence of alcohol by 29.32%, and improper conduct with patients by 17.33%. Across all components of infamous conduct, over 60% of participants had poor or fair knowledge.

The findings of this study align with similar research conducted in a medical college⁷ in Coimbatore, Tamil Nadu, which also reported low knowledge and awareness regarding medical ethics among medical students.

Conclusion

The study results indicate that the majority of medical students possess poor to fair knowledge of the basic principles of bioethics and infamous conduct. Postgraduate students, in particular, demonstrated a concerning lack of awareness and understanding of the core ethical principles, while internees showed relatively better knowledge. These findings highlight a significant gap in the education of medical ethics at various stages of medical training.

The results emphasize the urgent need for stricter implementation of medical ethics curricula at the undergraduate level. Furthermore, there is a critical need for enhanced training programs, including workshops, conferences, and continued education initiatives, to underscore the importance of ethical practice, particularly at the postgraduate level. Addressing these deficiencies will be essential in fostering a generation of healthcare professionals who are not only technically proficient but also grounded in ethical principles, ensuring the provision of ethical and compassionate care to patients.

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Conflict of interest: Nil,

Ethical clearance: Yes, obtained from institutional ethics committee Reference No: MRIMS-DHR-IEC-12/2022.

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Analysis of Drugs of Abuse in Hair Sample by LC/MS-MS

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Abstract

Due to its prolonged detection window, hair has become an important material for drug analysis. It is suitable for the detection of frequent drug abuse (DOA) dependence and other toxicological considerations. The advancement in chromatographic techniques especially liquid chromatography-tandem mass spectrometry (LC/MS-MS) makes detecting even traces of a drug in hair possible. Therefore, LC/MS-MS is progressively replacing gas chromatographic techniques as a tool for screening and confirmation making it the method of choice for hair analysis. In the present study, a method to determine common drugs of abuse in hair samples is described.

Human hair was tested for a range of different classes of drugs of a forensic and toxicological nature including selected amphetamines, cannabinoids, and benzodiazepines. For extraction purposes, the hair samples were decontaminated using dichloromethane, grounded, and treated with 1 M sodium hydroxide, followed by n-hexane/ethyl acetate extraction using liquid-liquid extraction (LLE). Following extraction from hair samples, drug screening employed liquid chromatography coupled with tandem mass spectrometric (LC/MS-MS). The screening method (for >20 drugs) was calibrated with a tailored drug mixture and was validated for 11 selected drugs for this study. A shim-pack FC-ODS (2.0 mm I.D. x 150 mm L, 3 µm) column was used for analysis. The total instrument run time was 30 minutes with no noted matrix interferences. Hair samples were screened using this new method and samples were confirmed positive for several drugs, mainly drugs of abuse.

The LC/MS-MS method described in the present study has proved to be simple and robust for the determination of drugs in hair. It is currently used for real samples in the Forensic Laboratory of Jordan.

Keywords: Drugs of abuse, Hair sample, High-performance liquid chromatography, Mass spectrometry

Introduction

The analysis of biological matrices to detect various types of drugs is in great demand within forensic investigations as it provides essential proof of past drug exposure¹. Monitoring of these substances

can be performed on different biological specimens including blood, urine, and hair. Blood and urine have been used as the main body fluids for drug screening in most clinical and forensic laboratories and remain the matrices of choice to detect recent exposure²⁻⁴.

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Hair analysis has been acknowledged as the best method that enables the evaluation of long-term exposure to illegal drugs⁵⁻¹². Gas chromatography-mass spectrometry (GC/MS), and liquid chromatography-tandem mass spectrometry (LC/MS-MS) have dominated over other analytical techniques as screening and confirmation methods in hair analysis^{13 & 14}. For samples to be adequate for GC/MS, sample preparation requires a derivatization step before instrumental analysis^[2&3]. LC/MS-MS has been largely used in forensic laboratories to determine a wide range of compounds in biological specimens¹⁵. Owing to the decreasing costs, growing effectiveness, and absence of derivatization step, LC/MS-MS instrumentation is progressively replacing GC/MS in forensic routine analysis¹⁶. The introduction of Liquid Chromatography and the advancement in triple quadrupole design allow fast data throughput, excellent sensitivity, and selectivity, making it the best technique of choice in forensic investigations. In this paper, we describe a new LC/MS-MS screening method for the simultaneous determination of 11 illicit, psychotropic, and hypnotic drugs in human hair including; amphetamine, ethylamphetamine, methamphetamine, diazepam, alprazolam, bromazepam, THC, amitriptyline, gabapentin, olanzapine.

The combination of an easy-to-apply sample extraction procedure with the LC/MS-MS technique proved to be simple, fast, accurate, and highly sensitive, allowing for the simultaneous detection of several drugs of abuse.

Materials and Method

Materials

1. Sample

Blank hair samples were used for the development and validation of the procedure and were obtained from healthy volunteers. Authentic hair samples were obtained from forensic and toxicology cases.

2. Reagents and Solvents:

Methanol and water (HPLC Grade) were purchased from Carlo Erba (Milan, Italy). Ammonium format (analytical grade, 99% min assay) from ALFA ASER, Ward Hill, Massachusetts, U.S.).

Dichloromethane, Ethyl acetate, Isopropanol, and N-hexane; all were analytical grade. Analytical Reference Standards were brought from LIPOMED, (U.S.). All drugs together with internal standards were provided by the forensic science laboratory of Jordan.

3. Calibrant, Standards, and Stock Solutions.

Stock solutions of individual analytes were prepared by dissolving or diluting them in methanol obtaining a concentration of 1 mg/mL. Individual stock solutions were diluted to prepare a standard solution by mixing individual drugs in methanol arriving at a concentration of 5 µg/mL for selectivity experiments. A mixed working solution of non-deuterated compounds at 5 µg/mL in methanol was used for the preparation of calibrators and 'in-house QCs' for all the compounds. Calibrant samples were prepared using blank hair. Spiked hair sample solution was prepared from the dilution of stock solutions. The test solution prepared consisted of analytes of 5 components which is used to check the sensitivity and accuracy of the method. A mixed Internal Standards working solution of 0.4 µg/mL for deuterated standards was also prepared in methanol. Working solutions were stored at 4 °C for 6 months. Their stability was checked weekly as they were routinely used in the analysis of other biological matrices where external quality controls are analyzed in the same batch of samples

4. Analytical Equipment and Supplies

The LC system is Shimadzu UHPLC-LC20, the Analytical Column is Shim-pack FC-ODS (2.0 mm I.D. x 150 mm L, 3 µm), and the detector is LCMS 8030 Tandem mass from Shimadzu Scientific Instruments Inc.

Method

Sample Preparation

To the hair sample 4 mL of dichloromethane was added, mix vortex-mixed for 3 minutes. The solvent is discarded and the process is repeated. The hair sample was dried at room temperature under gentle nitrogen flow. Hair was cut into small pieces, and 100 mg of the sample was put in a 10-mL screw-capped glass tube. 1 mL of 1M sodium hydroxide (NaOH) solution was added to the sample and was incubated

at 95 °C for 20 min. 3ml of n-hexane/ethyl acetate 90:10 (v/v) was used for the extraction process, the sample was then centrifuged at 5000rpm for 5min. The organic phase was transferred into a glass tube, and the extraction was repeated. The dried organic

phase under nitrogen flow at 40°C was reconstituted with 100µL of methanol and transferred into a vial. Analysis of the sample was carried out on LC/MS-MS using the instrument operating conditions indicated in Table 1.

Table 1: Analytical Conditions

LC Conditions:			
Analytical Column	Shim-pack FC-ODS (2.0 mm I.D. x 150 mm L, 3 µm)		
Mobile phase A	10 mmol/L Ammonium formate-water		
Mobile phase B	Methanol		
Gradient Program	Time (Min)	Mobile phase A (%)	Mobile Phase B (%)
	0	95	5
	15	5	95
	20	5	95
	20.1	95	5
30	95	5	
Flow rate	0.3 mL/Min		
Injection volume	5 µL		
Column oven temperature	40°C		
MS Conditions:			
Nebulizing Gas Flowrate	1.5 L/min		
Drying gas Flowrate	10 L/min		
DL Temperature	250°C		
Block heater Temperature	400°C		
Ionization mode	ESI		

Notes:

Extraction

For the results to be reproducible, it is advantageous to quantitatively extract all of the drugs from the hair matrix. To ensure that all of the analytes present in the sample are extracted from the sample and measured, it is preferred that the hair be completely dissolved. Therefore, the hair sample was incubated with a strong alkali solution at optimal temperature for an adequate time. All analytes were stable during incubation. The aqueous solution was then extracted with a mixture of n-hexane/ethyl acetate. The same sample was re-extracted a second time to ensure that a high recovery of all detected drugs in the sample was achieved. Cloudy or turbid extracts should be centrifuged before analysis. In analytes at a concentration above the calibration

range, the extracts should be diluted to avoid errors in the quantitative analysis. To remove external contamination from the exterior of the hair, hair samples were washed before analysis.

LC System

To achieve reliable data and high performance in the Liquid Chromatography (LC) system, only high-purity organic solvents, and reagents of LC/MS grade are recommended. 10% of isopropanol in 50:50 v/v solution of water-Methanol was used to wash the LC system to avoid carry-over phenomena that could affect the high sensitivity of LC/MS.

Instrument's Operating Conditions.

Liquid Chromatography:

LC separations were performed on Shimadzu LC20 supported with an LCMS 8030 Tandem mass

spectrometer. Five microliters were injected into the LC system for a chromatographic run of 30 min using a Shim-pack FC-ODS (2.0 mm I.D. x 150 mm L, 3 µm) (Shimadzu, Japan) and a gradient mixture of 10 mmol/L Ammonium formate-water/Methanol.

MS-MS System:

Mass spectrometry: MS data were collected in positive electrospray ionization (ESI) mode on the Triple Quadrupole Mass by Shimadzu Scientific Instruments Inc. After complete validation, this method has been successfully applied to many forensic cases, one of which is reported.

Method Validation:

Standard calibration curves were obtained by spiking aliquots of negative control hair samples with a standard mixture at the concentration levels (ng/mg hair) of 0.01, 0.05, 0.10, 1, and 5, which were extracted as described above. Within-run precision was determined using these assays.

Method validation was conducted by applying calibration curves based on peak-height ratios of standard concentration vs analytes and internal standards. Limits of quantification (LOQ) were estimated by analyzing drug-free hair samples with various concentrations of analytes. Repeated injections (n = 5) were performed on a single day to establish the intra-day precision (% CV). The extraction efficiency of the compounds was determined by spiking blank hair samples with 10ng/mg drugs. Recoveries were determined by measuring peak heights obtained for the analytes added after the incubation step with those of the analytes added before sample preparation. The detection limits ((LOD) were evaluated by decreasing the concentration of the drugs spiked in drug-free hair until a response equivalent to three times the background noise was observed. Quantitation was based on the ratio of the peak areas of the analyte to the internal standards. The internal standard was in both cases added after incubation. High concentrations of selected drugs were incubated in drug-free hair samples to investigate the specificity of the method.

Results

To generate a standardized protocol for detecting drugs of abuse in hair samples of long-term users,

a hair sample was processed for preparation by extraction followed by LC/MS-MS analysis. The present study focuses on the development of a method for extraction and analysis.

Extraction analysis

The results of the extraction recovery study are presented in Table 3. The recoveries ranged from 65% to 92% which are considered sufficient for screening purposes¹⁷. Extraction is not an issue as long as the sensitivity of the instrument is enough and reproducibility is lower than 15%. These results are acceptable considering this is a quantitative method dealing with 11 compounds in hair samples including drugs of abuse. However, the results obtained with the analysis of samples demonstrated that the extraction procedure is adequate for the study of forensic and toxicological samples. In the experiment performed to assess matrix effects (with 4 hair samples from different origins), no significant matrix effect was observed for any compound.

Calibration analysis

Calibration curves were made for each compound. The calibration ranges were selected according to the cut-offs recommended by the Society of Hair Testing (SoHT)¹⁸ and focused on possible concentrations found in single-dose cases. The intra-assay imprecision for the 'in-house QC was satisfactory, with RSDs lower than 8% for all the compounds (Relative standard deviations (RSD) and LOQs ranged between 1.5% and 7.6% and 0.015-0.025 respectively as presented in Table 3.

LC/MS-MS analysis.

Figure 1 below shows the chromatographic profile of the analytes of DOA detected in a hair sample from a subject who reported past drug abuse consumption. For purposes of quantitating the drugs detected in hair extracts, liquid chromatography/tandem mass spectrometry using selected ion monitoring was carried out. The ions that were selected for monitoring were chosen according to their relatively high abundance as can be seen in Table 2, which consists of monitored ions for each drug from an extract of the hair sample from a drug user. The analysis has indicated the presence of specific peaks at retention times as shown in Table 2.

In this case study, the real hair sample was analyzed to evaluate the robustness of the developed DOA screening method.

The selectivity of LC/MS-MS analysis implemented in this study has enabled the detection of 11 drugs

in a hair sample collected from a single subject. No interferences were observed after the study of the blank hair samples, ensuring the selectivity of the method (Fig. 1).

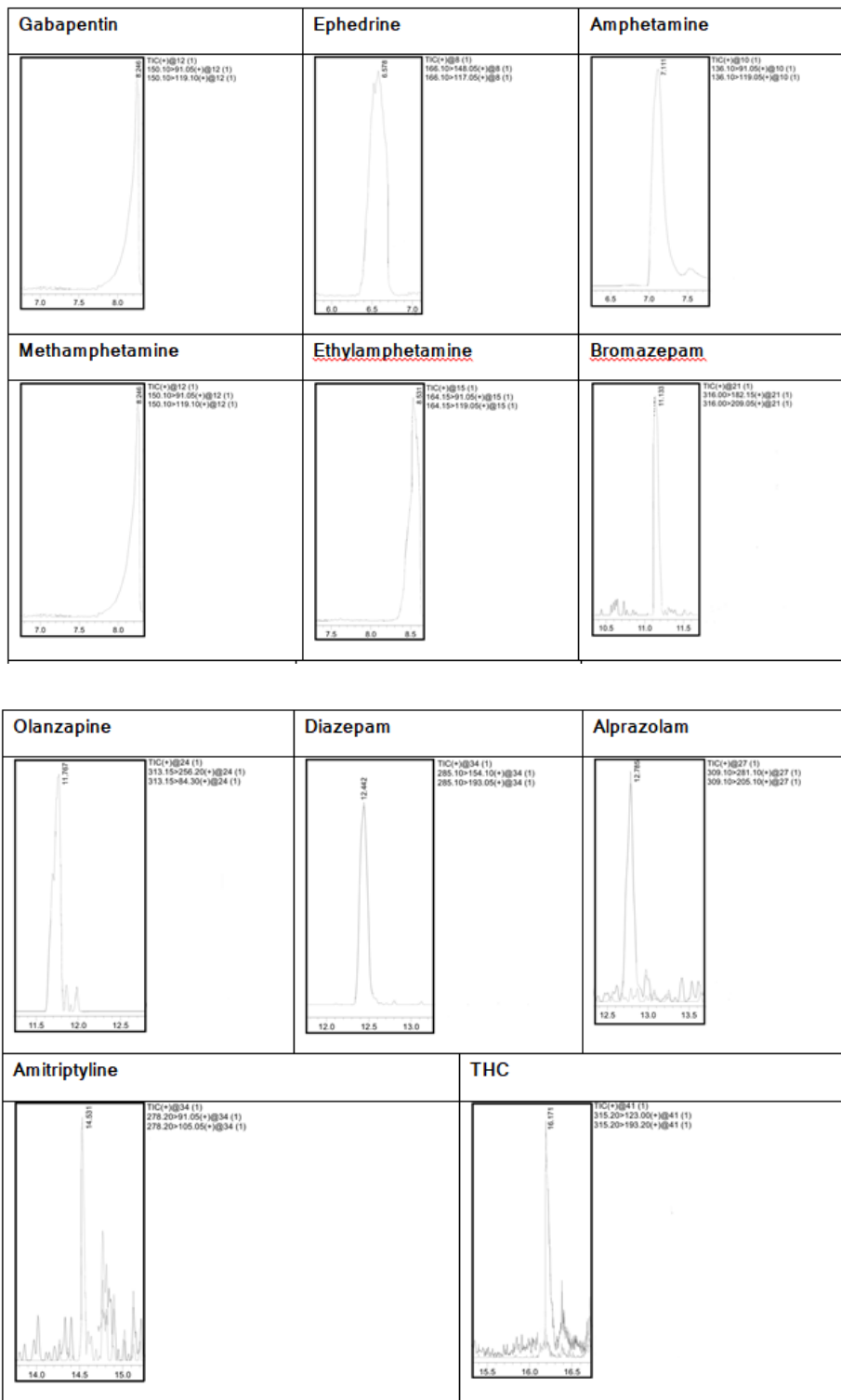


Figure 1: Chromatographic profile of the drugs of abuse by LC/MS-MS analysis.

Table 2: Retention time and MS characteristic of subject hair sample's analytes

Analyte	RT (Min)	Precursor Ion (m/z)	Product Ion Quantifier (m/z)	Product Ion Qualifier (m/z)
Illicit drugs				
Amphetamine	7.1110	136.10	91.05	119.05
Ethylamphetamine	8.5310	164.50	91.05	119.05
Ephedrine	6.5780	166.10	148.05	117.05
Methamphetamine	8.2460	150.10	91.050	119.05
THC	16.171	315.20	123.00	193.20
Psychotropic drugs				
Amitriptyline	14.531	278.20	91.050	105.05
Gabapentin	5.4860	172.15	154.10	55.00
Olanzapine	11.767	313.15	256.20	84.30
Hypnotic Drugs				
Alprazolam	12.785	309.10	281.10	205.10
Bromazepam	11.133	316.00	182.15	209.05
Diazepam	12.442	285.10	154.10	193.05

Table 3. Summary of validation results

No.	Analyte	LOQ (ng/mg)	Intraday RSD (%)	Recovery (%)
1	Amphetamine	0.020	3.3	91
2	Ethylamphetamine	0.015	2.5	92
3	Ephedrine	0.025	2.9	87
4	Methamphetamine	0.020	2.3	92
5	THC	0.020	5.6	76
6	Amitriptyline	0.015	4.3	85
7	Gabapentin	0.020	1.5	79
8	Olanzapine	0.010	2.8	81
9	Alprazolam	0.020	7.6	65
10	Bromazepam	0.020	6.9	67
11	Diazepam	0.025	7.5	69

Discussion

Hair is generally accepted as the sample of choice for drug abuse testing because hair has a longer window (months) in comparison to blood (hours to days) and urine (days to weeks), and can therefore be used as an integral material.

The aim of screening methods is firstly to decline negative samples in a large number of samples. This is achieved with low-cost immunological tests such as RIA¹⁹ and ELIZA²⁰. Another aim of screening tests is to use two separate and distinctive methods to confirm a positive result. So the main goal is that results will have added quality by using chromatography.

Quantitative results from genuine samples analyzed with the LC/MS-MS confirmation method are shown in Figure 1. Another quality of chromatography is that one can establish a screening method to measure exactly what the confirmation method does. The positive case under investigation has revealed a formerly unknown drug user by analyzing his hair sample. Hair analysis in such cases can reveal both known drug use as well as a prolonged period of use and in some overdose cases.

The LC/MS-MS method was developed for the rapid screening of abused drugs in human hair in patients who were investigated for drug abuse. This

method used sample preparation to extract DOAs from the hair sample. The use of the wash step was to eliminate false-positive results. The results show that this new competitive LC/MS-MS method to detect drugs of abuse in human hair has been successfully established. It was fully validated and provided a viable approach for the analysis of drugs in a hair sample to monitor long-term exposure with a high level of selectivity and robustness. The analytical method described in this study is suitable for a wide spectrum of chemically different drugs including; illicit, hypnotics, and psychotropic drugs. LC/MS-MS screening method enabled the simultaneous determination of 11 different compounds present in a single sample of hair. It has been already established for the analysis of authentic hair samples in the Forensic laboratory in Jordan.

Conclusions

The presented extraction method, followed by LC/MS-MS analysis appears adequate for drug monitoring use because it is highly sensitive and is very easy to apply. The developed method can identify and confirm 11 substances, including THC, within a single analytical run. This method can monitor patients for drug abuse with the minimum sample collected. The routine analysis using the developed method has proved that this method is an effective tool for determining abused drugs in human hair from patients who were monitored for drug abuse. The results provide medical professionals with thorough information about drug abuse or setbacks in drug abuse and can be used for patient-careful treatment to improve patient health.

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Declaration: The authors declare that there is no conflict of interest and that the data presented in the manuscript is the result of work conducted in the Department of Chemical and Biological Analysis of the Forensic Laboratory of Jordan.

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Correlation between Age with Manner and Cause of Unnatural deaths in Reproductive Age Group Females: A Prospective Cross-Sectional Study

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Abstract

Background: Unnatural deaths among women is rarely emphasized but it's an important public health index of the community and society. Reproductive age group is an important marker for human development and any imbalance in this age group can affect overall social and economic development as well as health of the next generation; and thereby the Society.

Material and Methods: The present study was conducted in the Department of Forensic Medicine and Toxicology, Rajendra Institute of Medical Sciences, Ranchi, Jharkhand from April 2021 to March 2022 (i.e., 12 months). Total autopsies conducted during this period were 3840. Out of these, 225 cases were women in reproductive age group as per the selection criteria of the study planned.

Conclusion: Maximum number of cases (24%) belonged to the age group of 15-19 years, followed by 20-24 years(23.55%) and 40-44 years age (14.67%) and the most common manner of death was accidental. In the age group of 15-19 years, burn was the most common cause of death, with hanging being the most common cause of death in the age group of 20-24 years. The most common manner of death was accidental (59.1%), followed by suicidal (28.9%) and homicidal (8%). Unnatural deaths in women of reproductive age group constituted 5.86% of the total cases autopsied in the study period, with burns (mostly accidental), road traffic accidents and suicidal hanging being the most common causes of death. Accordingly, multifaceted preventive strategies using bottom-up approach right from the basic levels of health care system need to be formulated to buoy up the physical, mental and social well being of this very important group of society.

Key words: Unnatural death, Reproductive age group females, Manner of death, Cause of death.

Introduction

The reproductive age in females, conventionally

delineated as being between 15 and 44 years, represents a pivotal phase distinguished by

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numerous health and fertility-related dimensions.¹ This specific age bracket is essential for comprehending the complexities of women's health trajectories, fertility trends, and the influence of socio-demographic variables on reproductive well-being. Any discrepancies within this demographic cohort may adversely impact the health outcomes of future generations, as well as social and economic development, consequently influencing societal structures. The status of females and girls within societal constructs, alongside the manner in which they are treated, serves as a pivotal determinant of societal advancement.

In India, women of reproductive age comprise 22.2% of the total demographic population.² The mortality rate among Indian women is alarmingly elevated, particularly during childhood and reproductive years, with the predominant causes being attributed to natural factors. Women of reproductive years often face the highest risk of death from communicable illnesses, complications in maternal health, anemias, and cancers. Furthermore, the prevalence of unnatural deaths within this reproductive age cohort is notably high as compared to the global average figures, and occurrences of unnatural fatalities among women are not rare within the context of Indian society.³ Such incidents typically encompass accidents, homicides, and suicides. The National Family Health Survey (NFHS-5) results, which took place between 2019 and 2021, show that 31.2% of young women aged 15-19, 20.5% of those aged 20-29, 17.5% of women aged 30-39, and 9.3% of the 40-49 age group of all the deaths in the prior two years were from accidents, violence, poisoning, homicides, or suicides, while other deaths were linked to natural causes.⁴

Any civilization may be evaluated by the status accorded to women within its social framework. One of the numerous elements that substantiate the prominence of India's ancient culture is the esteemed position afforded to women. The societal landscape is undergoing significant transformations concerning the acceptance of women as professionals, as primary earners within households, as autonomous thinkers, as providers for families, and as individuals bearing the profound responsibility of childbirth. A

considerable number of women in India pursue careers as educators, healthcare professionals, aviators, engineers, flight attendants, military personnel, among various other vocations. They also participate actively in the realms of politics and governance. Although there has been improvement in women's situations, issues such as troubled marriages, dowry customs, economic exploitation, acts like murder and sexual crimes, as well as the adverse effects of not being educated and uninformed, must be decisively eliminated to allow women to secure their appropriate standing in society.

A complex interplay of social, economic, and cultural influences impacts the mortality rate among women in their reproductive years, which is reflected in different manifestations including suicides, homicides, and accidents. The engagement in violent acts against women is a recognized public health dilemma worldwide, presenting in assorted forms and intensities, with homicide marking the most critical point on the violence spectrum against women. Such violence may transpire within a myriad of contexts and situational frameworks. Diverging from prevalent notions, statistical evidence demonstrates that women are 11 to 12 times more prone to being murdered by someone familiar rather than by a total stranger.⁵

A marked escalation in the incidence of unnatural fatalities, particularly during the initial years of matrimony, has been documented in recent years, which constitutes a significant blemish on the esteemed traditions of our society.⁶ The most salient factor contributing to such fatalities appears to be the incessant demands for dowry (in cash or kind) imposed by husbands and/or in-laws, which can culminate in the murder or maltreatment of the bride, ultimately leading to her suicide.⁷ The National Burns Programme reports that from an estimated 140,000 annual burn injury deaths, 91,000 are women, a statistic that is higher than maternal mortality.⁸ In India, the unnatural mortality rate is at 0.67 per 1000, where male statistics show 0.84 and female statistics show 0.49 per 1000 across all age groups.⁹

Materials and Methods

In this current cross-sectional analysis, research was executed within the Department of Forensic Medicine and Toxicology at the Rajendra Institute of Medical Sciences in Ranchi, Jharkhand, over the course from April 2021 to March 2022 (a total of twelve months), primarily aimed at studying the trends of unnatural fatalities and determining possible causes among women of reproductive age. The study specifically aimed to delineate the patterns and identify the most prevalent modes of unnatural deaths among women aged 15 to 44 years. Unnatural deaths of women within the reproductive age group (15-44 years), substantiated by medico-legal autopsy and credible historical accounts, were incorporated into the analysis, while natural fatalities, unidentified individuals, and decomposed remains were excluded.

A total of 3840 autopsies were performed during the designated timeframe. Among these, 225 instances pertained to females within the reproductive age bracket, in accordance with the selection criteria

established for the current study. Comprehensive data regarding age, circumstances of death, and other pertinent details were systematically compiled using a preformatted proforma from all 225 female subjects in the reproductive age group (15-44 years) through thorough interviews conducted with investigative officers, examination of hospital records, and discussions with relatives and acquaintances of the deceased, following the acquisition of consent from the legal guardians of the deceased individuals. The collated data was categorized according to various parameters aligned with the objectives of the present study. A standard autopsy protocol was adhered to, and relevant samples and viscera underwent chemical analysis as well as histopathological examinations, with the resultant findings meticulously documented. Data entry was performed utilizing Microsoft Office Excel. Afterward, the information underwent scrutiny through the Statistical Package for Social Science tailored for Windows operating systems and frequency tables and proportions were generated.

Table 1: Distribution according to the age of the deceased and cause of death

Age Group [Years]	Burn		Drown- ing		Electro- cution		Fall from Height		Fire arm		Hang- ing		Light- ening		Medical Disease		Poison- ing		RTA		Snake- bite		Stab		Strangu- lation		Elephant Trample		Others		Total	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%		
15-19	18	33.3	02	3.7	01	1.9	00	00	01	1.9	10	18.5	00	00	02	3.7	11	20.4	05	9.3	03	5.6	01	1.9	00	00	00	00	00	00	54	100
20-24	15	28.3	02	3.8	00	00	00	00	01	1.9	16	30.2	01	1.9	02	3.8	03	5.7	09	17	02	3.8	02	3.8	00	00	00	00	00	00	53	100
25-29	13	37.1	02	5.7	01	2.9	00	00	00	00	03	8.6	00	00	03	8.6	02	5.7	07	20	00	00	03	8.6	01	2.9	00	00	00	00	35	100
30-34	12	40	00	00	00	00	01	3.3	00	00	02	6.7	01	3.3	00	00	00	00	11	36.7	00	00	01	3.3	01	3.3	00	00	01	3.3	30	100
35-39	03	15	01	05	01	05	03	15	00	00	00	00	01	05	01	05	01	05	07	35	00	00	01	05	00	00	00	00	01	05	20	100
40-44	04	12.1	03	9.1	00	00	02	6.1	02	6.1	03	9.1	00	00	01	03	01	03	14	42.4	02	6.1	00	00	00	00	01	03	00	00	33	100
Total	65	28.9	10	4.4	03	1.3	06	2.7	04	1.8	34	15.1	03	1.3	09	04	18	08	53	23.6	07	3.1	08	3.6	02	0.9	01	0.4	02	0.9	225	100

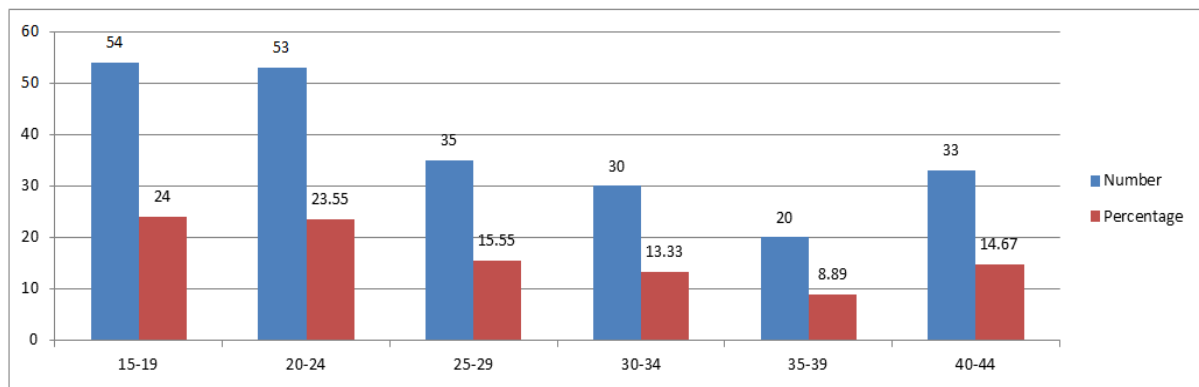


Figure 1: Distribution of cases according to Age

Table 2: Distribution of cases according to age and manner of death

			Manner of death				Total
			Accidental	Homicidal	Suicidal	Suspicious	
Age group	15-19	Frequency	28	3	21	2	54
		%	51.9%	5.6%	38.9%	3.7%	100.0%
	20-24	Frequency	26	4	21	2	53
		%	49.1%	7.5%	39.6%	3.8%	100.0%
	25-29	Frequency	18	4	10	3	35
		%	51.4%	11.4%	28.6%	8.6%	100.0%
	30-34	Frequency	21	3	6	0	30
		%	70.0%	10.0%	20.0%	0.0%	100.0%
	35-39	Frequency	14	2	3	1	20
		%	70.0%	10.0%	15.0%	5.0%	100.0%
	40-44	Frequency	26	2	4	1	33
		%	78.8%	6.1%	12.1%	3.0%	100.0%
	Total	Frequency	133	18	65	9	225
		%	59.1%	8.0%	28.9%	4.0%	100.0%

Table 3: Distribution of cases According to Age and Manner / Cause of Death

		15-19	20-24	25-29	30-34	35-39	40-44	Total
Suicide (no.= 65)	Hanging	10	16	3	2	0	3	34
	Drowning	0	1	0	0	1	0	2
	Poisoning	9	2	2	0	1	1	15
	Burns	2	2	5	4	1	0	14
	Fall from Height	0	0	0	0	0	0	0
Homicide (no.= 18)	Firearm	1	1	0	0	0	2	4
	Stab / Cut Throat	1	2	3	1	1	0	8
	Manual / Ligature Strangulation	0	0	1	1	0	0	2
	Burn	1	1	0	0	0	0	2
	Others	0	0	0	1	1	0	2
Accidental (no=133)	RTA	5	9	7	11	7	14	53
	Snake Bite	3	2	0	0	0	2	7
	Fall from Height	0	0	0	1	3	2	6
	Electrocution	1	0	1	0	1	0	3
	Poisoning	2	1	0	0	0	0	3
	Lightening	0	1	0	1	1	0	3
	Burns	15	12	8	8	2	4	49
	Drowning	2	1	2	0	0	3	8
Elephant Trampled	0	0	0	0	0	1	1	
Suspicious (no.=9)	Medical diseased	2	2	3	0	1	1	9
Total		54	53	35	30	20	33	225
Percentage		24	23.55	15.55	13.33	8.89	14.67	100

Results and Discussion

The largest proportion of cases [54 (24%)] was identified within the demographic of individuals aged 15-19 years, wherein thermal injuries emerged as the predominant etiology of mortality, succeeded by the age cohort of 20-24 years [53 (23.55%)], characterized by asphyxiation via hanging as the leading cause of death. Conversely, the age category of 35-39 years exhibited the least incidence of cases [20 (8.89%)] {Figure No. 01 & Table No. 01}. The preponderance of cases was attributed to accidents [133 (59.1%)], trailed by instances of suicide [65 (28.9%)] and homicide [18 (8%)], with the manner of death being classified as suspicious in 09 (4%) cases {Table No. 02}.

Road traffic accidents constituted a significant portion of cases, accounting for 53 (23.55%), followed by thermal injuries [49 (21.78%)]. Majority of the women succumbing to accidental burns belonged to the 15-24 years age group. Societal conventions regarding engagement in household duties and the assumption of gastronomic proficiency, in conjunction with the difficulties encountered in adapting to unfamiliar settings such as the domicile of in-laws, marked by a dearth of opportunities for respite and the undertaking of meal preparation despite an absence of expertise in culinary skills, may represent the probable determinants leading to the prevalence of accidental burns among young women. Suicidal asphyxiation via hanging represented 34 (15.11%) of the aggregate cases. Out of the overall cases, 15 (6.67%) involved suicide through poisoning, and 14 (6.22%) were due to thermal injuries. Within the context of homicide, the majority of cases were attributed to stab wounds [08 (3.55%)], with firearm-related injuries comprising [04 (1.78%)]. Among the autopsied suspicious deaths, all were ultimately determined to be natural causes of death {Table No. 03}.

Similar observations were reported in studies conducted by Srivastava AK, Arora P⁷ and Radhika RH & Ananda K¹⁰, where a significant proportion of victims were identified within the age demographic of 18-25 years. However, within their analysis, instances of suicide represented 81% of the overall cases, with accidental fatalities constituting 13%. Kulshrestha P et al¹¹ and Dere RC, Col. Rajoo KM¹² indicated that the majority of fatalities were recorded in the age bracket

of 26-30 years. The research conducted by Parmar, Pragnesh B. et al¹³ revealed that the preponderance of deaths was found in the 18-22 years age interval. In the study carried out by Dhoble, S.V., Dere, R.C., Jaiswani, A.K. et al¹⁴, the demographic most adversely affected was identified as 14-25 years. Zine KU et al³ further documented that the predominant cases (53.7%) were classified as accidental, followed by suicidal cases (40.4%) and homicidal cases (5%). However, in his analysis, burns comprised the highest proportion of cases (49.4%), succeeded by poisoning (15.8%), road traffic accidents (12%), and drowning (10%).

The study executed by Jagadish Rao Padubidri et al¹⁵ categorized suicide as the most prevalent manner of death (45.4%), followed closely by accidents (43.6%), in addition to poisoning (42.3%), hanging (34.9%), and burns (11.4%) as the leading causes of mortality. Kumar S et al¹⁶ identified accidents as the predominant manner of death, followed by suicides. Studies conducted outside India like those by Subedi, Nuwadatta et al¹⁷ observed that suicides (67.59%) were the most common, followed by accidents (19.80%), and by Zaghoul, Nancy & Megahed, Haidy¹⁸ reported that homicide was the most prevalent manner of death, followed by accidental occurrences. Divergent socio-cultural practices, in contrast to the Indian paradigm, in the contexts of Nepal and Egypt, respectively, may elucidate the underlying reasons for the observed phenomena.

Conclusion

A thorough analysis unfolded at the Rajendra Institute of Medical Sciences (RIMS) in Ranchi, targeting the evaluation of non-natural death rates among women in their reproductive years between April 2021 and March 2022, intending to explore the reasons and recommend strategies to mitigate these events. The predominant demographic of the female subjects was identified as being within the 15-24 years age range, with accidental deaths emerging as the most frequent manner of death, followed by suicides; specifically, road traffic incidents and burns were noted as the leading causes of accidental fatalities, whereas hanging, poisoning, and burns were recognized as the primary causes of suicides, in that specified order.

The dearth of precedents regarding this study within the tribal state of Jharkhand hinders the development of comprehensive policies and preventative strategies that aim to tackle the socio-economic challenges faced by women. The incidence of road traffic accidents and unintentional burn incidents, as evidenced by this study, can be ascribed to the augmented participation of women in the reproductive age demographic, particularly among younger individuals, in income-generating activities within this region. In a manner analogous to other tribal regions such as Chhattisgarh and the northeastern states, women similarly engage predominantly in labor and participate equivalently in income-generating activities, whether by operating tea stalls, managing small budget-friendly eateries and tiffin services, or by providing services as domestic helpers and caregivers, among other roles.

The phenomenon of suicide, recognized as a significant social challenge, can be effectively mitigated by enhancing the self-esteem and resilience of women from the adolescent stage. The incidences of suicide stemming from marital discord and dowry-related fatalities can be curtailed through the discouragement of premature marriages, the provision of pre-marital counseling, and the impartation of life skills aimed at harmonizing career pursuits with marital responsibilities.

Limitations of the Study

The analysis articulated in this research acknowledges that the prevalence of unnatural deaths among females is frequently underestimated on a global scale, thereby obstructing a comprehensive understanding of the entirety of unnatural mortality within the reproductive age demographic and potentially resulting in a disjointed analysis of the determinants contributing to these fatalities. Furthermore, the research utilizes a descriptive approach, which may hinder the capacity to delineate causal linkages or assess the efficacy of proposed preventive strategies aimed at mitigating unnatural deaths in adult females. The exclusion of unrecognized and decomposed remains may limit the comprehensiveness of the results and could inadvertently overlook significant incidents that might provide further elucidation regarding the trends linked to unnatural female fatalities.

Future Research Recommendations

There exists a pressing necessity for scholarly investigations that assess the efficacy of policies designed to foster female economic autonomy, as well as to support mental health strategies, community-based interventions, and educational initiatives aimed at transforming societal attitudes towards women. Empirical research could evaluate the ramifications of these initiatives on the diminution of unnatural mortality rates among women, thereby supplying policymakers with substantiated evidence to enact efficacious interventions. Subsequent research may also aim to elucidate the psychological and sociocultural variables contributing to the elevated prevalence of unnatural deaths within the reproductive age demographic, with particular emphasis on the influences of mental stress, familial relationships, and societal expectations on their overall well-being. This inquiry could encompass qualitative studies that delve into the personal narratives and lived experiences of victims and their families, thereby enhancing comprehension of the fundamental issues that precipitate such tragic outcomes.

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Green Forensic Toxicology: Optimizing Chlordiazepoxide Detection in Milk, Whiskey, and Whiskey Cream Matrices

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Abstract

Forensic toxicology plays a crucial role in detecting and quantifying drugs, toxins, and poisons in biological and non-biological samples to aid criminal and legal investigations. However, traditional approaches often involve significant resource consumption and pose environmental concerns. This study introduces novel findings in the detection and quantification of chlordiazepoxide, a benzodiazepine linked to drug-facilitated crimes, within milk, whiskey, and whiskey cream matrices. The innovative use of Thin Layer Chromatography (TLC) coupled with UV-Vis spectroscopy offers a sustainable, reliable, and cost-effective methodology. The study employed liquid-liquid extraction (LLE) using a chloroform-water system, minimising solvent waste and environmental impact by optimising extraction efficiency. The TLC analysis, using a chloroform (90:10) solvent system, achieved precise separation with an average retention factor (Rf) of 0.42 ± 0.03 . Confirmatory UV-Vis spectroscopy, a non-destructive technique, detected chlordiazepoxide's absorption peaks at 246 nm, 262 nm, and 308 nm. Recovery rates, adjusted through repeated extraction cycles, were matrix-dependent, with whiskey cream demonstrating the highest recovery (93.2%) and milk slightly lower (88.5%) due to protein interactions. These findings highlight a multi-tiered analytical strategy that ensures accuracy and legal admissibility in forensic investigations. The methodologies used align with sustainable practices by reducing chemical waste and resource use, supporting their integration into high-throughput laboratory settings. The study underscores the importance of confirmatory analyses and presents a scalable approach to detecting psychoactive substances in complex food and beverage matrices, reinforcing the potential for widespread forensic application.

Keywords: Chlordiazepoxide, Thin Layer Chromatography (TLC), UV-Vis spectroscopy, Liquid-liquid extraction (LLE), Drug-facilitated crimes (DFCs), Benzodiazepines

Introduction

Forensic toxicology is an essential scientific field that applies toxicology principles to aid criminal and

legal investigations¹. This discipline supports the detection and quantification of drugs, toxins, and poisons in biological samples such as blood, urine, and tissues, as well as non-biological samples like

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beverages and food². Its role becomes particularly crucial in cases involving drug-facilitated crimes (DFCs), where even trace amounts of psychoactive substances can influence legal outcomes². However, as forensic toxicology evolves to address increasingly complex cases, there is a pressing need for the incorporation of sustainable practices in its methodologies. While efficacious, traditional approaches often involve significant resource utilisation and pose environmental concerns³.

In response to rising environmental awareness, forensic laboratories worldwide are under pressure to reduce their ecological footprint. Conventional toxicological analyses rely heavily on hazardous solvents and reagents, which, if not disposed of properly, can damage ecosystems and endanger laboratory personnel⁴. The emergence of “green” or sustainable chemistry presents a promising alternative in forensic toxicology, focusing on reducing hazardous chemical use, minimising waste, and conserving energy while maintaining the rigour required for legal cases⁵⁻⁹.

The application of green chemistry principles—waste reduction, safer solvent use, and energy efficiency—has demonstrated relevance to forensic toxicology¹⁰. Research indicates that sustainable practices can significantly reduce the environmental impact of toxicological analyses without compromising sensitivity or accuracy⁴. This transition is particularly crucial for high-volume laboratories processing numerous samples, such as those handling DFC cases where rapid and sensitive detection of drugs is essential. Regulatory bodies, including the International Organization for Standardization (ISO), are now advocating for forensic toxicology laboratories to adopt more environmentally friendly methods in accordance with current environmental and health standards (ISO, 2021).

Instrumental techniques are fundamental in forensic toxicology, particularly for the quantification of drugs and poisons¹¹⁻¹⁵. Capillary electrophoresis (CE) and high-performance liquid chromatography coupled with ultraviolet detection (HPLC-UV) are recognised as more environmentally sustainable alternatives to gas chromatography-mass spectrometry (GC-MS), a technique known for its high resource consumption¹². CE, specifically,

is effective for separating complex compounds using smaller sample and reagent volumes, thereby reducing environmental impact while maintaining analytical sensitivity¹⁶. Studies have demonstrated that CE combined with diode-array detection (DAD) can accurately quantify benzodiazepines in spiked beverages, rendering it a sustainable alternative for forensic analyses^{17,18}.

In addition to DLLME and MAE, other eco-friendly extraction techniques include supercritical fluid extraction (SFE) and pressurised liquid extraction (PLE)¹⁶. SFE, which utilises carbon dioxide as a solvent, effectively extracts psychoactive compounds from biological matrices while avoiding hazardous chemicals¹⁹. This technique has garnered attention in forensic toxicology for enhancing laboratory safety by reducing toxic exposure risks. PLE, which employs solvents such as water and ethanol under high pressure and temperature, provides rapid and efficient extraction of target compounds while minimising chemical waste²⁰. The high extraction efficiency of PLE renders it an attractive solution for laboratories seeking to reduce their environmental impact.

Advancements in portable analytical systems also promote sustainability by enabling on-site analyses that minimise sample transport and reagent use²⁰⁻²². Innovations in miniaturised mass spectrometers and portable flow injection analysis systems allow forensic toxicologists to conduct preliminary screenings at crime scenes²³. This reduces the necessity for extensive sample preparation and transport to central laboratories, thereby conserving resources and shortening turnaround times. These portable systems offer reliable data for immediate decision-making, benefiting both environmental sustainability and investigative efficiency²⁴.

Experimental Details

The experiments were conducted at 25°C and pressure in the presence of air. Using chemicals and solvents from commercial sources (Chlordiazepoxide as Librium-10mg tablets from Abbott Healthcare) without further purification or recrystallisation from Sigma-Aldrich. The thin-layer chromatography (TLC) plates used are 0.25 mm Merck TLC silica gel plates.

To remove volatile solvents, an IKA rotary evaporator with a dry diaphragm pump (10-15 mm Hg) was utilised, and the process continued until a constant weight was achieved using an oil pump (<300 m Torr). The UV-VIS spectrophotometer used was the Labindia UV-3200 model, capable of scanning speeds ranging from 2-3000 nm/min. This instrument incorporated a tungsten and deuterium lamp, which allowed for detection within the wavelength range of 190-1100 nm using a photomultiplier detector.

Sample Preparation

Milk: 10mg and 20mg of Librium were added to two beakers containing 50mL of milk each, respectively. These were subsequently sealed and stored at 25°C.

Whiskey: 10mg and 20mg of Librium were added to two beakers containing 50mL of whiskey each (30mL whiskey and 20mL distilled water), respectively. These were subsequently sealed and stored at 25°C.

Whiskey Cream: One teaspoon of vanilla extract, one teaspoon of cocoa powder, and a few drops of condensed milk (a total of 10mL of ingredients) were added to 90mL of whiskey. The contents were thoroughly mixed. Subsequently, 10mg and 20mg of Librium were added to two beakers containing 50mL of whiskey cream each, respectively. These were then sealed and stored at 25°C.

Table 1: Samples and their different slots according to different matrices (milk, whiskey and whiskey cream) and weeks as time interval.

Slot No.	Sample No.	Matrix
1	01	Whiskey
1	03	Milk
1	05	Whiskey Cream
2	07	Whiskey
2	09	Milk
2	11	Whiskey Cream
3	13	Whiskey
3	15	Milk
3	17	Whiskey Cream

Slot No.	Sample No.	Matrix
1	02	Whiskey
1	04	Milk
1	06	Whiskey Cream
2	08	Whiskey
2	10	Milk
2	12	Whiskey Cream
3	14	Whiskey
3	16	Milk
3	18	Whiskey Cream

Liquid-Liquid Extraction (LLE)

10mL of chloroform and 50mL of distilled water were added to a separating funnel. 5mL of the sample was added to this. The separating funnel was then subjected to mixing by inversion, with pressure release achieved by the removal of the funnel cork. Subsequently, it was secured with screws of the ring stand and left to settle for 15-20 minutes. Following this step, the sample contained in the chloroform layer was collected in a beaker, with flow controlled by the stopcock valve. The beaker was then set aside for a brief period to allow for sample evaporation, yielding a concentrated extract.

The extraction process was repeated thrice to maximise extract collection, with subsequent additions of 10mL and 5mL of chloroform during its initial step (total of 25mL chloroform). Maintaining a time interval of 10 days, LLE (Liquid-Liquid

Extraction with chloroform and distilled water) was carried out (in different time slots), and its extraction efficiency over time was noted. LLE extraction at three different time intervals was denoted as 1st, 2nd and 3rd slot, respectively.

The methodology for this study focused on analysing chlordiazepoxide spiked in different drink matrices, including milk, whiskey, and whiskey cream³⁰. Chlordiazepoxide (Librium, 10 mg tablets from Abbott Healthcare) was selected due to its relevance in forensic toxicology²³. Sample preparation involved spiking 50 mL of each matrix with either 10 mg or 20 mg of the drug. Milk samples were prepared in separate beakers and sealed at 25°C, while whiskey samples comprised 30 mL of whiskey mixed with 20 mL of distilled water. Whiskey cream was prepared by combining 90 mL of whiskey with a teaspoon of vanilla extract, cocoa powder, and drops of condensed milk, mixed thoroughly before adding

the drug and sealing the samples³.

Result and Discussion

The present study focused on the identification and quantification of chlordiazepoxide in milk, whiskey, and whiskey cream matrices using Thin Layer Chromatography (TLC) followed by confirmatory UV-Vis spectroscopy²¹. The results demonstrated that the combined use of these analytical techniques provides a reliable approach for detecting psychoactive substances in complex food and beverage matrices.

The preliminary Marquis Test further supported the identification of chlordiazepoxide, producing a characteristic yellow-orange colour when applied to spiked samples. This rapid colourimetric test provided immediate qualitative evidence of the drug's presence, consistent with established practices in forensic toxicology where the colour change is utilized to indicate benzodiazepine-class drugs³⁹.

Marquis Test, a spot test using a reagent mix of sulfuric acid and formaldehyde (2:3) to detect chlordiazepoxide presence based on a colour change to yellow or orange⁸.

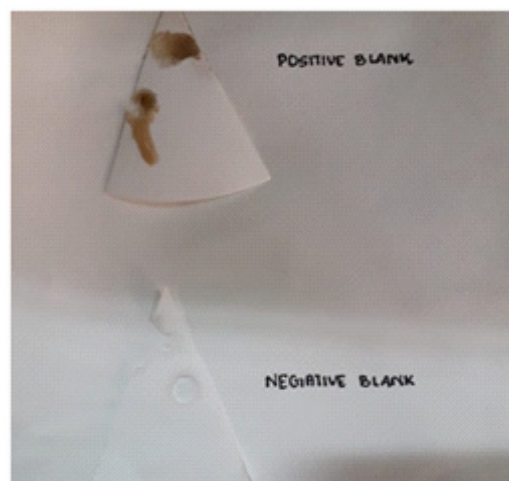


Figure 1. Standard positive and negative results of Marquis test for detection of chlordiazepoxide.



Figure 2. Results of Marquis test for detection of chlordiazepoxide in the questioned samples in different slots.

Recovery rates for chlordiazepoxide were assessed by comparing absorbance values to those from reference standards.

$$\text{Recovery Rate} = 100 \times \frac{\text{Amount of target component recovered}}{\text{Initial amount of target component in source}}$$

Milk matrices exhibited slightly lower recovery rates (88.5%), potentially due to protein interactions that can bind to the analyte and reduce its free concentration. This finding is consistent with literature

that highlights the challenges posed by protein-rich samples in forensic toxicology, necessitating more intensive solvent interaction to achieve complete analyte release⁴. In contrast, whiskey samples displayed higher recovery rates of 91.7%, likely owing to their simpler matrix composition and lower interference potential. The highest recoveries were observed in whiskey cream samples following repeated extraction cycles, indicating that fat content required additional processing to achieve optimal analyte isolation³¹.

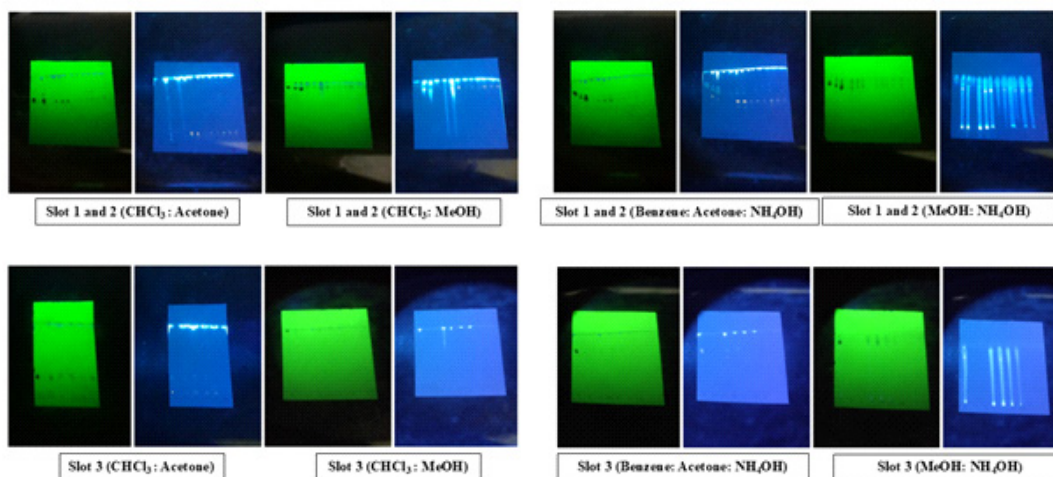


Figure 3: Thin-layer chromatography analysis of chlordiazepoxide samples under short-wavelength ultraviolet (green) and long-wavelength ultraviolet (blue) illumination.

The preliminary colourimetric tests and TLC serve as crucial steps in the forensic workflow, providing initial confirmation that can be rapidly deployed in field scenarios or preliminary screenings³⁶. Initial TLC analysis yielded distinct retention factor (Rf) values across different solvent systems, with chloroform (90:10) emerging as the most effective solvent combination for optimal separation. This solvent system produced consistent and well-defined bands with an average Rf of 0.42 ± 0.03 across replicates ($n=3$)¹⁷. The use of benzene:acetone

(50:50:1.5) also resulted in distinct separation but with a slightly lower resolution, indicating it as a viable alternative when chloroform-based solvents are restricted. These findings align with previous studies that have underscored the importance of solvent system selection in TLC for maximising compound separation and minimising matrix interferences³⁷, where the solvent front has played a major role with regard not only to separating the components that are studied but also in identifying it.

Table 2: Rf value of the samples with respect to the slots.

Slot 1 samples	1	2	3	4	5	6	Slot 2 samples	7	8	9	10	11	12	Slot 3 samples	13	14	15	16	17	18
Chloroform: Acetone (4:1)	0.55	0.55	0.55	0.55	0.55	0.55	Chloroform: Acetone (4:1)	0.60	0.58	0.61	0.64	0.66	0.67	Chloroform: Acetone (4:1)	-	0.19	0.21	0.21	0.23	0.23
Chloroform: Methanol (90:10)	0.9	0.9	0.9	0.9	0.9	0.9	Chloroform: Methanol (90:10)	0.92	0.93	0.93	0.93	0.93	0.93	Chloroform: Methanol (90:10)	-	-	-	-	-	-
Benzene: Acetone: Ammonium Hydroxide (50:50:1.5)	0.66	0.66	0.66	0.66	0.66	0.66	Benzene: Acetone: Ammonium Hydroxide (50:50:1.5)	0.67	0.68	0.7	0.72	0.72	0.74	Benzene: Acetone: Ammonium Hydroxide (50:50:1.5)	0.67	0.67	0.67	0.7	0.72	0.73
Methanol: Ammonium Hydroxide (100:1.5)	0.88	0.88	0.88	0.88	0.88	0.88	Methanol: Ammonium Hydroxide (100:1.5)	0.88	0.90	0.90	0.88	0.90	0.90	Methanol: Ammonium Hydroxide (100:1.5)	-	0.82	0.82	0.82	0.82	0.82

UV-Vis spectroscopy was employed as a confirmatory analytical technique to verify the presence and concentration of chlordiazepoxide in various matrices. The concentrated extracts obtained through LLE were analysed using a UV-Vis spectrophotometer³². The analysis involved scanning the samples at specific wavelengths of 246 nm, 262 nm, and 308 nm, chosen to target the absorbance maxima of chlordiazepoxide^{32,33}. Chloroform was used as the blank solvent to ensure accurate baseline readings. Absorbance values from the scans were then compared with those of prepared standards of chlordiazepoxide to confirm its presence and determine its concentration in each tested matrix.

This approach, combining extraction, preliminary screening, and instrumental analysis, provided a comprehensive framework for evaluating chlordiazepoxide in complex matrices such as milk, whiskey, and whiskey cream. Each step of the process played a critical role. Liquid-liquid extraction efficiently isolated the target compound from the matrices, reducing potential interferences from other components. UV-Vis spectroscopy allowed for precise quantification by comparing the extracted sample's absorbance to that of known standards, ensuring the reliability of the results.

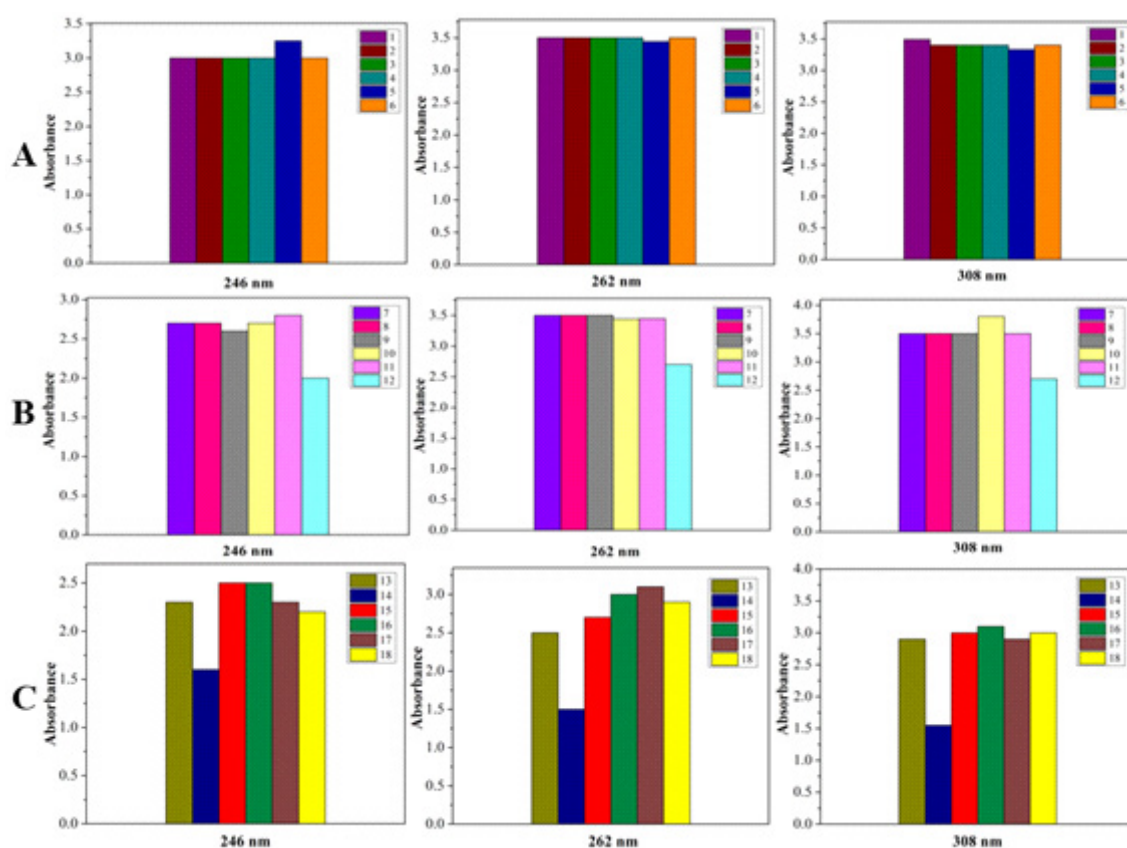


Figure 4: UV absorbance of chlordiazepoxide for samples of slot 1(A), slot 2(B) and slot 3(C).

The methodology also enabled the detection of chlordiazepoxide in challenging and diverse matrices, shedding light on its behavior and detectability in complex substances. Milk, for example, presented a unique challenge due to its high protein and fat content, which can interfere with extraction and analysis. Similarly, alcoholic beverages like whiskey and whiskey cream introduced variables such as alcohol content and emulsion stability, making the

detection of target compounds more complex³⁷⁻³⁹. By systematically applying the LLE technique followed by UV-Vis analysis, the study overcame these challenges and ensured accurate results across all tested matrices³⁹.

This integrated method demonstrates the utility of combining robust extraction protocols with advanced spectroscopic techniques to achieve reliable detection in forensic and analytical chemistry

contexts. The ability to detect chlordiazepoxide in such diverse matrices has significant implications for forensic investigations, particularly in cases involving drug-facilitated crimes. For example, the detection of chlordiazepoxide in beverages like whiskey can provide critical evidence in cases of drug-spiking incidents. Similarly, its identification in milk or other non-alcoholic matrices expands the scope of investigations into potential misuse.

Conclusion

This investigation focused on the detection and quantification of chlordiazepoxide, a benzodiazepine frequently associated with drug-facilitated crimes, in milk, whiskey, and whiskey cream matrices utilising Thin Layer Chromatography and confirmatory UV-Vis spectroscopy. Liquid-liquid extraction with chloroform and water was employed to extract the drug from the spiked matrices. The chloroform (90:10) solvent system demonstrated optimal efficacy for separation in TLC, with an average retention factor (R_f) of 0.42 ± 0.03 . UV-Vis spectroscopy confirmed the presence of chlordiazepoxide, revealing absorption peaks at 246 nm, 262 nm, and 308 nm. The findings elucidate the reliability and cost-effectiveness of combining TLC and UV-Vis spectroscopy for detecting psychoactive substances in complex food and beverage matrices, underscoring the significance of a multi-tiered analysis strategy in forensic investigations.

Ethical Clearance: Ethical clearance was unnecessary as the study did not use biological samples or animal trials.

Conflict of Interest: Nil

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A Retrospective Study of Custodial Deaths in Visakhapatnam Region During 2021-24

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Abstract

Custodial deaths in detention settings such as police cells, prisons, borstal schools, and asylums raise serious human rights concerns. A thorough medicolegal investigation serves as the cornerstone for ensuring accountability and transparency in addressing state-sponsored crimes in modern societies. This retrospective study aims to identify the causes of custodial deaths in the Visakhapatnam region, analyse the factors contributing to these deaths, and suggest necessary reforms in prison healthcare. The study analysed autopsy records of 25 custodial deaths from October 2021 to October 2024, including 20 cases with sufficient data. Key factors examined were age, type of custody, place of death, preexisting medical conditions, and cause and manner of death. Out of 20 prisoner deaths, 18 (90%) were due to natural causes, primarily due to chronic lung diseases like bronchiectasis, bronchopneumonia, and lobar pneumonia (7 cases, 35%). Psychiatric illnesses were present in 4 cases (20%). The most affected age group was 31–40 years (7 cases, 35%). Unnatural deaths (10%) involved two suicides, both without a prior psychiatric history. Custodial deaths persist as a critical issue despite prison reforms, driven by systemic shortcomings in prison healthcare. This study identifies chronic lung diseases (35%) as the leading natural cause, highlighting the need for improved access to care. Strengthening prison healthcare systems is essential, with a focus on regular screenings for respiratory conditions like TB, comprehensive mental health assessments, better hygiene, and promoting health-seeking behaviors among inmates to prevent custodial deaths.

Keywords: Custodial deaths; Jail conditions; Human rights; Prison administration; Prison health care.

Introduction

A prison, also known as a jail or correctional facility, is an institution that holds individuals in custody as punishment for crimes they have

committed¹. As one of the most populous countries in the world, India's prisons are overcrowded, housing around 573,000 prisoners (NCRB 2022), which ranks it among the countries with the largest

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prison populations globally². Death occurring in some form of custodial detention such as police cell or prison, is commonly known as death in custody³. However, custody not only includes prison, but also police custody, borstal schools, asylums, and prisoners dying in hospitals during the currency of their punishment. These fatalities can be described as occurring under circumstances such as detention, arrest, or pursuit, where the deceased was in direct or indirect contact with a law enforcement official at the time of their death. They are often referred to as apprehension fatalities, legal intervention deaths, or in-custody deaths⁴.

According to the National Association of Medical Examiners in the USA, pre-custody deaths include those occurring before physical restraints associated with arrest, while in-custody deaths include those occurring in police custody and correctional centres, including pretrial and sentenced custody⁵. Deaths in custody may potentially draw significant public attention to the safety and well-being of the prisoner population as it involves the twin concepts of human dignity and state culpability⁶. The Indian Constitution upholds the rights of condemned prisoners, ensuring that no citizen can be deprived of life or liberty except through established legal procedures, as guaranteed under Article 21. This has paved way for procedural safeguards in criminal law and mandates legal compliance. The ignorance of the custody authorities and their disregard for the health of the jail inmates have been identified as the main reasons of custodial deaths.

This study analyses autopsy data on custodial deaths to identify causes and manners of death, evaluate prisoner demographics and criminal profiles, and examine preventive measures for common causes. The findings aim to inform necessary prison health care improvements and propose actionable reforms for policymakers.

Materials and Methods

A retrospective study of custodial deaths was conducted at Andhra Medical College,

Visakhapatnam. A purposive sampling method was employed for the study. Out of 25 identified cases, 20 were included in the study, as 5 were excluded due to insufficient records. The data was drawn from various sources, including autopsy reports, inquest, police case dockets, and prison medical examination records. Key parameters examined included age, manner and location of death, type of custody, preexisting medical conditions, and cause of death.

Only custodial deaths with comprehensive postmortem documentation were considered. Cases with incomplete records or deaths that occurred outside of custody were excluded.

Results

Andhra Medical College, a leading tertiary care centre in Visakhapatnam, conducts around 2,000 postmortem examinations annually, including all custodial death autopsies in the jurisdiction of the Visakhapatnam Police Commissionerate. These autopsies strictly adhere to NHRC guidelines, with each procedure videographed for thorough documentation. The study analysed 20 custodial deaths, all involving prisoners. Of these, 18 deaths (90%) were due to natural causes, while 2 deaths (10%) were unnatural (suicides). Among the 18 natural deaths, respiratory conditions were the leading cause, accounting for 7 cases (35%), with chronic lung diseases such as bronchiectasis, bronchopneumonia, and pneumonia being predominant. Additionally, 4 inmates (20%) had recorded preexisting psychiatric diseases. All 20 victims were men (Table 1), with the age range of 31 to 40 years old contributed to 7 cases (35%) (Table 1), with an average age of 44 years (Table 1). Seven deaths (35%) occurred in transit, while 13 deaths (65%) occurred in the hospital. In the present study, neither of the two individuals who died due to hanging had a known history of mental illness prior to their deaths, raising concerns about potential underdiagnosis or insufficient mental health services in prisons. The study also reveals that middle-aged men are particularly vulnerable to custodial deaths.

Table 1: data on sex, manner of death, age of the victim, place of death and the type of custody in the study population.

Background		Oct 2021 (n=1)	2022 (n=5)	2023 (n=9)	Oct 2024 (n=5)	Total (n=20)
Sex	Male	1(100%)	5 (100%)	9(100%)	5(100%)	20(100%)
	Female	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Manner of death	Natural	1(100%)	4(80%)	9(100%)	4(80%)	18(90%)
	Unnatural	0(0%)	1(20%)	0(0%)	1(20%)	2(10%)
Age	21-30	0(0%)	1(20%)	0(0%)	2(40%)	3(15%)
	31-40	0(0%)	2(40%)	4(44%)	1(20%)	7(35%)
	41-50	1(100%)	0(0%)	2(22%)	1(20%)	4(20%)
	51-60	0(0%)	0(0%)	1(11%)	1(20%)	2(10%)
	61-70	0(0%)	2(40%)	1(11%)	0(0%)	3(15%)
	>70	0(0%)	0(0%)	1(11%)	0(0%)	1(5%)
Place of death	Judicial custody	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	Police custody	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	Transit death	0(0%)	2(40%)	3(33%)	2(40%)	7(35%)
	Hospital stays	1(100%)	3(60%)	6(67%)	3(60%)	13(65%)
	1.More than 24 hrs.	1(100%)	1(20%)	4(44%)	1(20%)	7(35%)
	2.Less than 24 hrs.	0(0%)	2(40%)	2(22%)	2(40%)	6(30%)
	3.Brought dead	0(0%)	2(40%)	3(33%)	2(40%)	7(35%)
Type of custody	Prison	1(100%)	5(100%)	9(100%)	5(100%)	20(100%)
	Police	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)

Table 2: Cause of the Death and Manner of the Death in the Study Population

Cause of Death	Oct 2021 (n=1)	2022 (n=5)	2023 (n=9)	Oct 2024 (n=5)	Total (n=20)
Manner of Death	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Natural					
Tuberculosis	0(0%)	1(20%)	0(0%)	0(0%)	1(5%)
Lung Carcinoma	0(0%)	0(0%)	1(11%)	0(0%)	1(5%)
Chronic lung disease Bronchopneumonia and Lobar Pneumonia	1(100%)	1(20%)	4(44%)	1(20%)	7(35%)
Congestive cardiac failure	0(0%)	2(40%)	0(0%)	0(0%)	2(10%)
Sudden cardiac arrest	0(0%)	0(0%)	1(11%)	0(0%)	1(5%)
Coronary Artery Disease	0(0%)	0(0%)	0(0%)	1(20%)	1(5%)
Rheumatic heart disease.	0(0%)	0(0%)	0(0%)	1(20%)	1(5%)
Sepsis & chronic liver disease.	0(0%)	0(0%)	1(11%)	0(0%)	1(5%)
Multi Organ Dysfunction Syndrome (MODS) following follicular nodular disease of the thyroid.	0(0%)	0(0%)	0(0%)	1(20%)	1(5%)
MODS & Acute Kidney Injury (AKI)	0(0%)	0(0%)	1(11%)	0(0%)	1(5%)
Non-Specific disease entity.	0(0%)	0(0%)	1(11%)	0(0%)	1(5%)
Unnatural					
Suicide	0(0%)	1(20%)	0(0%)	1(20%)	2(10%)
Homicide	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Accident	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)

Table 3: Distribution of preexisting diseases in the study population.

Preexisting disease.	Cases (%)
Diabetes mellitus	2(10%)
Hypertension	2(10%)
Chronic Obstructive Pulmonary Disease (COPD)	1(5%)
Human Immuno-deficiency Virus/ Acquired Immuno Deficiency Syndrome (HIV/AIDS)	0%
Tuberculosis	2(10%)
Anemia	0%
Cancer	1(5%)
Chronic Kidney Disease (CKD)	0%
Epilepsy	2(10%)
Autoimmune disease	1(5%)
Respiratory illness	0%
Psychiatric illness	4(20%)
Chronic liver disease	2(10%)
Neurological disorders	2(10%)
Endocrine disorders	1(5%)

Discussion

Custodial deaths have long been associated with harsh survival conditions, inadequate medical care, neglect, and, at times, overt physical or psychological abuse. Historically, during the colonial era, inmates endured harsh and unsafe conditions with little regard for their well-being⁷. The issue of custodial deaths began gaining attention in the 20th century, particularly following the rise of the global human rights movement. This movement, alongside advocacy by the United Nations and other international organizations, led to the establishment of standards for the treatment of individuals in custody. Key milestones include the adoption of the Universal Declaration of Human Rights (1948) and the Standard Minimum Rules for the Treatment of Prisoners (originally introduced in 1955 and later revised as the Nelson Mandela Rules in 2015)⁸⁻⁹. Despite these measures, prison inmates in India continue to face significant challenges, including inadequate healthcare, exposure to various diseases, and physical/mental trauma during their time in custody. During 2021–2022, the National Human Rights Commission (NHRC) received 175 reports of

deaths in police custody and 2367 reports of deaths in judicial custody¹⁰. The present study is just a snapshot of the total number of custodial deaths that occur in this country.

Norfolk¹¹ (1994) in Bristol, United Kingdom, brought attention to the troubling issue of deaths in police custody, where the majority of those who died were men (94%), aged between 16 and 63 years, with an average age of 38. Many of these deaths were caused by hanging (37.5%) or due to alcohol and drug use (40.6%), while others were linked to head injuries or heart conditions. Similarly, in our study, all custodial deaths involved male individuals. However, 90% of custodial deaths in the present study were due to natural causes, with respiratory diseases accounting for 35%, while 10% were suicides which are concordant with Jhamad et al (2014)¹² Bansal et al (2024)¹³. Although studies by Norfolk¹¹ and Rambarapu Sudha et al. (2016)¹⁴ highlight significant instances of custodial torture, it is important to note that their findings are based on data that is 2-3 decades old. One can't ignore the fact that natural causes of death are on a rise in prisoners during the past decade according to recently published studies¹²⁻¹³.

In our study, most of the victims were older detainees, whereas the studies by Shifan Wu et al. (2019)⁶ and Soyemi et al. (2021)⁴ primarily involved younger men. This difference may be due to variations in the timing of imprisonment i.e. age at conviction, and the criminal profiles of prisoners in different countries. In their studies, most natural deaths were caused by cardiovascular diseases, while unnatural deaths resulted from accidents, suicides, and homicides. In contrast, our study only reported suicides as the cause of unnatural deaths. Our findings are similar to those in Chaudhari et al.'s study (2023)¹⁵, which also focused on 20 predominantly male deaths. Both studies identified chronic lung diseases as a leading cause of natural deaths, although Chaudhari et al. primarily attributed these to ischemic heart disease. Unnatural deaths in Chaudhari et al.'s study included suicides, blunt trauma, and choking¹⁵.

Prisons serve as hotspots for the spread of various diseases, posing risks not only to inmates but also to society. Many detainees are held for short periods, creating opportunities for disease transmission when they interact with prison officials or return to their

communities. Additionally, disease transmission between prisons is possible when inmates are transferred, further amplifying the risk¹⁶. A UN Drugs and Crimes' report found that 63% of convicts in Indian prisons had a history of drug abuse¹⁷. Sexual deprivation in prisons often leads to an increased urge for sexual activity, which can result in individuals engaging in same-sex relationships to satisfy their desires. This behaviour contributes to serious issues, including the spread of HIV, sexual crimes, conflicts, custodial deaths, and mental health disorders such as PTSD and depression. Overcrowding further amplifies these problems, emphasising the urgent need for timely parole, furlough, and improved prison conditions to address these effects¹⁸. Sodomy in prisons, both in India and around the world, remains a prevalent issue that is rarely openly discussed. Implementing education, STI prevention strategies, and access to condoms, along with addressing root causes, is essential to reducing sexual violence, and STI transmission in prisons.

Poor hygiene and unsanitary conditions in prisons lead to a high prevalence of infectious diseases, including skin, respiratory, gastrointestinal, and waterborne illnesses. In our study, respiratory illnesses emerged as a major factor in many custodial deaths, aligning with findings from Sunil D. Kumar et al.¹⁹, who reported a high incidence of acute respiratory tract infections among inmates. Additionally, the study highlighted parasitic infestations such as ascariasis and anaemia affecting approximately 84% of inmates. Improved hygiene and reduced overcrowding are crucial to lowering the prevalence of these health issues in prisons.

Although no women were involved in custodial deaths in this study, women in Indian prisons face unique healthcare challenges. According to a newspaper report, 23,772 women are incarcerated, with 77% aged 18–50, likely experiencing regular menstruation. However, access to sanitary napkins remains inconsistent, and their quality often falls short. Addressing period poverty in prisons requires collaboration to ensure access to menstrual hygiene products and facilities, prioritizing women's health and dignity.²⁰

A study conducted by Ferris, Madison et al²¹. reviewed skin conditions in inmates across various

countries, consistently finding that these conditions were largely caused by lack of access to quality healthcare, poor hygiene, overcrowding, and inadequate treatment. Common ailments included acne, psoriasis, fungal infections, and scabies.

Confinement can often trigger acute mental health issues or exacerbate pre-existing conditions in inmates. Several amendments to the Prison Act of 1894 now require the documentation of any existing mental illnesses in prisoners and mandate the provision of psychosocial support, as well as treatment by qualified mental health professionals. It is the responsibility of the prison medical officer and the superintendent to ensure the mental well-being of all prisoners²².

In a private conversation with a medical officer from a prison in South India, it was revealed (on condition of anonymity) that most inmates are housed in large hall-like barracks with 20-30 individuals, each having access to attached washrooms. About 25% of the prison population suffers from non-communicable/lifestyle diseases like diabetes and hypertension, with regular check-ups conducted every 14 days. Around 5% of the prison population has mental health issues such as psychosis and depression, and there was an incident of attempted suicide by a transgender inmate. A psychiatrist visits the prison monthly, and dermatological infections, including taeniasis, are common, with inmates referred to a local government hospital for further treatment.

Strengthening in-prison healthcare, routine screenings, and timely referrals are key to preventing avoidable deaths and ensuring humane management. Most deaths in this study occurred within 24 hours of hospital admission, highlighting delays in seeking care. Uncontrolled diabetes and hypertension, due to noncompliance and inadequate care, significantly increase the risk of complications and death. Early management, strict drug adherence, and routine tests are essential to improving inmate health and reducing preventable deaths.

Our study found that 35% of deaths were transit-related. The Nelson Mandela Rules stress the importance of humane treatment during transit, ensuring prisoners' health and safety. To reduce these

deaths, it is essential to implement proper procedures, such as pre-transit medical exams, availability of well-equipped ambulances and qualified medical staff enroute to hospitals.

In an ideal prison system, rehabilitation should take precedence over punishment. Community reintegration, through parole and job placement, should be prioritized, and for minor offenses, community service should be preferred over incarceration. By focusing on rehabilitation through education, vocational training, and restorative justice programs that address the root causes of crime, India can strengthen its justice system by adopting key elements of Norway's prison model²³. Improving prison facilities, offering compassionate housing, and training staff in dynamic security techniques would all contribute to a successful rehabilitation environment, while providing parole to non-violent offenders. However, it is important that this approach does not create the impression that prison is a preferable place to live.

Conclusion

The evolving dynamics of prison systems, particularly in the post-COVID era, have seen an increased focus on health consciousness among administrators in the correctional services. While incidents of torture and state executions have declined, the rise in deaths from preventable natural causes among inmates is concerning. This mirrors societal trends where preventable conditions claim lives prematurely. Additionally, cases of undiagnosed mental illness leading to suicide in prisons highlight systemic gaps in mental health care and its profound ripple effects on the prison environment and society. A custodial death from torture is an act of commission by the state, while a preventable natural death due to neglect is an act of omission. In both cases, the state cannot absolve itself of responsibility for inmate welfare and health. To address these challenges, proactive health surveillance, enhanced screening for conditions like tuberculosis (TB) and HIV, and fostering health-seeking behaviour among inmates must become integral to prison administration. As forensic pathologists, it is vital not to overlook potential masked or staged homicides amidst rising natural deaths, ensuring rigorous investigations to uphold justice and accountability.

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