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Method Validation and Quantitative Estimation of Ethanol using n-Propanol as Internal Standard in whole Blood by Gas Chromatography - Headspace (GC-HS)

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Abstract

Detection and quantification of ethanol in drunken driving cases is of immense importance inforensic toxicology. Despite several analytical methods being available for identification of ethanol in blood, an accurate quantification with minimum sample preparation and rapid analysis is still an ongoing task. The present study evaluates the suitability of Headspace-Chromatography with a capillary column and Flame Ionisation Detector as a method for determining the ethanol content in whole blood samples received for the blood alcohol concentration analysis. An internal standard 'n-propanol' was added to the sample to authenticate the results. The peaks areas were measured and calculations were carried out considering peak ratios of analyte to internal standard. The total run time of GC and HS was 20.68 min. The validation study for the method resulted in linearity for a range of 7.9 mg-237 mg/100 ml, coefficient of variance (R^2) was 0.999. Recovery of more than 96% was achieved in spiked samples. LOD and LOQ were 0.20 mg/100 ml and 1.0 mg/100 ml respectively. Blood ethanol measurement by this method is an easy, simple, reliable and reproducible.

Keywords: GC-HS; Alcohol; ICH Guidelines; FID; Whole Blood etc.

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Introduction

Chemically, ethanol (C_2H_5OH) or ethyl alcohol belongsto a group of chemical compounds known as alcohols. An alcoholic beverage is a drink which contains substantial amount of this psychoactive drug, ethanol (informality called alcohol) in low doses causes euphoria, reduced anxiety and sociability and in higher doses causes intoxication (drunkenness), stupor and unconsciousness [1].

When ethanol is ingested, usually in form of an alcoholic beverage, it is readily absorbed in the blood by the process of simple diffusion by stomach lining. According to Fick's law, the rate of diffusion across a membrane is proportional to the concentration gradient on either side of the membrane [2]. Therefore more the concentration of alcohol in stomach, more with be the absorbtion. This process can be hastened by many other factors like presence of kind of food in stomach, like oily and fatty food will delay the absorbtion whereas light and sugar rich would hasten the absorbtion.

The temperature of drink, quantity of water, aerated or nonaerated nature of drink will affect the rate. With its absorbtion, majority of the alcohol is broken down or metabolised in liver through the portal venous system. Its main metabolisation is done with the "Alcohol Dehydrogenase system" yielding carbon dioxide and water as end products and acetaldehyde and acetic acid as the intermediaries. Rest of the ethanol is excreted via the kidneys unchanged in urine. With time the ethanol in blood stream is continuously removed by metabolising in liver. A formula (Widmark's) has been created to predict the amount of ethanol and individual has consumed based on time and the measured BAC [3].

Due to its use and easy abuse, ethanol is significantly associated with violent acts, drunken driving, suicides etc [4]. Due to these acts of abuse there are stringent laws associated with their consumption and conduct. Therefore determination and quantification of ethanol is perhaps the most important routine analysis done in toxicology laboratories [5]. The number of samples received and their relatively short hold time, has nowadays resulted in labs requiring methods that are fast, accurate, reliable, and have less chances of human error.

Traditional ethanol analysis is done by methods like Widmark's method, enzymatic reactions, and cuvette test, although these procedures are fast, they lack the accuracy of quantitation. In contrast, Gas Chromatography is qualitative (retention time) as well as quantitative (peak area). Latest technologies of GC with manual injection and GC with mass determination even though yield excellent separation and quantification, are known to include small inaccuracies. These inaccuracies can be in form of presence of interfering compounds and other component in biological samples during traditional methods of sample preparation and volatile extraction. On the other hand use of static headspace analysis with GC offers limited to no sample preparation, less contamination risk, higher sensitivity, complete automated analysis. Static headspace works by concentrating the volatile prior to analysis and examining the concentration of these analytes directly from the vapour phase in the sealed vial with sample. The concentration of vapours is done by sealing the vial before heating [6–10]. For laboratories pursuinghighly reliable results of volatile analysis GC-HS is the preferred technique due to its simplicity and the high number of repeated analysis in an normal daily run. This technique offers diminished inlet and column maintenance, highest sample throughput, reduced interfering artefact or sample degradation, robust and trouble free design.

In this article an attempt has been made to analyse blood samples using Gas Chromatography instrument from cases brought to laboratory for the analysis of blood ethanol concentration in cases of drunken driving, and other cases where query if for qualitative and quantitative estimation of ethanol.

Experimental

Materials and Method

Instrument: Gas Chromatography system, Model No. 7890A and Headspace Sampler, Model No. 7697A from Agilent, U.S.A. were used.

Column: DB-624 length 30m, diameter 0.530 mm, film 3.00 µm, Temperature Limits from 20^0C to 260^0C was used

Software: ChemStation® for the data analysis of the signals was used.

Reagent / Chemicals: Ethanol (GC grade) and n-propanol (GC grade) from Merck, Germany and Ultra-pure water from Rions India were used.

Glassware: 20 ml Head space vial from Agilent Technologies U.S.A. were used.

Miscellaneous: Micropipette of volume 100-1000 µl and 20-200 µl from Corning, U.S.A., septa (PTFE) and aluminium crimpfor sealing the HS vial from Agilent, U.S.A. were used.

Preparation of Ethanol Standard Solution

The stock solution of concentration, 790 mg/100 ml was prepared from absolute ethanol, by dissolving 1 ml of standard ethanol with ultrapure water in 100 ml volumetric flask.

Five working dilutions of concentration 19.75 mg/100 ml; 39.5 mg/100 ml; 79 mg/100 ml; 158 mg/100 ml; 237 mg/100 ml were prepared from the stock solution by dissolving volumes of 250 µl; 500 µl; 1000 µl; 2000 µl; 3000 µl in 10 ml volumetric flask with ultrapure water.

Preparation of Internal Standard Solution

Three hundred (300) µl ofinternal standard (n-propanol) was dissolved in 100 ml of ultrapure water in a volumetric flask.

Preparation of Calibration standard

One (1) ml of each from 19.75 mg/100 ml; 39.5 mg/100 ml; 79 mg/100 ml; 158 mg/100 ml; 237 mg/100 ml standard were taken in five different HS vial and 90 µl of internal standard was added in each vial. Each glass vial were sealed with septa and metallic crimpusing crimper.

Preparation of Sample

One (1) ml of blood sample was taken in HS vial and 90 µl of internal standard was added to it. Vial was sealed with septa and metallic crimp using crimper.

Instrumentation conditions

GC conditions: GC cycle time was set at 20.00 min. A constant Nitrogen flow of 8 ml/min was used. The injection port temperature was maintained at 250^{0}C with a 5:1 split injection of the Headspace and a septum purge flow of 3 ml/min. The initial GC oven temperature of 50^{0}C was held for 5 min and then ramped at 35^{0}C/min to a final temperature of 200^{0}C held for 1 min. Total GC runtime was for 10.286 min per sample.

Headspace conditions: Headspace oven temperature was set at 70^{0}C. The HS Loop and Transfer Line Temperature were set at 80^{0}C and 90^{0}C resp. Vial equilibration was set at 10.00 min. Injection, loop fill and total cycle time were set at 0.50 min, Default, 16.00 min respectively.

Detector conditions: Flame Ionisation detector was used for the detection of analytes. The FID temperature was maintained at 250^{0}C with Hydrogen (40 ml/min), Zero Air (400 ml/min) and makeup flow of 25 ml/min. the FID signal was zeroed at 0.01 min with data collection rate of 20Hz.

Result and Discussion

Ethanol was detected in the spiked samples of blood and DDW. GC is the routinely utilised instrumentation for ethanol estimation in forensic laboratories. Before utilisation of the developed method for quantification of ethanol in biological fluid, the developed method was fully validated for specificity, linearity, accuracy, precision, repeatability, detection limit and quantification limit according to ICH guidelines [11].

Specificity

The method demonstrated excellent chromatographic specificity with no endogenous interference at the retention times of ethanol (1.624) and n-propanol (2.900). Specificity was confirmed by analysing a known standard and overlapping its graph with that of an unknown blood sample as shown in Figure 1.

Linearity

The five serial dilution ranging from 19.5% mg to 237% mg (v/v) were selected to plot the calibration curve. Linearity of the curve was calculated using concentration (x-axis) vs peak area (y-axis) as shown

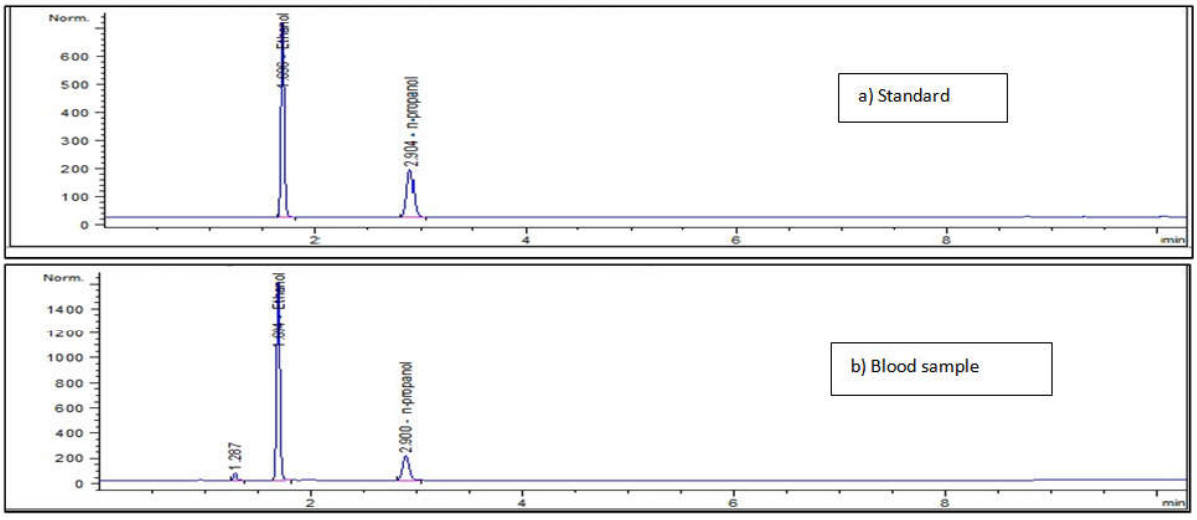

Fig. 1: GC-HS Chromatograms of a) standard sample of ethanol in DDW with n-propanol as Internal Standard, b) unknown sample of ethanol in blood with n-propanol as Internal Standard

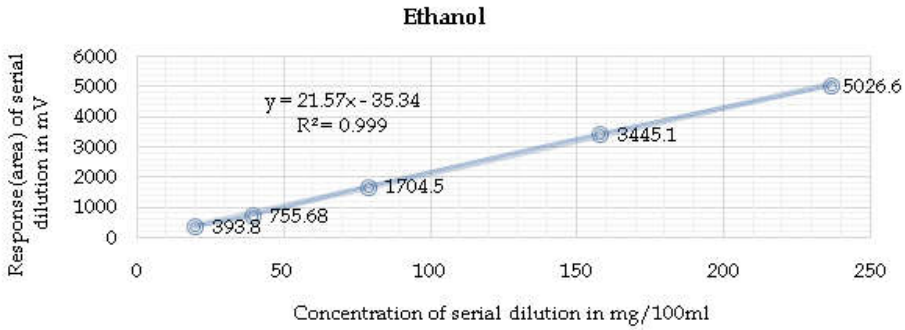

Fig. 2: Five point calibration curve of ethanol

Table 1: Inter and Intra- Day precision and accuracy of standards spiked in blood and water

Matrix	Solvent	Conc (mg/100 ml)	Intraday			Interday		
			Estm Conc. Mean ± SD (mg/100 ml)	Precision (RSD)	Accuracy	Estm Conc. Mean ± SD (mg/100 ml)	Precision (RSD)	Accuracy
Water	Ethanol	39.5 mg	39.6 ± 0.2	0.4	0.25	38.95 ± 0.70	1.81	1.39
		79.0 mg	80.15 ± 0.65	0.81	1.43	79.57 ± 0.67	0.85	0.71
		158.0 mg	159.55 ± 0.72	0.45	0.97	158.44 ± 1.21	0.77	0.27
Blood	Ethanol	39.5 mg	39.46 ± 0.11	0.29	0.10	39.22 ± 0.61	1.58	0.71
		79.0 mg	79.4 ± 0.1	0.13	0.50	79.75 ± 0.69	0.87	0.94
		158.0 mg	159.22 ± 0.16	0.10	0.76	158.85 ± 1.15	0.73	0.53

Table 2: Recovery of spiked concentration of standards in blood and water

Matrix	Concentration (mg/100 ml)	Range (mg/100 ml)	Mean	Recovery %
Water	7.9	7.9 - 8.0	7.95	100.63%
	19.75	19.50 - 19.90	19.7	99.74%
Blood	7.9	7.3 - 7.9	7.6	96.20%
	19.75	19.25 - 19.56	19.40	98.22%

in Figure 2. This resulted in correlation coefficient (R2) of 0.999. The concentration of an unknown sample was calculated using this calibration curve.

Accuracy and Precision

Intraday assays were performed using five replicates during a single day and interday assays on 3 different days. For 3 different concentrations 5 replicates were run to determine Accuracy and Precision. The results are shown in Table-1. Reproducibility of method in cases of blood sample were calculated by taking the same sample for every analysis and storage under optimal conditions to avoid degradation. Percentage accuracy is calculated as-

$$\% \text{ Accuracy} = \frac{(\text{Calculated Concentration of analyte} - \text{Actual Concentration}) \times 100}{\text{Calculated Concentration}}$$

Recovery and carry over effect

Recovery test were performed by spiking a known concentration (7.9 mg/19.75 mg) of standard to blood and water. A recovery rate of more than 96% was obtained as shown in Table 2. As evident from the data of table 2, recovery of ethanol increased with increased concentration of standard because at higher concentration the matrix effect reduces.

To study that no carryover of the previous sample remained in the head space sampler or the column after the analysis of a high concentration biological sample, a blank was run. No residual peak in the blank run confirms no carryover with this method.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

To determine the sensitivity of the method, the calibrator of the solvent with the lowest

concentration (7.9 mg/100 ml) was progressively diluted to determine the lowest limit of detection (LOD) and quantification (LOQ). The concentration to give signal to noise ratio of 3 was considered acceptable for estimating LOD. LOQ was estimated based on the signal to noise ratio of 10 obtained by diluting the standard to such extend that all compounds are detected with sharp, symmetrical chromatographic peaks. Limit of detection (LOD) was found to be 0.20 mg/100 ml and limit of quantification (LOQ) 1.0 mg/100 ml.

Discussion

Blood alcohol concentration of an case sample helps the investigator to know about the of ethanol at presence of ethanol at the time of offence. The developed method was used to analyse real life samples of blood alcohol. Quantitative method for the analysis of real samples of blood was validated for GC-HS-FID according to the ICH guidelines. To overcome the risks of any artefact during analysis an internal standard was used. Thus, the peaks areas were measured and calculations were carried out considering peak ratios of analyte to Internal Standard.The use of internal standard and flame ionisation detector in the above method for GC-HS sample testing proves effective for the proper detection of the components in the sample provided as well as in obtaining the near accurate quantity of the analyte in the sample. Using this method, desired calibration of ethanol was performed with 0.999, coefficient of variance. The peaks are obtained of the standard (GC grade ethanol) and the sample ethanol from blood with the internal standard, and is plotted against the retention time v/s the response from the FIDdetector.By evaluating the chromatograms, it is seen that the given blood sample contains ethanol as it shows similar retention (1.624 min) with that of the standard ethanol (1.624 min). Another small peak is obtained in the sample chromatogram having retention time of 1.287 min, which might be of any other analyte in the given sample or may be some impurities present in it. The quantity and the concentration of the analyte present in the sample was obtained by calculating the area of the peak.

Conclusion

The method developed is easy, economical, and rapid. It shows reasonable specificity with small sample volume. Analysis is not interfered by other volatiles in the environment. Technique is very useful in cases of driving under influence, drug-facilitated sexual assault, workplace drug monitoring, or where query is for the qualitative and quantitative estimation of ethanol in blood. Since the instrumentation is fully automated with sample preparation is done using headspace technique, manual error is significantly reduced. Therefore, this method can be routinely utilised for the said purpose of ethanol estimation in real life cases.

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Depletion Studies on Different Fluorescent Powder Compositions

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Abstract

A depletion series was used to evaluate the relative sensitivity of a number of fluorescent powder compositions on a non porous surface. A set of exemplar finger impressions of one particular finger successively impinged on to aluminium foil without recharging the sweat secretions was considered in this investigation. Amongst the studied fluorescent dye based compositions, Rhodamine B and Fluorescein based compositions were adjudged to give best results in terms of ridge clarity of depletion marks.

Kewords: Fluorescent powder; Depletion series; Fingerprint; Sensitivity.

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Introduction

Latent fingermarks are a source of positive evidence and are indispensable for establishing identity. The detection and subsequent development of latent fingermarks is thus necessary in criminal investigations [1]. Therefore it is pertinent to render the latent impression visible by an optical, physical or chemical technique. The powder dusting technique is one of the oldest and most common methods of latent print detection, with one of the earliest references dating back to 1891 [2]. This is a physical method of developing latent fingermarks and is based on the premise that the powder particles adhere to the moisture and oily components of sweat residue. For evaluating the efficacy and sensitivity of the powder method, the quality of developed prints is assessed by visual examination [3].

Fresh as well as aged fingermarks have been developed for different time intervals with these formulations. A series of fingermarks is deposited by successive contacts of the same finger with the surface. Each successive contact leaves progressively less residue on the surface and therefore as one proceeds down the depletion series, the quality of developed print concomitantly decreases [4]. The number of fingermarks in a specific depletion series that develop to optimum quality depends on the nature of surface and the constituent (s) of sweat that the development process targets. In general, on a porous surface the sweat residue depletes consistently till the 5th impression, while the 6th one leaves too meagre an amount to affect visualization by chemical techniques [5].

On non-porous surfaces the absorption of sweat constituents is less and some of the techniques develop even the 10th impression of the depletion series. In the present investigation, we have used a non-porous surface, aluminium foil, to develop latent impressions by fluorescent compositions and thereafter examine the results of depletion studies.

We have done the quality and sensitivity check for all the studied fluorescent powder compositions. These studies are published separately.

Materials and Method

In the present study, Starch (Sigma Aldrich) and talc or hydrated magnesium silicate (commercial grade) was taken as an adhesive material. The following fluorescent dyes have been used for the preparation of different formulations coded as indicated.

1. Brilliant Blue (Sigma Aldrich) ($\lambda_{max\text{-}abs}$ 596 nm); code ST-B
2. Acridine orange (Sigma Aldrich) ($\lambda_{max\text{-}abs}$ 489 nm); code ST-A
3. Eosin Y (Sigma Aldrich) ($\lambda_{max\text{-}abs}$ 524 nm); code ST-E
4. Rhodamine B (S.D.Fine Chem.) ($\lambda_{max\text{-}abs}$ 543 nm); code ST-R
5. Fluorescein (Sigma Aldrich) ($\lambda_{max\text{-}abs}$ 494 nm); code ST-F

Brilliant blue R is a triphenylmethane dye that is used in the textile industry, as well as for staining proteins in analytical biochemistry [6]. Rhodamine B is a bluish red, fluorescent, amphoteric dye used generally as a biological stain along with osmic acid to fix and stain blood [7]. Acridine orange is a metachromatic, fluorescent, cationic dye, commercially used in lithographic applications and dyeing leather [8]. Eosin Y is a water soluble dye, used in textile dyeing and ink manufacturing. Fluorescein is a synthetic organic dye, slightly soluble in water and alcohol. It has an absorption maximum at 494 nm and emission maximum of 521 nm (in water) [9].

The experiments were carried out in the months of December/January when the temperature varied from 20 to 250C and the relative humidity between 40% and 50%. To 25 mL distilled water, a mixture of 4 g starch, 1g hydrated magnesium silicate and 50 mg of a fluorescent dye were added. The contents were allowed to dry under natural conditions. The solid mass was ground to a fine powder and stored in glass beakers covered with aluminium foil. The dye content in the compositions was 1.0-1.5%.

We used 15 aluminium foils (approx. 0.3 * 25m, thickness 11micron MFM HomewrappTM for fingermark deposition. The detection of the latent fingermark by fluorescent powder compositions was performed using powder dusting method [10]. The powder mechanically adheres to the residue defining the ridge pattern of the fingertips.

First, a forensic light source, in which oblique white light, was used to visually scan for fingermarks on the surface of foil prior to fingermark deposition. No traces were detected on the examination site (aluminium foil). Then, the site was labelled with number sequence. One male donor was chosen for deposition of fingermarks on foil. The participant deposited fingermarks on to aluminium foil, exerting medium pressure. One depletion series prepared for each fluorescent powder compositions. His hands were washed prior to finger mark deposition. During the deposition of latent fingermarks the contact time was around 1 second without recharging it with latent secretions. The procedure took 1 h as there were short intervals between each deposition. Thus, a total 75 latent fingermarks were deposited on foil. Fifteen successive latent fingermark of the same finger were impinged on to aluminium foil, to obtain a depletion series of progressively decreasing sweat residue. The same procedure was followed for each composition to be tested. The depletion series were constructed, using the same finger, and the impressions were marked from 1 to 15, 1 having the maximum and 15 having the least sweat deposition. Fingermarks were developed by powdering method immediately after deposition. There was no time lapse between the deposition and development of fingermarks.

Development Methods

Visual Assessment

Visual examination was used prior to other methods. The latent prints were examined using white light [11,12].

Powder/Brush

A few grams of each fluorescent powder composition was taken on a clean and dry watch glass and spread out as a thin layer. The latent fingerprint was developed by picking the powder with the aid of a brush and applying it over the latent impression. The excess of powder was removed by gently tapping the surface. For each fluorescent powder composition, a camel hairbrush was used, all of them of same brand (SIRCHIE, Standard Size Fiberglass Brush, 122L). Application of powder to the print by brushing is a simple and an easy technique. It took 1-2 minutes to develop each impression.

Photographs were taken using a digital camera (Canon power shot A1100IS-14.2MP-100 mm macro

lens) in the auto mode. All the photographs were taken with full resolution to capture finer details. However, when it comes to the actual fingermarks images, the resolution is 480 pixels x 290 pixels. The images were stored in the jpeg format for record.

Results and Discussion

When comparing the fingermarks for the first seven depletions, the quality of development was almost equal, with performance unable to be differentiated in term of quality (Figure 1). The ridges were clearly defined in terms of detail, with observable upto 7th impression (pores and ridges). Differences in terms of sensitivity emerged beyond 7th impression (Graph 1). Images of fingermarks 1, 3, 5, 7 and 10 recorded on to aluminium foil by each composition are depicted in Figure 1.

The ST-B, ST-A and ST-E compositions developed impressions up to depletions 8, 9 and 10 respectively. Brilliant blue and Rhodamine B based compositions exhibited fluorescence in the multiple wavelength range while acridine orange based composition worked well only in the short range.

Graph 1: Depletion series of latent fingermarks on aluminium foil using (a) Brilliant Blue code-ST-B, (b) Acridine orange code-ST-A, (c) Eosin Y-code-ST-E, (d) Rhodamine B- code-ST-R and (e) Fluorescein code-ST-F compositions. The grades were scored in colour mode.

The results show that ST-E, ST-R and ST-F compositions give constant results up to the 10th

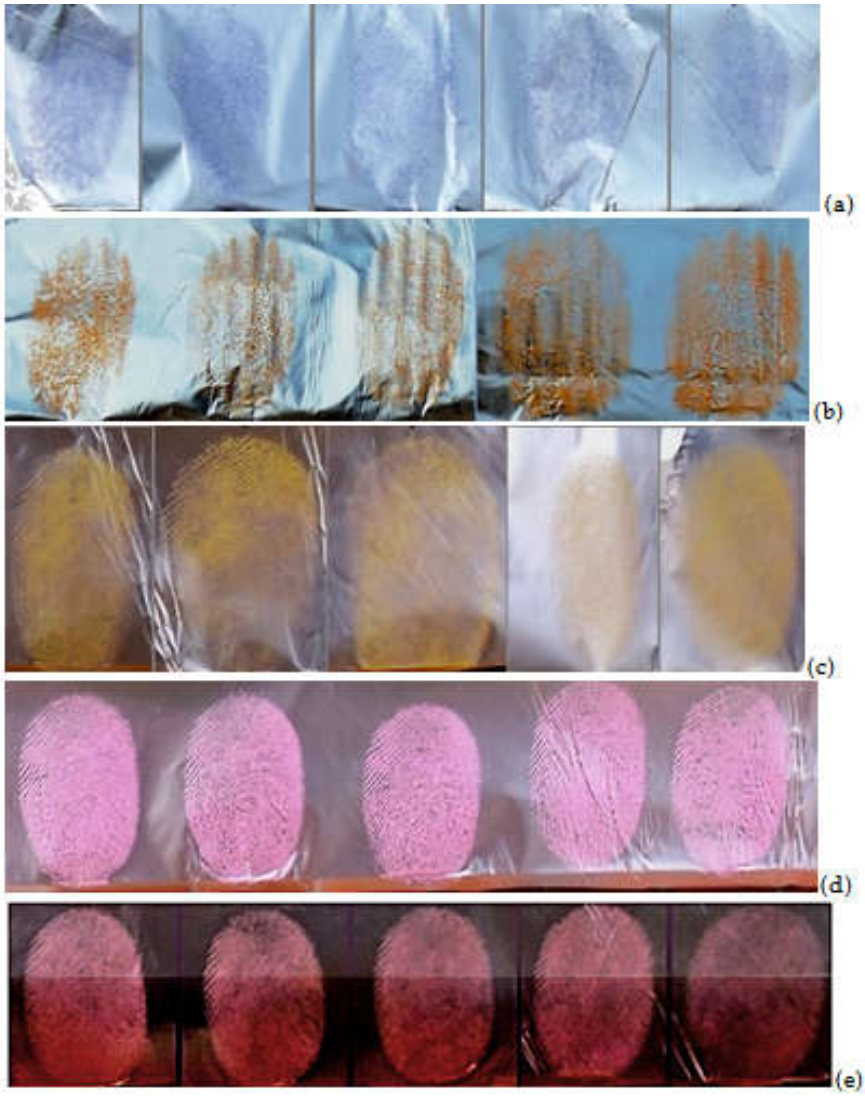


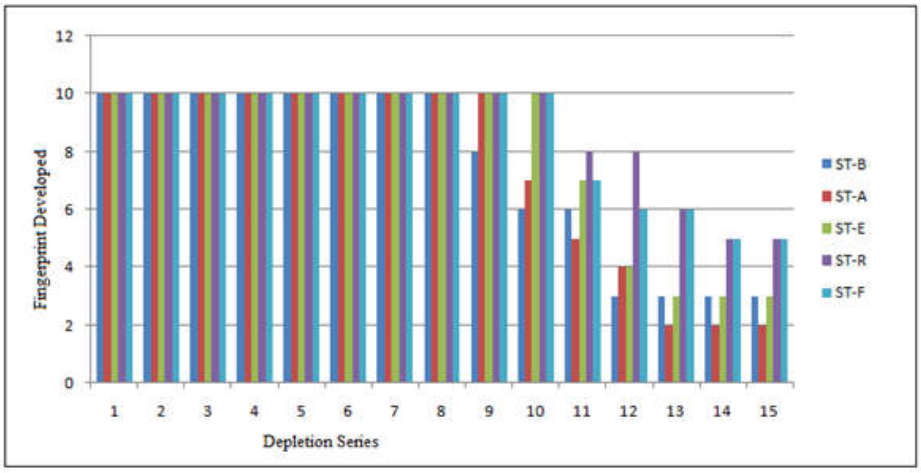
Fig. 1: Latent Fingermarks 1, 3, 5,7 and 10, developed on to aluminium foil using (a) Brilliant Blue code-ST-B, (b) Acridine orange code-ST-A, (c) Eosin Y code-ST-E, (d) Rhodamine B code-ST-R and (e) Fluorescein code-ST-F compositions

depletion mark. However, ST-A show quality differences of latent fingermarks after 12th depletion, whereas ST-R and ST-F compositions continued to give good results in terms of ridge pattern and minutiae detail even up to the last depletion (15th). These scores have been obtained by examining the fingermarks in visible mode, and that results could be improved further by examining under fluorescence.

Figure (2) shows the comparative study of latent fingerprint development using white light and fluorescent light. We also developed latent fingerprint on various porous and non porous substrates respectively. In this paper, we are focusing on depletion studies using these formulations, so we assessed results using Crime-lite® 2.

Conclusion

Latent fingermark depositions were made on to aluminium foil using five different fluorescent powder compositions. Rhodamine B and fluorescein based compositions showed optimum results in terms of detection of latent fingermarks, as well as of their depletion counterparts. The raw materials used to prepare fluorescent powder compositions are cost-effective and non-hazardous. The shelf life under ambient temperature was found to be



Graph 1: Depletion series of latent fingermarks on aluminium foil using (a) Brilliant Blue code-ST-B, (b) Acridine orange code-ST-A, (c) Eosin Y- code-ST-E, (d) Rhodamine B- code-ST-R and (e) Fluorescein code-ST-F compositions. The grades were scored in colour mode

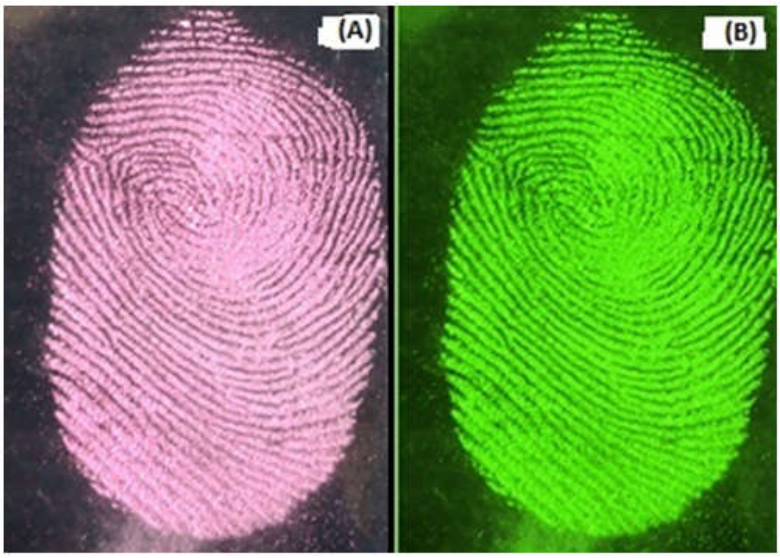


Fig. 2: Fingerprints developed on glass using ST-R (A) under White light, (B) under Fluorescent light

upto 12 months, when the reagents were stored in aluminium foil covered beakers.

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Post Mortem CT (PMCT) in Forensic Medicine and Toxicity

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Abstract

Background: The Postmortem in Computed tomography (PMCT) had been started from since 1990. PMCT advantages are more relevant in full-body examination (FBE) through scan is quite helpful to assess the whole body scan with clear evidence and computed recorded data.

Material and methods: Here trying to justify the PMCT application, analysis and its interpretation in cadaveric, CT examination test before autopsy test to know the causes of death (COD), cause of injury (COI) and other additional information related to image study.

Inclusion Criteria: The original contribution selected form (1990 to 2019) is included in study.

Exclusion Criteria: An irrelevant study or any case study subject excluded from this study.

Statistical analysis: Here simple statistics used from the available database for interpretation.

Results: PMCT in analysis given more than (70-90%) better picture than the autopsy test. It clearly analyzed the cause of death (COD), manners of injury (MOI), and personal of identification (POI) through the postmortem examination (PME) and its development in the field of forensic medicine and toxicological research (FMTR).

Conclusion: PMCT technology newly added and highly recommendable for correct information through their slice images. So many types of variables (TOVs) may easily compare and predict through the different informative slice images (DISIs) to use the accurate application on forensic medicine and toxicological angle.

Keywords: Forensic radiology; PMCT; Post-mortem computed tomography; Virtual autopsy; Research

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Introduction

Currently, Post-mortem CT (PMCT) technology is leading as the best diagnostic imaging techniques and toxicological point of view [1]. PMCT is now advanced diagnostic imaging techniques and gaining popularity in forensic biology, pathology, medicine and toxicology [2]. PMCT scan and their settings are using in post-mortem imaging machine (PMIM) employed as 8/16-slice spiral CT [3]. PMCT also observed with or without contrast to enhancement the CT-guided biopsies, post-mortem ventilation (PMV), and PMCT angiography (PMCTA)[4]. PMCT is able to guide the forensic pathologist, researchers, and clinicians in the Worldwide with respect to evidence in validation for medico legal issues [5].

Material and methods

Sample study: justifying the PMCT application,

analysis and its interpretation in cadaveric, CT examination test before autopsy test to know the causes of death (COD), cause of injury (COI), pattern of death (POD) and other additional information related to the image study.

Image processing: The location, shape, size, and essential parameters of different tissues in organs, are directly estimated through the given volume (GV). The SSM-guided expectation-maximization (EM) standardization process with respective large deformation and intensity changes.

Image analysis: The authentic segmentation performance (ASP) is measured through Jaccard index (JI) between the segmentation result and within the true label. The relevant findings documented from the obtained reports from and divided into two categories like anatomical location and tissue specific characteristics. The findings of obtained data's are calculated quantitatively in binary fashion analysis (BFA) through the basic simple mathematics.

Inclusion Criteria: The original contribution selected form the available database Pubmed/ Medline; SCOPUS/The wave of Sciences, Cochrane review, and EMBASE (1990 to 2019) is included in study.

Exclusion Criteria: An irrelevant study or any case study subject excluded from this study.

Statistical analysis: Here simple statistics used from the available database for interpretation.

Results

The majority of PMCT reports (70-90%) noted for the skull fractures (SFs), intraventricular- and subarachnoid hemorrhages (IVSAHs), bullet trajectories (BTs), intracranial shrapnel (ICS) correlation of brain atrophy (COBA) hernia ion, and facial and the soft tissues [6-7]. The sensitivity and specificity in the brain edema (BE), brain atrophy (BA), brain injury (BI), presence of gases (POG) at tissues, cavities are quite better values according to their detection rates (DRs) [8]. The spinal cord, spinal column, spinal canal, joints, lumbar discs, ligaments, coronary atherosclerotic stenosis (CASS) and other additional tissues gives better images with their significant values [9].

PMCT angiography (PMCTA) provides excellent consistency values with immuno histopathological (IHC & IHP) findings in diagnosis of coronary atherosclerotic stenosis degree (CASSD) [10]. Now automated liver segmentation (ALS) from PMCT volume is great challenging in larger deformation and intensity (LDI) changes due to severity in pathophysiology amongst postmortem changes report noticed [11]. Novel segmentation process approach (NSPA) helps in statistical shape model (SSM) in postmortem liver (PML) through an automated liver segmentation (ALS) at their appropriate PMCT volume (PMCTV), intensity, values for calculation and interpretation for the correct observations [12].

Discussion

The SSM-guided EM suggested that location, shape, size and variable, parameters of liver in given volume (GV), given intensity (GI), effectiveness of actual postmortem CT volumes (APMCTVs) [13]. Postmortem radiology discipline (PMRD) is developing specialty, used as one unique substitute for conventional autopsy (SFCA) [14].

PMCT goal is to find out patterns of death (POD), and its causal factor of death (CFOD), with evidence based approach is more important [15]. PMCT images are light processing of decomposition (LPOD), so that radiologists are presently, unfamiliar within the majority of postmortem (MOPM) changes and there specific regimen still a research question [16].

Ideally, formation of gas (FOG), edema, atrophy should not be any mistaken in pathological processes (PP) in the living and non-living persons [17]. The importance of PMCT and postmortem thoraco-abdominal (PMTA) changes on the FMCT images are differentiating these clinical findings, including with their pathological processes [18].

According to the Detector's eye view (DEV)-based subsets of expectation maximization (SOEM) report is giving an accurate reconstruction on the benchtop x-ray fluorescence computed tomography (XFCT) images [19]. There is at least two data sets obtained from XFCT imaging like gold nanoparticle (GNP)-containing at phantom imaging for the postmortem and others [20].

Interventions, Outcomes and Lessons: PMCT contrast-enhances an isolation of the intestinal tract dissection (ITD) of the postmortem body and suggested that the contrast agent flowed out through the ruptures [21]. In autopsy and histological examination Hematoxilin and Eosin (H&E), immune histo chemistry (IHC) reported that perforated crevasse, and confirming their cause of peritonitis (COP) and other additional probabilities [22]. PMCT contrast also one effective

technique for the interpretation in gastrointestinal tract rupture (GITR) and objectively served as a non-invasive tool (NIT) to identify the injury and infections both sides [23].

PMCT and PMCTA is both of the combination technology (CT) and helpful for forensic pathologists to determine the cause of death (COD) and if the cases involving with the traumatic vascular injury (TVI), and traumatic brain injury (TBI) [24]. The multidetector CT (MDCT) represents and used in several forensic departments in different institutes, for there numerous applications for their better diagnostic tool for increasingly benefit in lowering the data acquisition faster (LTDAF) and revalued at the any point of time (POT) [25].

Limitations: FMCT angiography examine the pathological changes of blood vessels, might have some limitations on the diagnosis for the cause of death (COD).

Conclusion

PMCT technology is beneficial and newly added and highly recommendable for correct information through their slice images in different aspects to observe tissues in organs. So many types of variables (TOVs) easily compare through the different informative slices images to use as an evidence for accurate application on forensic medicine and toxicological angle as a standard for decision making in future and present routine practice in laboratory.

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Original Article

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Derivative UV-Vis Spectrophotometric Analysis of Caramel in Some Common Liquor Samples

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Abstract

In recent years, awareness regarding the quality of food has gradually increased as per the increase in consumer demands. One of the most important parts of the food is alcoholic beverages. Many additives such as caramel, sugar, water, etc. are added to improve the aroma volume, and color of alcoholic beverages. Caramel colors are dark brown substances often added in alcoholic beverages to give the more alluring appearance. However, many metabolites of caramel colors such as 5-hydroxymethyl furfural (5 HMF) are toxic in nature. Many studies show that in controlled concentrations, caramel colors don't pose any threat. Therefore, it is exigent to monitor the concentration of such additives. Moreover, these colors are present in trace amounts and require extensive sample preparation methods. In the present study, an attempt has been made to identify and detect caramel colors in various factory-made and commercial liquors using derivative Ultraviolet-visible (UV-Vis) spectrophotometry. It was observed that peak maxima of 281 nm of zero order spectra; in first-order spectra peak maxima of 260nm and peak minima of 290 nm can prove to be very effective in the identification of caramel colors. Derivative UV-Vis spectrophotometry thus, has proved to be an effective, non-destructive technique which requires minimal sample preparation.

Keywords: Alcoholic beverage; Caramel; Derivative UV-Vis spectrophotometry; 5-Hydroxymethyl furfuryl (5HMF).

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Introduction

Alcohol has been an integral part of human society since ancient times. For a chemist, it refers to a group of organic compounds containing –OH group. WHO has classified alcoholic beverages into two categories depending on whether the data pertaining to their production, sales, and consumption is available or not [1]. These categories are a) Recorded alcoholic beverages (those alcoholic beverages for which such data is available) and b) Unrecorded alcoholic beverages (those alcoholic beverages for which such data is not available). The composition of different alcoholic beverages depends on the raw material used, the methodology used during the production, and other factors such as environmental factors and additives which have been added to increase the aroma and coloration of the alcoholic beverages [2,3].

Alcoholic beverages often contain various types of additive, which are intentionally added to elaborate their aroma profile as well the appearance. One of the most commonly used additives is caramelcolor. Caramel constitutes one of mankind's oldest and

most important dietary material. Caramel colors include a family of distinct red to dark brown materials (liquid and powders) often used as additives in foods and beverages. Caramel colors are used to produce eye-pleasing colors in foods and beverages [4,5]. Caramel color is produced by the process of caramelization; the process of thermal treatment of carbohydrates. Industrial caramel colors are classified into four classes (I, II, III, IV). Class I (Plain caramel) caramel color is used in whiskey and other high proof alcohols. Class II (Sulfite caramel) caramel color is used in cognac, sherry, and vinegar. Class III (Ammonia caramel) caramel color is used in beers, whereas, class IV (Sulfite ammonia caramel) caramel color is used in non-alcoholic soft drinks [5,6].

The main use of caramel colors is to impart colors to beverages, however, they also serve other important functions. Caramel colors facilitate flavor retention and retard flavor changes in various alcoholic beverages [7]. Each class of caramel color are physically and chemically different and pose safety implications. Industrial caramel is composed of residual sucrose along with other mono-, and oligosaccharides such as glucose and fructose. And several degradation compounds mainly 5-hydroxymethyl furfural (5HMF) [8]. Such degradation compounds are present in trace amounts; as a result, it is very difficult to detect their presence in alcoholic beverages. Moreover, many of the degradation compounds show adverse effects on human health. Although caramel color containing ammonia often produce metabolites which pose health hazards, caramel colors meeting specifications can be used without any adverse effects. Therefore, it is important to detect, identify and quantify the additives in alcoholic beverages [8,9].

Many techniques including HPLC [8,10], UPLC – MS/MS [11], have been used to identify components of caramel color. Although these techniques provide reliable, sensitive, and reproducible results, they suffer from the limitation on part of their destructive, and expensive nature. Moreover, these techniques require extensive sample preparation which makes them time-consuming. Spectroscopic methods have emerged as an effective outcome for such problems in the last few decades. One such technique is Derivative UV-Vis spectrophotometry; which can be used as a technique of choice for qualitative and quantitative screening of various alcoholic beverages for caramel colors. It requires minimal sample preparation and can be used to separate unresolved bands, and for eliminating the effects of baseline shifts and baseline tilts by differentiating the normal zero order spectra [12]. In the present study, an attempt has been made to identify the caramel color in nine samples of whiskey, rum, and other commercial alcoholic beverages using Ultraviolet-visible (UV-Vis) spectrophotometry. Zero-order and first-order spectra were obtained to identify the maxima and minima peaks of caramel in various alcoholic beverages.

Materials and Methods

Collection of samples – Nine samples of alcoholic beverages were collected from the local markets of Chandigarh and Patiala. Table 1 illustrates the samples collected for the present study.

Table 1: Details of Samples Analysed

S. No.	Brand/ Description	Details				Source
		Batch No.	Mfg. Date	Proof	Distilled Blended and Bottled	
1.	Sun Caramel Ammonium Sulphate process food grade 15-4467, CML-L8156879, Colour int. 0.10-0.60 Manufacture- Sun Food Tech, Distt. Alwar, Rajasthan, India	4192 Type IV	Dec 2008			Local market, Chandigarh
2.	Officer's Choice Whiskey	72	Jan 2009	75^0	Batra Breweries	Local Market Chandigarh
3.	Officer's Choice Deluxe XXX Rum	OCR	Dec 2008	75^0	Rana Sugars Ltd. Village Louhka, Tehsil Patti, Distt. Tarn Taran, Punjab	Local Market, Chandigarh
4.	Officer's Choice Deluxe Whiskey	OCW 32	Jan 2009	75^0	Rana Sugars Ltd. Village Louhka, Tehsil Patti, Distt. Tarn Taran, Punjab	Local Market, Chandigarh

5.	Old Monk Rum	283	Jan 2009	75^0	Mohan Meakin Ltd. Mohan Gram, Bhankarpur, SAS Nagar (Mohali), Punjab	Local Market, Chandigarh
6.	Tohfa	----	----	50^0	Khasa Distillery Co. Gursimran Distilleries. KhasaDistt., Amritsar, Punjab	Local Market, Chandigarh
7.	Malwa	57	Dec 2008	50^0	Chandigarh Distilleries & Bottlers Ltd. BanurDistt. Punjab	Local Market, Patiala
8.	Lal Gulab	216	Dec 2008	50^0	Pioneer Industries Ltd. Pathankot, 145001, DisttGurdaspur, Punjab	Local Market, Patiala
9.	Santra	B122	Dec 2008	50^0	Ashoka Distilleries & Chemicals Pvt. Ltd. HathnDistt. Palwal, Haryana 121103	Local Market Patiala
10.	Jagadhri	81	Jan 2009	50^0	Haryana Distillery, Yamuna Nagar, Haryana	Local Market, Patiala

Table 2: pH of standard caramel solution and different alcoholic beverages

Code	Type	pH
1.	Standard Caramel Solution	4.48
2.	Officer's Choice Whiskey	4.85
3.	Officer's Choice Deluxe Rum	4.65
4.	Officer's Choice Deluxe Whiskey	5.02
5.	Old Monk's Rum	4.10
6.	Tohfa	4.34
7.	Malwa	4.26
8.	Lal Gulab	4.25
9.	Santra	4.28
10.	Jagadhri	8.00

Table 3: Maxima and Minima of Zero and First Order Spectra for Standard Caramel Solution and various alcoholic beverages

S. No	Spectrum for Standard Caramel Solution	Maxima (in nm)	Minima (in nm)
1.	Zero Order	281.0, 225.5	---
	First Order	260.0, 215.5	295.0, 237.5
2.	Zero Order	276.5, 217.5	---
	First Order	263.5, 214.5	295.0, 239.5
3.	Zero Order	279.5, 221.0	---
	First Order	264.5	290.5, 230.5
4.	Zero Order	280.0, 222.0	---
	First Order	342.5, 265.0	381.0, 295.0, 220.5
5.	Zero Order	279.5, 223.0	---
	First Order	264.5, 225.5	295.0, 234.5
6.	Zero Order	284.0, 235.5	---
	First Order	265.5, 225.5	299.0, 243.0
7.	Zero Order	285.0, 235.0	---
	First Order	265.5, 224.0	300.5, 243.0
8.	Zero Order	284.0, 219.0	---
	First Order	265.5, 214.5	299.0, 238.0
9.	Zero Order	284.5, 246.0, 235.0, 217.0	---
	First Order	349.0, 265.5	399.0, 299.0, 243.0
10.	Zero Order	284.0, 235.0	---
	First Order	344.5, 265.5, 223.0	375.5, 299.5, 243.0

Preparation of Standard Sample of Caramel - 500 µg of Caramel powder (manufactured by Sun Food Technology) was dissolved in a mixture of ethanol: water (1:1). Since ethanol and water have cut off wavelength at 210 and 190 nm respectively, therefore, they were used as a solvent in the present study. The pH of standard caramel solution and liquor samples were recorded using pH/Ion 510 manufactured by Eutech Instruments. The respective pH of standard caramel solution and liquor samples are provided in table 2.

Instrumental parameters and analysis – All the samples and standard caramel solution were scanned to obtain zero-order spectra in the region of 210 nm – 700 nm using Shimadzu UV-Vis 1700, Pharmaspec (Japan) spectrometer equipped with UVProbe software (Version 2.0). The samples were diluted to ten times using distilled water. Samples were analyzed in absorbance mode with a recording range of 0.00A – 1.00A and 3 scans were taken for each recording. Once the zero order spectra were recorded they were converted into first-order derivatives of their respective zero order spectra.

Results and Discussion

In the present study, an attempt has been made to identify the caramel in different types of alcoholic beverages (including whiskey, rum, and other local commercial liquors). The standard caramel solution and nine samples of different alcoholic beverages were analyzed using derivative UV-Vis spectrophotometry. Table 3 summarizes the maxima and minima results for zero order and first order analysis of standard caramel solution and nine samples of various alcoholic beverages.

a) Results of Zero Order Uv-Vis spectrophotometry.

Zero-order spectra of standard caramel solution and all samples show two maxima without any minima except for the sample of Santa which shows four maxima. Zero-order spectra of standard caramel solution demonstrate maxima at 281 nm and 225.5 nm. Officer's choice whiskey showed maxima at 276.5 nm and 217.5 nm, whereas an officer's choice deluxe whiskey showed maxima at 280 nm and 222 nm. Officer's choice deluxe rum shows maxima at 279.5 nm and 221 nm, meanwhile, old monk rum shows maxima at 279.5 nm and 223 nm. The spectra of commercial liquors show a greater variation in the wavelength at which maxima are observed. Tofa liquor displayed maxima at 284 nm and 235 nm; Malwa displayed maxima at 285 nm and 235 nm; Lal Gulab displayed maxima at 284 nm and 219 nm; Santra displayed maxima at 284.5 nm, 246 nm, 235 nm, and 217 nm; Jagadhri displayed maxima at 284 nm and 235 nm.

From the comparison of zero order spectra of standard caramel solution and zero order spectra of alcoholic beverages, it is evident that the maxima of 281 nm and 225 nm from standard caramel solution can be used for identification of caramel colors. The maxima of 281 for other alcoholic beverages fall in the range of 281 ± 5 nm, however, the maxima of 225 nm shows more variation with a range of 225 ± 10 nm. Therefore, it is safe to say that in the case of zero-order spectra, maxima 281 nm is a better peak for identification of caramel color.

b) Results of First Order Derivative UV-Vis spectrophotometry

First order spectra of standard caramel solution and nine samples of alcoholic beverages show two maxima and two minima with the exception of Officer's choice deluxe rum (one maxima and two minima), Officer's choice deluxe whiskey and Santr (two maxima and three minima each), and Jagadhri (three maxima and three minima). First order spectra of standard caramel solution showed maxima at 260 nm and 215.5 nm with minima at 295 nm and 237.5 nm. First order spectra of Officer's choice whiskey showed maxima at 263.5 nm and 214.5 nm with minima at 295 nm and 237.5 nm and Officer's choice deluxe whiskey showed maxima at 342.5 nm and 265 nm with minima at 381 nm, 295 nm, and 220.5 nm; Officer's choice deluxe rum showed maxima at 264.5 nm and minima at 290.5 nm and 230.5 nm, whereas, Old Monk rum showed maxima at 264.5 nm and 225.5 nm; Tofa showed maxima at 265.5 nm, and 225.5 nm with minima at 299 nm and 243 nm; Malwa showed maxima at 265.5 nm, and 224 nm whereas the minima was at 300.5 nm, and 243 nm; Lal Gulab showed maxima at 265.5 nm, and 214.5 nm with minima at 299 nm a, and 238 nm; Santra showed maxima at 349 nm, and 265.5 nm with minima at 399 nm, 299 nm, and 243 nm; Jagadhri showed maxima at 344.5 nm, 265.5 nm, and 223 nm with minima at 375.5 nm, 299.5 nm, and 243 nm.

Standard caramel solution shows two maxima (260 nm, and 215.5 nm) and two minima (295 nm, and 237.5 nm). The maxima at 260 nm show a range of 260 ± 1 nm for the samples, whereas, the 215.5 nm maxima show a range of 215 ± 10 nm. However, the maxima at 215.5 nm are absent in Officer's choice deluxe rum, Officer's choice deluxe whiskey, and Santra commercial liquor. In the case of minima of 295 nm, the observed range lies within 295 ± 5 nm, whereas, the range for maxima of 237.5 nm lies within 238 ± 5 nm.

Conclusion

The present study was conducted as an attempt to use derivative UV-Vis spectrophotometry to identify caramel colors in various factory-made and commercial alcoholic beverages. It can be concluded that derivative UV-Vis spectrophotometry can be used successfully to detect the caramel colors. Moreover, it is a non-destructive technique that requires minimal sample preparation. It is an easily available technique having a low per sample analysis cost.

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None to declare

Conflict of Interest:

None to declare

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Study of Characteristic Burn Patterns Formed by Three Different Accelerants on Plastered Wall

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Abstract

One of the important aspect of arson investigation is to study the burn patterns or characteristics resulting from various accelerants. In India, the basic accelerants which are used to set fire to a scene include petrol, diesel and kerosene. This research article is based on the different types of burn patterns on plastered wall resulting from the use of three different accelerants which are being commonly used to set fire to a scene. Fire was initiated by spilling the accelerant on the plastered wall to simulate the scene of arson. An equal amount of each accelerant was taken for experimental purposes. The burn patterns provided by the three accelerants namely petrol, diesel and kerosene on the plastered wall shows some varying characteristics which help in differentiating the patterns caused by these accelerants. It is expected that results provided by this research will help the arson investigators in the investigation process.

Keywords: Arson; Fire; Accelerant; Burn pattern.

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Introduction

The word **"arson"** is often employed to specify that a crime has taken place whereas the word **"incendiary"** is used to denote that a fire was deliberately or intentionally set. Though it is probable for incendiary fires to be the consequence of imprudent but not criminal acts, there is generally a large connexion between incendiary and arson fires. In general, fires due to arson are not hard to identify. Arsonists normally set fire to a scene by the use of an ignitable liquid, which is identifiable once the fire has completely burnt out. Lentini (2006) stated that in few cases where the author was asked to investigate and check the claims that a specific fire was deliberately set, most of the determinations were carried out by evaluating the burn patterns or the existence of definite artefacts, how ever were not based on the presence of residues from ignitable liquid in the fire debris as all the samples tested negative [1]. The examination of fire scene should be investigated by an arson investigator for signs of arson immediately after the fire has subsided. Most of the arson cases are initiated with the usage of petroleum or petroleum based products for example, kerosene or gasoline. Hence, existence of containers with the possibility of holding accelerants or ignitable liquids rouse

suspicions of arson [2]. Today, few researches have been carried out to study the specific burn patterns created by the use of gasoline or petrol on various flooring materials [3, 4-8]. Liang and Liu (2010) carried out a study based on the impact of amount and spilling style of gasoline or petrol on the burnt traces found on different types of flooring materials [4, 7-8]. Moreover, when a fire starts, number of factors affect the burn characteristics after the sustained fire has subsided either naturally or by manual means. In order to help investigator apart from collection of evidences from the scene of crime and also in identification of the presence of possible accelerant at the scene of crime, it is required to study the pattern formed very precisely so as to pin point the type of accelerant used.

In this paper, three accelerants have been considered for the purpose of study – petrol, diesel and kerosene because of their usage in wide number of arson cases in India and to study the burn pattern characteristics, plastered wall has been considered. Patterns were characterised and studied by simulating the scene of fire. Fire was let to subside naturally and no mechanical means was used to douse the fire.

Materials and Methods

Accelerant samples

50 mL of each of the three accelerant samples viz. petrol, diesel and kerosene have been considered for the study of pattern characteristics.

Volume of the accelerant samples taken, were kept constant for each set of study.

To study the differences and carry out comparative studies, a set of three observations was taken with each accelerant sample.

Surface/ area

A well ventilated space was chosen for the simulation of scene of arson.

The surface considered for study of characteristics of burn patterns was carried out on a plastered wall.

Way of ignition

The method of ignition with the use of accelerant was chosen to be the method of spillage. This way of ignition was selected purporting to the established fact that an arsonist will use the common technique of pouring or spillage to set the scene on fire and destroy the evidences or for that matter the scene.

Photography

A DSLR camera was used to clearly record the burn patterns created after the complete ignition had subsided naturally.

Results and Discussions

Patterns were studied on plastered wall by three accelerants viz. petrol, diesel and kerosene. The burn patterns were studied after the fire subsided naturally and without the use of any mechanical means to douse the fire. The inferences thereby obtained for each type of accelerant has been summarised systematically.

a) Patterns formed by petrol

The burn patterns obtained by petrol on plastered wall has been shown in Figure 1, Figure 2, and Figure 3. Set of three observations were obtained on three different areas of the same wall using the same volume of accelerant (petrol) i.e. 50 mL by spillage method. The observations thereby obtained has been summarised in Table 3a.

a) Patterns formed by petrol

Fig. 1: First pattern formed on the vertical wall after petrol was spilled and then burned.

Fig. 2: Second pattern formed on the vertical wall after petrol was spilled and then burned.

Fig. 3: Third pattern formed on the vertical wall after petrol was spilled and then burned.

b) Patterns formed by diesel

The burn patterns obtained by diesel on plastered wall has been shown in Figure 4, Figure 5, and Figure 6. Set of three observations were obtained on three different areas of the same wall using the same volume of accelerant (diesel) i.e. 50 mL by spillage method. The observations thereby obtained has been summarised in Table 3b.

c) Patterns formed by kerosene

The burn patterns obtained by kerosene on plastered wall has been shown in Figure 7, Figure 8, and Figure 9. Set of three observations were obtained on three different areas of the same wall using the same volume of accelerant (kerosene) i.e. 50 mL by spillage method. The observations thereby obtained has been summarised in Table 3c.

Table 3a: Observations obtained from burn pattern formed by petrol on plastered wall

Accelerant used	Method of extinguishment	Observations (for three set of readings)
Petrol	Naturally	• Soot formation was observed being dense and concentrated near the origin of fire and as the fire moved in upward direction concentration of soot formation and accumulation goes on decreasing. • No complete or partial charring of wall or plaster was observed in all the subsequent experiments carried out. • Plaster was intact in all the three subsequent experiments carried out in three phases. • No additional features or characteristics found.

b) Patterns formed by diesel

Fig. 4: First pattern formed on the vertical wall after diesel was spilled and then burned.

Fig. 5: Second pattern formed on the vertical wall after diesel was spilled and then burned.

Fig. 6: Third pattern formed on the vertical wall after diesel was spilled and then burned.

Table 3b: Observations obtained from burn pattern formed by diesel on plastered wall

Accelerant used	Method of extinguishment	Observations (for three set of readings)
Diesel	Naturally	• Dense soot formation was found at places where the fire moved in upward direction and grey colored soot formation resulted near the origin of fire indicating that soot concentrated mostly all over the fire travel. • Marked distinction between the two regions i.e. grey to black colored soot formation can be seen in Figure 2 thereby leading to discoloration of plastered wall ranging from dark black to ash grey. • Figure 2 and 3 shows plaster being swollen at some parts. This characteristic was not observed while studying burn characteristics by petrol. • No additional features or characteristics found.

c) Patterns formed by kerosene

Fig. 7: First pattern formed on the vertical wall after kerosene was spilled and then burned.

Fig. 8: Second pattern formed on the vertical wall after kerosene was spilled and then burned.

Fig. 9: Third pattern formed on the vertical wall after kerosene was spilled and then burned.

Table 3c: Observations obtained from burn pattern formed by kerosene on plastered wall

Accelerant used	Method of extinguishment	Observations (for three set of readings)
Kerosene	Naturally	• Formation of dense soot near the origin of fire and all over the fire travel in upward direction. • No complete or partial charring of plastering noticed. • Discoloration of the plastering is found ranging from dark black to ash grey. • Figure 8 and 9 shows characteristic swelling at some parts of the plastered wall. • The swollen plastered parts made cracking noise during the process of burning. • More profound effects (swelling of plaster) are seen near the origin of fire. • No additional features or characteristics found.

Conclusion

The burn patterns presented by different accelerants on plastered wall shows few characteristics which easily differentiates them apart. Few of the characteristics mention in the observation tables of patterns produced by petrol, diesel and kerosene are quite similar for example the formation of soot and absence of any characteristic charring of the plastered wall. No spallation of plastering was seen in this experimental case. Moreover, the burn patterns are dependent upon the volume of accelerant or ignitable liquid used, direction of wind, method of ignition, etc. The differentiating characteristics found in case of kerosene is that the characteristic swelling of plaster at some points and becoming more profound near the origin of fire. Also, these swelled up plaster broke down with characteristic noise during the process of burning. The cracking noise was absent in case of burn patterns formed by diesel and swell up of plaster was not so profound. Both these characteristics have been found to be absent in case of burn pattern caused by petrol.

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Forensic Discrimination of Fake and Genuine Mobil Oils

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Abstract

The major functions of lubricating oils is the reduction of friction and wear by the separation of surfaces, metallic or plastic, which are moving with respect to each other. The few Indian infringers are engaged in cheating, substandard and duplication cases forensic scientist and police facing problems with rapid increase in different types of Mobil oils having hydrocarbons and other additives. Which can be used for comparison purpose keeping in this view a protocol for rapid screening and analysis of these type of material by using physical parameters, thin layer chromatography and FT-IR spectral analysis have developed to differentiate fake (used) Mobil oil and genuine unused branded Mobil oils.

The present work describes the discrimination of fake and genuine Mobil oils on the basis of physical parameters, thin layer chromatography and FT-IR analysis.

Keywords: Mobil oils; TLC; FT-IR analysis.

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Introduction

The major functions of lubricating oils is the reduction of friction and wear by the separation of surfaces, metallic or plastic, which are moving with respect to each other. Petroleum base Mobil oils mainly consist of Paraffinic, Naphthenes and aromatics. The lubrication property of the particular lubricant depends on the distribution of these hydrocarbons. It consists of C_{28} to C_{40} hydrocarbons. Identification of the lubricating agent is difficult undertaking because of the broad range of products and the possibility of contamination and degradation, which may occur during use in the vehicle [1-3].

There has been regular emergence of new brands of Mobil oils due to lifting the restriction on the marketing by private companies. The prices obviously vary depending on the purpose of use; grading etc. the adulteration problem has also come to the surface like other commonly used petroleum products viz. petrol and diesel oils. Not all engine oils sold in the market are genuine. With today's technology, synthetic engine oil syndicates are able to imitate the well-known brands. Examples of well-known brands in the market are Castrol, Shell, Motul and Pennzoil. Subsequently, there are three categories of lubricants in the market namely mineral, semi synthetic and fully synthetic. For synthetic oils, it is formed through some complex additional chemical processes to ensure the formation and production of a good liquid lubricant. Engine oil function is allows smooth steel movement in the engine. The usage of fake engine oils will result in the engine oils unable to absorb the heat generated. This cause the engine quickly

heating up and the shift will become rugged and wear and eventually cause overheating of the engine. Therefore, the type and grade of engine oils should be suitable to maintain engine resistance. Subsequently, car user also needs to follow the scheduled change of engine oil to prevent component wear as the engine oil viscosity level would already reduce significantly.

One of the ways to recognize or detect the fake engine oil is by comparing the smell with the original engine oil. Alternatively, you are recommended to buy directly from a legitimate distributor. This is because the risk of buying fake engine oil is higher when buying from any third party. Besides that avoid from buying any engine oil at a lower prices than the market prices. Using the right or appropriate oils to our vehicles will not only protect and extend the engine life, but also increase the engine performances while helping to save on the car maintenance and the fuel costs. Large circulation of fake (used) Mobil oil in some major cities of M.P. assumed alarming situation for both police and public. The few state infringers were engaged in cheating, substandard and duplication cases forensic scientist and police facing problems with rapid increase in different types of Mobil oils having hydrocarbons and other additives. Which can be used for comparison purpose keeping in this view a protocol for rapid screening and analysis of these type of material by using physical parameters, thin layer chromatography and FT-IR spectral analysis have developed to differentiate fake (used) Mobil oil and genuine unused branded Mobil oil.

Materials and Methods

The fake Mobil oil samples physical parameters compared with genuine Mobil oil sample furnished by authenticated source (castrol).

Table: Physical parameters

S. No.	Physical parameter	Fake Mobil oil	Genuine Mobil oil
1	Fluorescence	Blue	Violet
2	Viscosity in secs /70°C	45 sec	98 sec
3	Specific gravity	0.8843	0.890
4	Flash point	210	240
5	Refractive index	1.488	1.476

Thin Layer Chromatography

A standard TLC plated was coated with slurry of silica gel G in water to a uniform thickness of 0.25 mm the plate was activated by heating in an oven at 100°C for about 1 hour an aliquots of genuine Mobil oil and fake Mobil oil were spotted on to the plate, which was developed with chloroform: benzene (50:50) in a presaturated TLC chamber to a height of 10 cm. The plate was removed from the chamber dried in air in which sprayed with 50% sulphuric acid. The brownish coloured spots appeared after one hour heating in oven at 160° in white background. The Rf values are as follows:

1. Fake Mobil oil 0.15, 0.45, 0.65 and 0.85.
2. Genuine Mobil oil 0.35, 0.49, 0.75 and 0.90.

FT IR Spectroscopy

FTIR Spectra studies were performed on the Perkin Elmer one spectrophotometer using universal ATR accessories, Spectrum recorded between 600-4000 cm-1 and the obtained spectrum compared which showed clear deviation in both the samples proved to be of different origin.

Peaks for fake (used) oil- 727.29, 1051.7, 1161.7, 1380.87, 1464.5, 2854.04

Peaks for genuine oil- 511.60, 720.45, 1046.57, 1161.51, 1380.87, 1459.12, 2848.66, and 2932.29.

Results and Discussions

Fake (used) mobil oils has emerged as one of the serious form of economic and cheating offences during past few years in India and caused a serious set back to economy of the nation and general public.

Mobil oils are costly commodity used in spark plug engines. The high cost of this oil tempts illegal syndicates to adulterate this with lower cost or used burnt processed oils sell under brand names. The easy availability of burnt oil and easy processing and higher profit attract these infringers to sell these products to fetch higher profit, which causes serious problems in spark plug engines. The effect of deterioration of engine can be seen in short period. Also this type of cheating causes serious environmental pollution.

The lower Specific Gravity value of fake oil indicates Presence of high boiling solvents or admixture of lower gravity oils. The Viscosity is the property of its resistance to flow. Viscosity is the most important single property of lubricating oil.

Conclusion

In this study the value of the viscosity of fake oil is lower than the genuine oil it clearly indicates

fuel dilution or admixture with low viscosity oils. Similarly different values of Rf in TLC and different peaks in both the oils clearly prove oils of different origin. The present method protocol is able to differentiate fake and genuine oils for criminalistic inference.

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Estimation of Zinc Levels in Blood, Liver and Stomach Contents using ICP-AES: A Cross Sectional Autopsy Based Study

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Abstract

Zinc is an essential component of the body, but its excess quantity is harmful and long term intake above upper limits causes acute or chronic toxicity. Normal levels of zinc in serum is 75-120 µg/dL while in blood it is 1200 µg/dL. A study was conducted to determine the level of zinc in various biological samples from the medicolegal autopsies conducted at mortuary, Department of Forensic Medicine & Toxicology, All India Institute of Medical Sciences, New Delhi. Biological samples were taken from 100 cases comprising of Blood, Liver & Stomach contents from each case. They were analysed using Inductively Coupled Plasma-Atomic Emission Spectrophotometry (ICP-AES). The data obtained were analysed using various demographic profiles and treatment history to know prevalence & distribution of these metals in general population so as to help later in investigation of alleged deaths due to metal toxicity & metallic compounds poisoning. The result showed mean blood zinc levels were 14.21 µg/ml (range 0-77.36 µg/ml), mean zinc levels in liver and stomach contents were 25.66 µg/g (range 0.72–127.03 µg/g) and 7.95 µg/ml (range 0–60.94 µg/ml) respectively. The data analysis on the basis of treatment history showed that mean zinc levels in blood, liver and stomach contents were higher in cases where treatment was not given i.e. 14.97 µg/ml, 15.25 µg/g and 8.18 µg/ml respectively.

Keywords: Zinc; Autopsy; Blood; Liver; Stomach Contents; ICP-AES.

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Introduction

Zinc is an essential component of the body, but its excess quantity is harmful it can be acute and chronic also. Acute adverse effects include nausea, vomiting, loss of appetite, abdominal cramps, diarrhoea, and headaches [1]. Intake of 150-450 mg of Zn per day is associated with chronic effects as low copper status, altered iron function, reduced immune function, and reduced levels of high density lipoproteins [2]. Chronically high intakes adversely affect some aspects of urinary physiology [3]. Hadla S. Ferreira et al. [4] in 2007 proposed a direct method based on slurry sampling of the determination of zinc and copper in human hair samples by multi-element sequential flame atomic absorption spectrometry. The slurries were prepared by cryogenic grinding and sonication of the samples. The optimization step was performed using univariate methodology and the factors studied were: nature and concentration of the acid

solution, amount sample/slurry volume, sonication time and particle size. The established experimental conditions were the use of a sample mass of 50 mg, 2 mol /l nitric acid solution, sonication time of 20 min. and slurry volume of 10 ml. adopting optimized conditions. This method allowed the determination of zinc and copper with detection limits of 88.3 and 53.3 ng/g, respectively, and precision expressed as relative standard deviation (RSD) of 1.7% and 1.6% (both, n=10) for contents of zinc and copper of 100.0 and 33.3 µg/g, respectively. The accuracy was checked and confirmed by analysis of two certified reference materials of human hair. The procedure was applied for the determination of zinc and copper in two human hair samples. The zinc and copper contents varied from 100-175.6 and from 3.2-32.8 µg/g, respectively. These samples were also analyzed after complete digestion in a closed system and determination by FAAS. The statistical comparison by t-test (95% confidence level) showed no significant difference between results. B Zerahn et al. [5] in 1999 conducted a study on thirteen soldiers (11 men and two women) who were exposed to zinc chloride smoke (ZCS) during a combat exercise. Even though their initial symptoms were modest, a prolonged follow up with lung function testing and blood samples was undertaken due to previous cases with fatal outcome after exposure to ZCS. Four weeks after exposure there were statistically significant declines from baseline values in lung diffusion capacity and total lung capacity of 16.2% and 4.3% respectively. At the same time plasma levels of fibrinogen and zinc were significantly elevated, though mainly within the normal range. All variables showed a tendency towards normalization at follow up 8 weeks and 6 months after exposure. These findings indicate an unexpected quantifiable damage to lung parenchyma with a remarkable delay after modest exposure to zinc chloride smoke despite sparse initial symptoms. JB Dawson et al. [6] in 1969 described a method for the determination of zinc in plasma diluted twenty fold with 0.1N HCl, in whole blood diluted one hundred times and urine diluted tenfold, using a Perkin-Elmer 303 atomic absorption spectrophotometer. Suppression of upto 15% of the apparent zinc content by inorganic components of the sample was overcome by the addition of the appropriate amounts of those ions to the standard zinc solutions used in the determination. The organic components of the samples had no significant effect on the apparent zinc content. Random contamination presented a problem which was best detected by replicate analysis. Studies of the plasma zinc level of 20 normal subjects (10 men, 10 women) showed a significant difference ($p<0.001$) between samples taken fasting at 9h and 5h later (14.00h) after the usual meals. The mean values were: 9h, men: 98 µg/100 ml, women: 96 µg/100 ml; 14h, men: 80 µg/100 ml, women: 83 µg/100 ml. the difference in whole blood values taken fasting at 9h and 5 hours later was not significant ($p> 0.6$) and the means of two samples were: men 584 µg/100 ml (range 414-794 µg/100 ml), women 582 µg/100 ml (range 342-700 µg/100 ml). The 24h urine excretions were men 585 µg (range 263 -817 µg) and women 414 µg (range 276-702 µg) this difference was significant ($p<0.05$).

Objective: To determine the level of zinc in blood, liver and stomach contents of post mortem bodies.

Material & methods

Chemicals and reagents: 69% Nitric Acid GR, 30% Hydrogen Peroxide, Ultrapure Water,

Biological Samples: Blood, Liver and Stomach Contents

Equipment used: Microwave Digester (MDS-10) from sineo company, ICP-AES (Inductively coupled plasma – Atomic Emission spectrophotometry) (JY2000) from Horiba Jobin YVON company.

Standard Preparation: Mix standard solution of zinc of 100 ppb, 500 ppb and 1000 ppb was prepared by diluting the 1000 ppm standard by using N1V1 = N2V2 Formula.

Sample collection: All samples (Blood, liver & stomach contents from each case) were collected from Mortuary, All India Institute of Medical Sciences, New Delhi.

Exclusion & Inclusion Criteria:

Inclusion Criteria: All medico legal autopsies without the history of metallic/metallic compounds poisoning.

Exclusion Criteria: All deaths with a history of metal/metallic compounds poisonings.

Procedure

Samples taken:

1. *Blood*: 10 ml of blood preserved in Sodium fluoride of 10 mg/ml and potassium oxalate of 30 mg/10 ml

2. *Liver:* 10 gms of liver preserved in saturated solution of sodium chloride (Common salt-36 gms/100 ml)

3. *Stomach Contents*: 25 gms stomach contents preserved in saturated solution of sodium chloride.

Digestion of sample

Various methods using different quantities of biological sample with varied reagent concentrations were tried but the valid sample composition is as follows:

0.5gms/ml of biological sample + 4.5 ml of 69% HNO_3 + 0.5 ml of H_2O_2

Sample loading

Prepared samples (total of 6 ml for each sample) were added to the vessels then the vessels were closed using a spencer. Vessel assembly was prepared and the vessels were put on the respective positions on the turntable inside the microwave digestion system by balancing the vessels.

Door was closed and digestion programme was started.

Microwave Digestion Programme

Various programmes of temperature ramping along with the sample composition (already mentioned) were tried among which the validated temperature programme is as follows:

Table 1: Programme showing ramping of temperature with respect to time

Temperature	Time in Minutes	Power(watt)
120	10	800
150	10	800
180	10	800
200	10	800

After completion of temperature programme the machine was left for cooling for 35 minutes. After cooling the door was opened and the vessels were taken out one by one with proper precautions. Vessels were then opened in the fume hood using the spencer and allowed to degas and then transferred to the tarson tubes of capacity 15 ml.

Fig. 1: Microwave Digestion System from Sineocompany

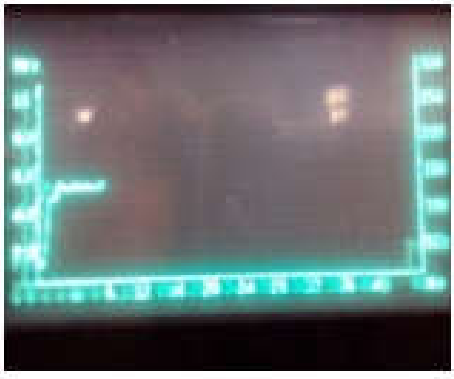

Fig. 2: Ramping of temperature programme shown in the form of graph on MDS 10

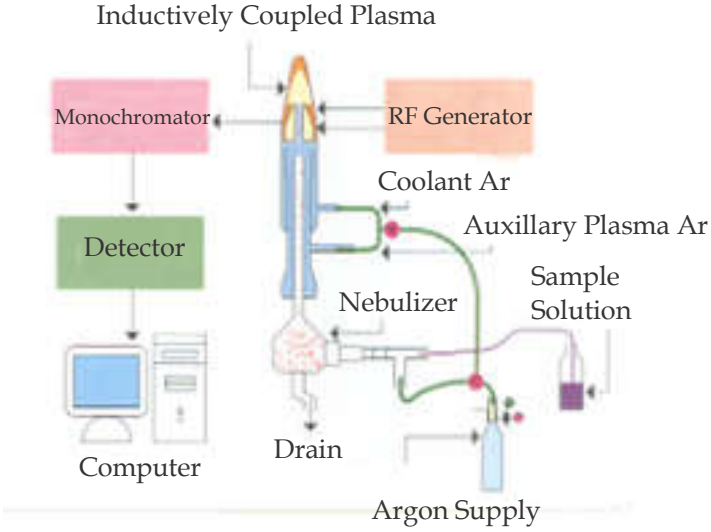

Fig. 3: Flow chart showing working of ICP-AES

Fig. 4: ICP-AES instrument

Analysis on Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP- AES) *[7]:*

ICP-AES is an emission spectrophotometric technique, exploiting the fact that excited electrons emit energy at a given wavelength as they return to ground state after excitation by high temperature Argon Plasma. The fundamental characteristic of this process is that each element emits energy at specific wavelengths peculiar to its atomic character. The energy transfer for electrons when they fall back to ground state is unique to each element as it depends upon the electronic configuration of the orbital. The energy transfer is inversely proportional to the wavelength of electromagnetic radiation, $E = hc/\lambda$ ... (where h is Planck's constant, c the velocity of light and λ is wavelength), and hence the wavelength of light emitted is also unique. Although each element emits energy at multiple wavelengths, in the ICP-AES technique it is most common to select a single wavelength (or a very few) for a given element. The intensity of the energy emitted at the chosen wavelength is proportional to the amount (concentration) of that element in the sample being analysed. Thus, by determining which wavelengths will be emitted by a sample and by determining their intensities, the analyst can qualitatively and quantitatively find the elements from the given sample relative to a reference standard. The method for analysis of zinc was created as its peak was found on the wavelengths of 213.856 nm. The machine was calibrated using standard solutions of mix standard of zinc with different concentrations of 100 ppb, 500 ppb and 1000 ppb which were prepared by dilutions of 1000 ppm standard solution obtained from Merck. After calibration the samples digested were run for analysis and each sample was run thrice with three replicates of each. The parameters used for analysis of zinc is as follows:

Table 2: ICP-AES parameters for zinc detection

Parameters	Conditions
ICP-AES software version	5.2
Wavelength for zinc	213.856 nm
Plasma flow	12 L/min
Sheath flow	0-2 L/min
Pump speed	20 rates/min
Nebulizer flow rate	0.34 L/min
Nebulizer pressure	2.76 bar
Detector	Photomultiplier tubes

Results and Discussions

The data obtained were analysed using various demographic profiles and treatment history

Table 3: Average distribution of Zinc

Biological Sample	Blood (µg/ml)	Liver(µg/g)	Stomach Contents (µg/ml)
Mean Values	14.21	25.66	7.95

Table 4: Age wise distribution of Zinc

Range (Years)	Blood (Mean)	Liver (Mean)	Stomach Contents (Mean)
0-10	38.27	107.36	32.38
11-20	20.47	31.85	8.45
21-30	14.44	23.23	5.25
31-40	15.59	22.84	10.49
41-50	14.98	11.07	6.84
51-60	6.65	17.97	6.69
61 & above	10.72	17.89	14.89

Table 5: Sex wise distribution of Zinc

Gender	Blood (Mean)	Liver (Mean)	Stomach Contents (Mean)
Male	13.96	20.58	8.46
Female	15.7	17.16	5.67

Table 6: Distribution of mean values of Zincaccording to treatment history

Treatment history	Blood	Liver	Stomach Contents
Treatment history present	10.35	14.93	5.87
Treatment history not present	14.97	15.25	8.18

This study was conducted to estimate the blood zinc levels in post-mortem cases of South Delhi area that was brought to the Department of Forensic Medicine and Toxicology, AIIMS, New Delhi. Hundred cases were studied as per the inclusion criteria during the period of march 2014 tomarch 2016. We found that the mean blood zinc levels in South Delhi population were 14.21 µg/ml and range was 0 to 77.36 µg/ml. The mean zinc levels in liver and stomach contents were 25.66 µg/g with a range of 0.72 – 127.03 µg/g and 7.95 µg/ml with a range of 0 – 60.94 µg/ml respectively. Also on the basis of age-wise distribution the mean zinc levels were higher in the age group of 00-10 years i.e. 38.27 µg/ml and in liver and stomach contents also the mean zinc levels were higher in the same age group i.e. 107.36 µg/g & 32.38 µg/ml respectively.

On the basis of sex-wise distribution the mean zinc levels in blood was higher in females i.e. 15.7 µg/ml while in both liver and stomach contents it was higher in males i.e. 20.58 µg/g and 8.46 µg/ml respectively.

We also classified the data on the basis of treatment history which showed that mean zinc levels in blood, liver and stomach contents were higher in cases where treatment was not given i.e. 14.97 µg/ml, 15.25 µg/g and 8.18 µg/ml respectively.Normal levels of zinc in serum is 75-120 µg/dL and in blood is 1200 µg/dL [8]. The Food and Nutrition Board has established upper limits for Zn (table 7). Long term intakes above upper limits increase the risk of adverse health effects [1].

Table 7: Tolerable Upper intake level of zinc

Age	Male	Female	Pregnant	Lactating
0-6 months	4 mg	4 mg		
7-12 months	5 mg	5 mg		
1-3 years	7 mg	7 mg		
4-8 years	12 mg	12 mg		
9-13 years	23 mg	23 mg		
14-18 years	34 mg	34 mg	34 mg	34 mg
19 + years	40 mg	40 mg	40 mg	40 mg

Conclusion

This study was carried out to know the prevalence & distribution of these metals in general population so as to help in investigation of alleged deaths due to zinc toxicity and its related compounds poisoning. The findings also suggested that treatment can reduce the traces of zinc. Although zinc is an essential element but its excess levels also causes harmful effects that may be acute or chronic in nature.

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Review Article

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Medical and Legal Interpretation of Injury report: A Physician's Dilemma

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Abstract

In India a registered medical graduate having MBBS degree is legally authorized to treat a medicolegal case and prepare the injury report. He is also required to opine about the nature of injury, possible weapon of offence and manner of injury to assist the crime investigation. Forensic Medicine is taught in the MBBS curriculum, but the studentsgenerally don't get enough practical exposure to deal with medicolegal cases of injury reporting and opining. Most of them learn while on service when situation compel them to handle medicolegal cases and are summoned by the court of law to depose the medical evidence. It has to be realized that medical evidence is animportant corroborative evidence for guiding the investigation and the court of law. It needs to be dealtin a prudent way, scientifically and professionally. This article is an attempt to address the dilemma faced by the physicians, dealing with medicolegal cases of injury reporting and giving expert opiningand evidence in the court of law for the deliverance of justice in the state legal system.

Keywords: Medico-legal; Hurt; Grievous hurt; 320 IPC; permanent privation etc.

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Introduction

Legal definition of hurt and interpretation

The word hurt generally means to cause bodily injury or pain to another person [6]. The legal definition of hurt is mentioned in Section 319 IPC. Hurt is defined asbodily pain, disease or infirmity caused to any person [1].

Based on this definition, the essential ingredients of hurt are: 1) Bodily pain, disease or infirmity must be caused. 2) It should be caused due to a voluntary act of the accused.

All bodily hurts, not only those which are serious but also those which are slight, are within the provisions inserted under this division. It covers all harm, except those which no person of ordinary sense or temper would complain of. The phrase causing bodily pain must be understood to mean the imparting of pain directly to the body, by the sense of touch, as by the use of force. In essence, the law requires the force or the threat of force must be physical harm in order to convert to an offence. So hurt requires physical harm. The hurt is generally not taken for mental agony, pain or mental injury.

But again there is nothing in the definition of hurt to suggest that hurt should be caused by

direct physical contact between the accused and his victim. A physical contact may not be necessary as in case of poisoning. The administration of drug is likely to cause hurt by causing bodily pain and infirmity. Where the direct result of an act is the causing of bodily pain, it is hurt whatever be the means employed to cause it.

A person communicating a particular disease to another would be guilty of hurt. The difference between the act and onset of disease should be small enough, so act and disease could be connected together.

Infirmity means the inability of an organ to perform its normal function which may either be temporary or permanent.Some judgment has mentioned that where serious mental derangement is caused by some voluntary act, a hurt is caused, even though there is no direct physical contact. So mental derangement and nervous shock may also be included in hurt under infirmity. It is also called mental or psychic injury as per medical literature. But mental injury orpsychic injury is difficult to prove in the court of law.

The law does not mention the word simple hurt anywhere, but simple hurt is understood to be hurt, which are not Grievous. Generally a simple hurt is one which is neither extensive nor serious and which heals rapidly without leaving any permanent deformity or disfiguration.

The authors of the IPC code observed that it would be difficult to precisely define or draw a line between those bodily hurts which are serious or grievous and those which are slight or simple, but it was important to draw a line even if it is not perfect so as to punish the cases which are clearly more than simple hurt.And only hurts described in section 320 IPC was considered serious enough to be called grievous hurt.

Under the doctrine of stare decisis, a lower court must honor findings of law made by a higher court. It is good for the doctors to be aware of the latest judgements made by a higher courtrelated to 320 IPC for knowledge and guidiance.

In day today medical practice it is observed that it has become very common to use the word injury synonymous with hurt. It is also observed that the law does not clearly differentiate between hurt and injury. The legal definition of injury is mentioned in section 44 IPC. The word injury is defined as any harm whatever illegally caused to any person, in body, mind, reputation or property. It has two components, firstly injury is an act contrary to law (illegal), secondly, injury includes body, mind, reputation, and property. Therefore it has a wider meaning than the term hurt as it also includes illegal damage to reputation or property of other. In other words, all hurts are injuries, but all injuries are not hurt. And also it is observed that the word assault is invariably used by doctors in the brief history part of the MLC injury report. Section 351 of IPC defines assault as – whoever makes any gesture, or any preparation intending or knowing it to be likely that such gesture or preparation will cause any person present to apprehend that he who makes that gesture or preparation is about to use criminal force to that person, is said to commit an assault. That means assault is an offer or threat or attempt to apply force on body of another in a hostile manner. It may be a simple assault or an intention to murder.

The medical fraternity has also been using the term wound conveniently to indicate the physical injury or hurt. Wound by medical definition is any break in the continuity of body structures caused by violence, trauma or surgery to tissues [5].

320 IPC and interpretations

Grievous hurt has been enumerated in Section 320 IPC. Eight kinds of hurt have been designated as grievous as per this section. There is no definition of grievous hurt mentioned in the section but the hurt which has been designated as grievous in the section would be considered grievous and all other would be simple. Hurts as per provision of the penal code are hurt and grievous hurt, but in practice hurts are simple, grievous or dangerous hurt.The following kinds of hurt are designated as grievous under Section320 IPC:

1. Emasculation.
2. Permanent privation of sight of either eye.
3. Permanent privation of hearing of either ear.
4. Privation of any member or joint.
5. Destruction or permanent impairment of the powers of any member or joint.
6. Permanent disfiguration of the head or face.
7. Fracture or dislocation of a bone or tooth.
8. Any hurt which endangers life or which causes the sufferer to be during the space of twenty days in severe bodily pain or unable to follow his ordinary pursuit.

Thus, to make out the offence of causing grievous hurt as per law, there must be some specific hurt, voluntarily inflicted, and should come within any of the eight kinds enumerated in this Section.

1. Emasculation

This clause is applicable only to males, they being the victim of the said offence. Emasculation means depriving a male of his masculine vigor or power, i.e., to render man impotent. The masculine power of male pertains to the ability of male to perform sexual act or intercourse which involves erection, penetration and ejaculation. Therefore, emasculation is to cause the male unable to perform normal sexual intercourse. The emasculation caused must be permanent. Some examples of emasculation are cutting of penis, trauma to lumbar vertebrae of 2^{nd} – 4^{th} region, causing damage to nerves responsible for erection. In patients with spinal cord injury, the degree of erectile dysfunction depends on the completeness and level of the lesion. Patients with incomplete lesions or injuries to the upper part of the spinal cord are more likely to retain erectile capabilities than those with complete lesions or injuries to the lower part. Although 75% of patients with spinal cord injuries have some erectile capability, only 25% have erections sufficient for penetration [2]. This clause does not speak about the sterility. A person may be sterile but retain masculine power or he may lack masculine power but not be sterile.

Castration is the excision of the gonads (testicles or ovaries) or destruction or inactivation of the gonads by chemical or medications. Castration will not be a grievous hurt under clause 1 as the masculine power remains after castration also. It will be grievous hurt under clause 4, as testis or ovary is a member of the body part. Even cutting of penis in male or clitoris in female will be a grievous hurt under clause 4 legal definition of grievous hurt. Testosterone is an anabolic steroid hormone secreted by the Leydig cells of the testes. It acts to increase libido, but their exact role in erectile function remains unclear. Individuals with castrate levels of testosterone can achieve erections from visual or sexual stimuli. Nonetheless, normal levels of testosterone appear to be important for erectile function, particularly in older males. Testosterone is required in adult males for maintenance and normal function of the primary sex organs [3]. Masculine, virile and potent are the common synonymous words used with similar meaning in the textbooks.

2. Permanent privation of the sight of either eye

Permanent privation of the sight of either eye means permanent loss or deprivationof sight or use of one or both eyes. The loss may be partial or complete. The test of gravity is the permanency of the injury. It is worth mentioning that permanent does not mean that, it should be incurable. The injury may be correctible by operation or surgical intervention, but law does not take into account operative interference. For example an injury on the eye may cause corneal opacity and affect the field of vision. But the corneal opacity may be curable by surgical procedurecalled corneoplasty. But since the corneal opacity caused by scarring in corneadue to injury to eye is permanent by itself it will be a grievous injury. The chance of its cure by corneoplasty does not minimize its gravity. The patient needs to be examined by ophthalmologist and the findings taken into account for opining the nature of injury. Other examples of grievous injury to eye can be injury leading to reduction of vision, retinal detachment, lose of eye sight due to adulterated alcohol (mixed with methyl alcohol), gouging of eye etc. These injuries may also fall under Section 320 (f).

3. Permanent privation of the hearing of either ear.

Permanent privation of the hearing of either ear means permanent loss or deprivation of hearing or use of one or both ears. The loss may be partial or complete. The test of gravity is the permanency of the injury. Again it is worth mentioning that permanent does not mean that, it should be incurable. The injury may be correctible by operation or surgical intervention, but law does not take into account operative interference. The loss of hearing due to ear drum perforation may be corrected by tympanoplasty, but does not minimize its gravity. The patient needs to be examined by ENT specialist and the findings taken into account for opining the nature of injury. The doctor may have to examine the patient for second time after the normal time of healing process and repeat the audiometric test. Some examples of grievous injury to ear are rupture of tympanic membrane due to slap over the ear or poking by stick while attempting to clean ear by local cleaners or quacks, pouring hot liquid into the ear, injury affecting the auditory nerve etc.Even if there is partial permanent loss of hearing it will be a grievous hurt.

4. Privation of any member or joint.

Section 320 IPC does not define what is member or joint in the body. As per medical dictionary the term member means anorgan or limb.And an organ is defined as any part of the body having a distinct function. But Section 2(h) of The Human Organ Transplant Act, 1994 defines human organ as any part of a human body consisting of a

structured arrangement of tissues, which if wholly removed, cannot be replicated by the body. The medical dictionary defines joint as the point of juncture between two bones. It is usually formed of fibrous connective tissue and cartilage. So we can make important derivation from these medical definitions for legal interpretation. Some examples of organs are eyes, ears, nose, mouth, fingers, hand, feet, genitals etc, and include all parts of the body having distinct function. Some parts like nail, hair, blood, body fluids etc cannot be called members/ organs as it can replicate or regenerate as per definition of organ in THOA, 1994. Therefore the term member is generally used to mean an organ or a limb.

The permanent privation of any member or joint implies that the injury has caused the permanent loss of the organ or joint depriving the normal function of the organ or joint of the body. Examples are Amputation of leg or fingers, chopping of nose, gouging of eyes etc.

5. Destruction or permanent impairing of the powers of any member or joint.

This clause differs from clause 4 in the sense that there is destruction or permanent impairing of any member or joint and not privation. The privation is depriving the person of the organ with its function like amputation of limbs, removal one kidney, chopping of the penis, ear, nose etc. The destruction or permanent impairing of powers of any organ is to damage the organ in a way that it permanently impairs its function even though it remains part of the body, like crushed finger or toe, incised injury to hand causing severing of nerves leading to loss of function of fingers or hand etc.

6. Permanent disfiguration of the head or face.

As per The Indian Penal Code book by Ratanlal and Dhirajlal - Disfiguration means a change of configuration and personal appearance of the individual by some external injury which detracts from a person's personal appearance. It may not weaken him or her. Some examples are, cutting of nostrils or ears, gauzing of the eyes, deep scars on the face etc. In simple terms it is spoiling the appearance of the person. As per law disfiguration of head and face part is enough to label as grievous injury. It does not differentiate it or discriminate it on the basis of the gravity or the effect of disfiguration on the person's personal life, livelihood, career or profession, age, sex etc. However there are judgments of different courts considering these factors, probably for compensations. But the medical officer should not consider these factors while opining about the nature of the injury and it is only court's discretion to decide whether to take these factors into consideration. Scar on the face of an actress, or young unmarried girl or an old women or laborer are grievous in nature as per law. Generally the superficial injuries like abrasions heal without scar and are simple but the deep injuries like laceration heal with scar and are grievous. Some common causes of grievous injury in India are throwing acid on the face (vitriolage), causing burn by throwing kerosene, cutting of the nose or ear as punishment etc. The surgeons may cause bad scar on the face. He has to explain it to the patient before the surgery while taking consent to avoid litigations.

7. Fracture or dislocation of a bone or tooth.

There is no legal definition of fracture mentioned in the penal code. As per medical definition, fracture is a break of a bone. Dislocation is the displacement of a bone from its normal position in a joint. The fracture or dislocation of a bone may not cause permanent disability as it can rejoin or set right by treatment. But the injury is labeled as grievous under this section probably due to the intense pain and suffering caused to the person, leading to severe sudden disability, though it may be temporary or cured later. Clinically the fracture type may be hairline fracture, greenstick fracture, incomplete fracture etc. All of them will be grievous. It is not necessary that a bone should be cut through and through or the crack must extend from the outer table to the inner table or there should be displacement of any fragment of the bone. If there is a break by cutting or splintering of the bone or there is a rupture or fissure in it, would amount to a fracture within this clause. On the other hand just a superficial scratch or cut which does not go across the bone surface cannot be called a fracture or grievous. What we have to see is whether the cuts in the bones noticed in the injury report are only superficial or do they effecta break in them. In all these situations the doctor has to opine prudently based on the clinical examination and X-ray report of the bone injury and consult medico-legal expert if needed. We need to interpret medically based on scientific evidence of break of bone. The medical opinion will help the court to decide the case based on the interpretation of law and their wisdom.

In case of fracture or dislocation of a tooth, the examination of a doctor or dentist is very important to determine the grievousness of the injury by any

offence. The doctor need to examine the condition of the oral hygiene, tooth, gum, any disease conditions and associated external injury over the corresponding facial area for corroboration. Before giving opinion the doctor has to rule out that there was no diseased and dislocated tooth already present in the cavity. Generally there will be signs of injury like fresh bleeding from gums with tear, loose tooth and also corresponding external or internal injury over the lips. The X-ray of the oral cavity will be helpful to interpret the findings. The opinions should be based on scientific facts without bias. While preparing report avoid using short forms or abbrevationsand write proper simple sentence. For example, I remember a case where the dentist had written – loss of tooth with corresponding dental chart diagram showing which tooth is missing. Somebody had manipulated it as –' no loss of tooth' by just adding 'no' infront of loss of tooth. If he had written the sentence – there is loss of tooth it could have been not easy to manipulate it.

8. Any hurt which endangers life or which causes the sufferer to be during the space of twenty days in severe bodily pain or unable to follow his ordinary pursuits.

This section has three components: a. Any hurt which endangers life or b. Any hurt which causes the sufferer to be during the space of twenty days in severe bodily pain orc. Any hurt which causes the sufferer to be unable to follow his ordinary pursuits for twenty days.

Any injury which qualifies for any of these three components is a grievous hurt as per this section.

a. Any hurt which endangers life

The clause - hurt which endangers life, is not elaborated in this section and is left to the opinion of the doctor and the interpretation of the court as per their wisdom. It can be explained that any injury which can be fatal or cause death will qualify this component. Here, there is a significant probability of the victim ending in death in its natural course, which means that the injury has put the life of the injured in danger. The endangerment may be for a short period only and the patient might have recovered after the medical intervention, yet it would qualify for this clause to be called grievous hurt. The injuries endangering life are those which cause imminent danger to life, either by involvement of important organs and structures or extensive area of the body [9]. If no surgical aid is available, such injuries may prove fatal. Many a times the doctors document the injury as dangerous injuryin the injury report which is synonymous with injury which endangers life. The word dangerous injury is not mentioned in the clause of 320 IPC, but when the doctor has used this word in the report he has to clarify in the court of law that his opinion meant or is consistent with first part of 320 IPC. It should not be interpreted in a different way because the exact word was not used if the meaning or interpretation is the same as injury endangering life. This matter was clarified in court judgments also.

In Atma Singh Vs State of Punjab, 1980, it was decreed that an injury which can put life in immediate danger of death would be an injury which can be termed as dangerous to life and therefore when a doctor describes an injury as dangerous to life, he means an injury as dangerous to life in term of clause of Sec. 320 IPC. Wherever a doctor describes an injury as dangerous to life and the nature of the injuries is such which could merit such a conclusion, then such an injury has to be treated as grievous hurt as per clause 8 of Sec. 320 IPC.

In Madan Lal Vs State of Himachal Pradesh, the court held thatdanger to life from an injury should be imminent to constitute it as a dangerous one.

Common example of injuries which endangers life:

1. Penetrating injury to the body cavities like peritoneal cavity, chest, skull etc.

2. Head injuries showing signs of intracranial compression.

3. Extensive Burn.

4. Bleeding due to injury to large vessels, liver, spleen, multiple bruise etc.

5. Fracture of skull.

Injuries inflicted on the vital parts of the body are generally dangerous, endangering the life of the person. The question whether a given injury is dangerous to life is relevant, but what is more relevant is how far it had placed the victim in danger of his life. A simple hurt cannot be designated as grievous simply because it was on a vital part of the body, unless the dimensions or the nature of the injury or its effects are such that (in the opinion of the doctor) it actually endangers life [8]. For the court to determine whether the hurt caused is grievous, the extent of the hurt and the intention of the offender are taken into account. The medical officer, must confine himself to only opining whether a given hurt is grievous or otherwise, as per the eight clauses of Section 320 IPC.

b. Any hurt which causes the sufferer to be during the space of twenty days in severe bodily pain.

As per this clause the person should be in severe bodily pain for 20 days. How much pain is severe is not defined in this clause. Pain is a subjective experience and will vary from person to person. Severe pain for one person may be bearable pain for the other and it is difficult to assess. Some injuries are more painful than the others depending upon the system or organ involvedand location of the injury. A burn may affect a lesser area but be very painful if it is a third degree burn. In this cases opinion of the doctor is necessary and important. It is generally agreed that technically 20 days need not be continuous. It can be with short pain free gap. The person may be in pain for two weeks followed by 3 pain free days and then again at pain for one week.

c. Any hurt which causes the sufferer to be unable to follow his ordinary pursuits for twenty days.

Again as per this clause the sufferer has to be unable to follow his ordinary pursuits for twenty days. What is ordinary pursuit is not defined in this clause. But it is more easier to prove than severe pain clause as it is more objective in nature. It is generally understood that ordinary pursuits are those daily routine activities which are necessary for day today survival of every human being. It may be brushing teeth, shaving, going to toilet, taking bath, taking food, wearing clothes etc. But mere remaining in the hospital for 20 days or more cannot be itself equated with patient remaining unable to follow his ordinary pursuits without any evidence to that effect.

Punishment for voluntarily causing hurt as defined in section 323 IPC is imprisonment of either description up to 1 year and a fine up to 1000 rupees, while punishment for voluntarily causing grievous hurt is imprisonment up to 7 years as well as fine.

Relevant amendments under criminal law ordinance, 2013

A recent amendment was made in 326 IPC, which has brought new insight into grievous hurt in relation to acid attack.

Section 326 A: Whoever causes permanent or partial damage or deformity to, or burns or maims or disfigures or disables, any part or parts of the body of a person or causes grievous hurt by throwing acid on or by administering acid to that person, or by using any other means with the intention of causing or with the knowledge that he is likely to cause such injury or hurt, shall be punished with imprisonment of either description for a term which shall not be less than ten years but which may extend to imprisonment for life and with fine which may extend to ten lakh rupees, provided that any fine imposed under this section shall be given to the person on whom acid was thrown or to whom acid was administered.

Section 326 B: Whoever throws or attempts to throw acid on any person or attempts to administer acid to any person, or attempts to use any other means, with the intention of causing permanent or partial damage or deformity or burns or maiming or disfiguration or disability or grievous hurt to that person, shall be punished with imprisonment of either description for a term which shall not be less than five years but which may extend to seven years, and shall also be liable to fine.

For the purposes of section 326 A and B, acid includes any substance which has acidic or corrosive character or burning nature, that is capable of causing bodily injury leading to scars or disfigurement or temporary or permanent disability. Permanent or partial damage includes deformity, or maiming, or burning, or disfiguring, or disability of any part or parts of the body of a person. And for the purpose of this section, permanent or partial damage or deformity shall not be required to be irreversible.

So, after this amendment, following changes can be derived. Earlier only permanent disfiguration of face was alone considered as grievous hurt, but now even disfiguration of any part of the body by throwing or administering acid is considered as grievous hurt. Even temporary or permanent disability due to throwing or administering of an acid is covered under grievous hurt. Moreover, the damage or deformity shall not be required to be irreversible. The punishment was enhanced and may extend to imprisonment of life and a fine which may extend to ten lakh rupees. Under section 326 A and b, even attempt to throw or administer acid on any person is punishable. The offences under Section 326 A and 326 B are cognizable and non bailable.

Other legal IPC Sections pertaining to injuries

Section 319 to 338 of IPC deals with the legal aspects of hurt in various forms which is beyond the scope of this article to discuss in detail.

Medicolegal duties ofmedical officer

The medical practitioner has the duty to examine the injury, document it carefully and treat the

patient. He should be careful in recording the injury report. First of all he should write the general information of the injured person like name, age, sex, address, exact time of the examination, Viz., hour, date, month and year and two marks of identification to enable him to recognize the injured person in the court. The name and belt number of the accompanying police person should be noted. He should also record the consent of the injured person for being examined by the medical officer and then proceed with examination proper. Proper preparation of MLC report in injury case is very important. The detailed guideline to prepare MLC report may be beyond the scope of this article. But the doctor is expected to document the following facts of the injury namely type of injury, location, dimensions or size (length, breadth, depth and shape if appreciable), by what weapon inflicted (sharp or blunt object), approximate age or duration of injury and nature of injury, grievous or simple. Regarding the nature of injury, it is not mandatory to opine it on the spot or the same day. If there is a need to observe the patient for some time or some investigation needs to be done or report of other specialty is essential then you can opine it after the required things are done. When asked for expert opinion on the nature of injury by police or court, he has to opine whether the injury is grievous or otherwise as per the eight legal clauses of Sec. 320 IPC. Many a times the medical practitioners are not confident or clear about the grievous injury section due to in experience in dealing such cases. In such situation, it is prudent to consult with the Forensic Medicine specialist or medico-legal experts to give correct opinion based on the medical facts. We should know that it is a piece of corroborative evidence for crime investigator and the court. The opinion should be scientific and consistent. However it may be revised or amended in later stage if needed, based upon the available new scientific factsand circumstance of the case. The medical opinion guides the investigator and the court to decide the case appropriately as per law. Therefore the medical opinion is very important and has significant role for legal system to administer justice. However the expert opinion or medical evidence of the medical officer is not final and indispensable for conviction. It is an important corroborative evidence for the investigating officer and guides the court for deciding the case. In the Judgement (Hadia Mia, 1988 Cr LJ 1459 (Gau)) the court said, medical evidence is of importance though it is not conclusive and the judgment must be of court. The final opinion on the nature of injury is left to the discretion of the court. The court can reverse the doctor's opinion also.

If an opinion regarding the nature of injury cannot be formed at the time of the examination, as in the case of a head injury where symptoms are obscure, the injured person must either be re-examined after 24-48 hours or admitted under observation until a definite opinion can be formed. A forensic doctor also should know about the legal definition of dangerous weapons or means. Many a times the investigating officer would be seeking for the expert medical opinion on the recovered weapon of offence and the injury findings for corroboration. As per section 324 and 326 IPC, dangerous weapons or means include any instrument for shooting, stabbing or cutting, or any instrument, which used as a weapon of offence, is likely to cause death; fire or any heated substance, poison or any corrosive substance; explosive substance or any substance which is deleterious to the human body to inhale, to swallow, or to receive into the blood, or by means of any animal. The medical officer can opine whether the alleged weapon of offence is dangerous weapon or mean. However the court will finally decide whether the assailant was armed with dangerous weapon or not, depending upon the circumstances of the case and expert medical opinion.

The medical testimony of doctor in the court of law is very important. We should be honest, scientific and consistent in our opinion. Sometimes the court may ask the doctor to dictate his whole findings in the report completely, especially in handwritten reports for clarity. As a medical person we have to guide the public prosecutor and the court in recording the right and relevant findings, even though it may be a negative finding. When evidence is read to be recorded in the court of trial, we have to highlight the important relevant findings of the report pertinent to the case. Sometimes one paragraph or even one part of the paragraph may be all that is necessary to substantiate the point you are making, e.g., mentioning the cause of death in case of road traffic accident, instead of dictating the whole report, which is time consuming. Evidence must be documented in the words of the author, i.e., doctor who prepared it. But if the court feels that the whole document has to be dictated as it is, we should do it respectfully, even though it may be time taking. Now a days the court admits most of the computer typed reports asit is, without dictating again, unlike the hand written reports where the handwriting has to be recognized and dictated for clarity of the court.

Misinterpretation of injuries and wrong opining

The wrong interpretation of injury and opinion can lead to serious injustice in the legal system.

It is not uncommon to come across a bad report or opinion of a doctor. There are various reasons for it. As per my 20 years of experience in the medicolegal service, I observed that the most common cause is the inadequate medicolegal training and exposure of the medical students during the MBBS course. It is well known that about 70% cases coming to the emergency department are medicolegal cases. In India a MBBS graduate is legally qualified to see all medicolegal cases and also authorized to conduct Medicolegal postmortem and give expertopinion in the case. He is also summoned in the court of law to give evidence as a medical expert. Quiet often it is seen that it is forced upon the government doctor posted in various hospitals of the sate to perform the medicolegal duty in spite of their in competency. These leads to compromise on the quality of medicolegal work and affect the crime investigation and justice system. Secondly there is inadequate qualified forensic expert (MD/DNB, Forensic Medicine) to guide the doctors and help them in dealing with the medicolegal cases. We have seen numerous cases where the investigation has been jeopardized due to bad or wrong medical reports, either due to ignorance or carelessness. The opinion had to be revised and rectified to come to a logical conclusion. When it comes to the higher referral centre for opinion it is good to speak to the first treating doctor who prepared the report and if possible make him part of the medical board. In doing so there will be a transparent and direct discussion leading to a logical and scientific opinion. The ignorant doctor should not be made a victim or scapegoat. In my experience, most of the mistakes were due to ignorance of the doctor without any malafide intention or motive and very rarely due to pressure from higher sources. As per my experience it is observed that the doctors are taught that they should stand by their opinion at any cost and not change or revise, otherwise they will be blamed. It is not so. The unintentional mistakes can be rectified by discussion with the experts in the larger board of experts and opinion rectified and revised in a scientific way and as per legal procedure for logical conclusion and justice in the case.

It isessential that all the medical officers posted in the emergency should attend an orientation program regarding handling of medicolegal cases before they are posted. Many a times the court has frequently summoned the medical superintendent of the hospital for explanation for lack of clarity and quality medical reports of the doctors handling medicolegal cases. I have deposed many such summons to medical superintendent of AIIMS, New Delhi in relation to bad medicolegal report of the emergency doctors. They should work under the supervision of medicolegal expertor senior consultant with medicolegal experience to provide quality medicolegal reports and to avoid mistakes and embarrassment to the institute.

Investigating officer

All injury case becomes a medico-legal case. A person may show himself or be brought by police for medical examination and treatment. In both situation the medical officertreats the patient and prepares the medicolegal reports. If the person comes by himself the doctor informs about the case to the nearest concerned police station. In big hospitals there is police post adjacent to the emergency and the information is passed on to the police on duty which subsequently conveys to the concerned police station. The medicolegal report is collected by the police for inquiry and investigation. If the opinion about the nature of injury is not mentioned, he will approach the concerned doctor for the same. He may also enquire about the likely weapon of offence. The investigating officer seeks expert opinion from the treating doctor to corroborate their investigation findings about the nature and manner of the injury to take the legal course of action as per law of the land.The nature of injury may be simple or grievous and the manner of injury may be accidental, self inflicted or homicidal. The medical officer must confine himself to only opining whether a given hurt is grievous or otherwise as per the eight clauses of Section 320 IPC and not make extra comments on the intention or knowledge part of the offender. The police officer takes the guidance of medical evidence, apart from the circumstantial evidence to charge sheet the accused in appropriate section of IPC. It is essential that the police also should provide the correct and relevant brief history of the case to the treating doctor, which will help in the treatment and preparation of good medicolegal report.

Judiciary System

The judge decides about the nature of injury based on the medical findings and the investigation reports. To determine whether the hurt is grievous or not, the court generally takes into account the extent of the hurt and the intention of the offender. Further it has to be proved that the offender intended to cause or had the knowledge that his act was likely to cause grievous hurt. Intention to cause grievous hurt is inferable from the circumstances of the case and the nature of injury caused. He can

summon the doctor to depose the medical injury report in the court of lawand admit the report as a corroborative evidence in the case. The defense lawyer can cross examine the findings. And also the public prosecutor or Judge may also ask queries to clarify any doubts on the report.

Important Court Judgments in relation to hurt and 320 IPC

1. Injury report does not prove injuries. Doctor must be produced and he must state all the injuries (Bawasala Mad. 1953 Cr. LJ, Kutch).

2. The medicolegal report can be admissible in evidence U/S 32(2) Evidence Act, if the doctor is dead or not available. The compounder or other person present at the time of examination and who can identify the writing of the doctor should be produced.

3. The expert must give his reasons. It is not, however, necessary, that he should give reasons at length in support of his opinion. It is sufficient if he gives reasons briefly. The expert should also give his qualifications (Prem Shankar Misra, 1957 Cr. LJ 108 All).

4. It is the duty of the prosecution, and no less of the court, to see that the alleged weapon of the offence, if available, is shown to the medical witness and his opinion invited as to whether all or any of the injuries on the victim could be caused with that weapon. Failure to do so may sometimes,cause aberration the course of justice. (Ishwar Singh Vs State of Uttar Pradesh, AIR 1976 SC 2423).

5. When there is conflict between the two Doctors, the opinion which supports direct evidence, be accepted (Piara Singh Vs State of Punjab, AIR 1977 SC 2274).

6. It was held in Atma Ram Vs The State of Punjab (D.B) 1882 (2) CLR 496, that the court is not absolved of the responsibility, while deciding a criminal case to form its own conclusion regarding the nature of the injury, expert's opinion not withstanding. The court has to see the nature and dimension of the injury, its location and the damage that it has caused. Even when an injury is described as to be one which endangers the life, the court has to apply its own mind and form its own opinion in regard to the nature of injury, having regard to the factors that should weigh with the court, already mentioned. It was also held that wherever a doctor describes an injury as 'dangerous to life' and the nature of the injuries is such which could merit such a conclusion, then such an injury has to be treated as 'grievous hurt' of the description mentioned in first portion clause (8) of section 320 IPC. In this case Injury inflicted by accused was opined by the doctor, as dangerous to life was declared grievous in nature. It was held that the injury which 'endangered life' must be held to be of grievous nature. It was held that the expression 'dangerous' is an adjective and the expression 'endanger' is verb. An injury which can put life in immediate danger of death would be an injury which can be termed as 'dangerous to life' and, therefore, when a doctor describes an injury as 'dangerous to life', he means an injury which endangers life in terms of clause (8) of section 320 IPC, for, it describes the injury 'dangerous to life' only for the purpose pf the said clause. He instead of using the expression that this was an injury which 'endangered life' described it that the injury was 'dangerous to life',meaning both the times the same thing.

7. It is not the duty of the doctor to enquire from the injured patient about the actual assailants and the inquiry would be confined as to how he received the injuries and the weapon used etc. (P. BabuVs State of A.P, 1994 SCC Cr. 424).

8. In Case, Raj Singh Vs State of Punjab, 1992, Suppl 15 CCR: 519, the High Court observed that [10]:

a. On a bare perusal of the injury, it left no doubt that the doctor had failed to mention the extent of the cut to the bone of the left shoulder, what to say as to stating as to which portion of the bonewas cut.

b. The injury was not subjected to X-ray examination for ascertaining the extent of the cut to the bone under this injury. Under these circumstances it cannot be said by any stretch of imagination that the bone was fractured or dislocated as provided under the clause seventhly to section 320 IPC.

c. The trial court had found this injury to be grievous in nature that it had endangered the life of the injured. In this regard it is noteworthy that injury was with sharp edged weapon on a nonvital part of the body like shoulder without extensive damage to the underlying bone, cannot be said by any stretch of imagination having endangered the life of the injured.

d. Under these circumstances the findings of the trial court regarding the injury being grievous in nature is not sustainable. On the other hand, the accused is guilty of the offence punishable under section 324 IPC.

9. In the case, Sreenivas Vs State of Kerala [10], (KER) 2006(3) AICLR:408, when the expert used the expression"Maxilla cut", the court was of the

opinion that it had to be understood reasonably. The expert did no say that there was a "cut on the maxilla", instead what was specifically stated was that the "maxilla was cut". The court expressed anguish against the manner in which the expert had tendered the evidence. If the medical expert is not experienced and does not tender the evidence on that aspect voluntarily, the prosecutor must lead evidence on that specific aspect. Even if an inexperienced prosecutor omits to lead to relevant evidence the court must seek clarification to elicit relevant evidence.

10. In the case, Lakhvirsingh Vs State of Punjab, 2004, RCR:829, an injury was described as "an incised wound 8cmx3cm with cut on the underlying radius bone measuring 3cmx1cm on posterior lateral aspect of the right forearm just above the right wrist joint. The wound was profusely bleeding". The injury was not considered as grievous under the clause seventhly to section 320 IPC as there was no fracture or dislocation of the bone in this case. The doctor had declared the injury grievous only on the basis of clinical examinations. The high court set aside the findings recorded by the learned trial court and lower appellate court that the injury was grievous. The court also observed that before declaring the injury as grievous, it must be seen whether the cut in the bone,as per the medical report, is superficial or it dislocates the bone or there is a fracture.

11. In case State of Punjab Vs Manga Singh, (P&H) 1992, (2) RCR:144, injury was caused by gandasi cutting the bones; but no X-ray was done to prove the nature of the injury. In medicolegal report it was mentioned that the underlying bone was cut along the direction of the wound. The injury was declared grievous without X-ray examination. The division bench held that the opinion declaring the injury grievous based on mere visual observation fell through and was ignored by the trial court.

Conclusion

It is the duty of the medical officer to know the law correctly and apply them in their strict sense while giving expert medical opinion. The medical expert witness is expected to put before the court all scientific materials and facts which led him to the conclusion. He also should enlighten the court on the technical/scientific aspects of the case by explaining the terms of science in simplified words, so that the court although not an expert in the field may form its own judgement on those material facts after giving dueregards to the experts opinion. Medical evidence is an important corroborative evidence in the court of law. Once the expert opinion is accepted by the court it is not the opinion of the doctor but of the court. The investigating officer has to thoroughly investigate the case and collect all the available scientific and circumstantial evidences to assist in the logical conclusion in the case. It is finally the judiciary which will interpret the law and apply according to the fact and circumstances of each case and deliver the justice.

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Some Forensic Aspects on Antidepressants Drugs

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Abstract

Antidepressants are the most prescribed for depression. The exact mechanism of action of antidepressant is unknown. The prevailing theory is that antidepressants increase the concentration of one or more brain chemicals (neurotransmitters) that nerves in the brain use to communicate with one another. The neurotransmitters affected by antidepressants are nor epinephrine, serotonin and dopamine. The different classes of antidepressants differ in the neurotransmitters they affect. This determines some of their side effects and potential drug interactions. All available antidepressants are effective and for most cases of depression there is no good evidence that any antidepressant is more effective than another. Side effects and potential drug interactions are major factors that influence selection of antidepressants. This article highlights some forensic aspects, their types, side effects and potential drug interactions of the major antidepressants classes.

Keywords: Antidepressants, Forensic detection

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Introduction

Antidepressant drugs are an effective medication in treating the severe alleviated depression and anxiety., including dysthymia, anxiety disorders, obsessive compulsive disorder, eating disorders, chronic pain, neuropathic pain and, in some cases, dysmenorrhoea, snoring, migraines, attention-deficit hyperactivity disorder, substance abuse and sleep disorders. They can be used alone or in combination with other medications. An antidepressant should be more efficacious than placebo to justify the risk associated with side effects [1]. Studies have shown that antidepressant-induced mania can occur in 20–40% of bipolar patients [2]. Antidepressant drugs are available under the different names and in different doses form such as injectable, inhaled and by oral preparation. Some studies have shown that the use of some antidepressants correlate with an increased risk of suicide in some patients and especially youth [3]. This problem has been serious enough to warrant government interventions in some places to label greater likelihood of suicide as a risk of using antidepressants [4]. Before the 1950s, opioids, amphetamine, and methamphetamine were commonly used as antidepressants. Their use was later restricted due to their addictive nature and side effects. These are of the following types

Tricyclic antidepressant

Tricyclic antidepressants are chemical compounds used primarily as antidepressants. They are named after their chemical structure, which contains three rings of atoms. Examples Imipramine HCl, Nortriptyline., Doxepin HCl., Amitriptyline HCl., Trimipramine, Dothiepin, Clomipramine, Nitroxazepine Amoxapine. The

side effects of these compounds are dry mouth, blurred vision, urinary retention, constipation, dizziness and emesis (or vomiting).

Tetracyclic

Tetracyclic antidepressants contain four cyclic rings as part of their structure. Tetracyclic antidepressants are similar to tricyclic antidepressant and act by inhibiting reuptake of neurotransmitters serotonin and noradrenaline in the brain, and elevate mood. They are effective antidepressants but are used less often now due to their side effect profile. Examples Mianserin HCl., Mirtazapine. The side effects of these compounds are anxiety, depression, hot flashes, insomnia, major depressive disorder, panic disorder and post traumatic stress disorder.

Monoamine oxidase

Antidepressants such as Monoamine oxidase ease depression by affecting chemical messengers (neurotransmitters) used to communicate between brain cells. An enzyme called monoamine oxidase is involved in removing the neurotransmitters norepinephrine, serotonin and Dopamine from the brain. Monoamine oxidase also affects other neurotransmitters in the brain and digestive system, causing side effects. Example Moclobemide. The side effects of these compounds are dry mouth, nausea, diarrhea or constipation, drowsiness, insomnia, skin reaction at the patch site, dizziness or lightheadedness and muscles aches.

Selective Serotonin Reuptake Inhibitors

Selective serotonin reuptake inhibitors and related drugs work by increasing a chemical called serotonin in the brain. Serotonin is a neurotransmitter (a messenger chemical that carries signal between nerve cells in the brain). After carrying a message, serotonin is usually reabsorbed by the nerve cells (known as 'reuptake'). Examples Fluoxetine, Sertraline. The side effects of these compounds are increasing risk of bone fractures, akathisia, suicidal ideation, photosensitivity, reduce sexual desire and insomnia, Diarrhea.

Serotonin Norepinephrine Inhibitor

Serotonin Norepinephrine Inhibitor work by changing the levels of one or more of these naturally occurring brain chemicals. Serotonin Norepinephrine Inhibitor block the absorbption (reuptake) of the neurotransmitters serotonin and norepinephrine in the brain. They also affect certain other neurotransmitters. Changing the balance of these chemicals seems to help brain cells send and receive messages, which in turn boosts mood. Medications in this group of antidepressants are sometimes called dual-action antidepressants. Example Venlafaxine. The side effects of these compounds are constipation, caugh, increased in sweating, sleep problems, weight loss, yellow eyes and skin (jaundice), and upper right abdominal pain.

Serotonin Reuptake Inhibitors or Triazolopyridine eg. Trazodone

All the antidepressants, discussed above contain Amitriptyline HCl, Trimipramine, Dothiepin, Clomipramine, Nitroxazepine, Amoxapine, Mianserin HCl, Mirtazapine, Moclobemide, Fluoxetine, Sertraline, Venlafaxine, Trazodone, Trianeptine, or formed by the combination of all these drugs and the Common Side-Effects are Hypertension, Pre-eclampsia, Suicidal ideation, Sexual drive, Failure to reach orgasm and Erectile dysfunction. These compounds also shows Common Withdrawal symptoms are Dependence, Nausea, Chills, Muscles aches, Dizziness, Anxiety, Irritability, Insomnia Fatigue.

Some Forensic Aspects

One of the challenges that chemist often have to contend with is separation of different compounds in mixtures. Forensic chemists, for example, are often asked to identify pills or powders found at the scene of crime or a drug overdose. Those pills or powders may be single pure substances, or more commonly mixture of pure substances. It is quite natural that every person must not be aware that what antidepressant actually is, and how it acts on our body as well as what are their side-effects. In India and outside countries, various case related to antidepressant can be seen. It may be homicidal, suicidal and accidental. In Homicidal cases, the culprit used to provide overdose of antidepressant to the subject. In homicidal cases, the culprit knows the diseases and problems of the subject, still he use to provide overdose of medicine intentionally. Consequently, death of the subject may occur. In suicidal cases, the subject himself uses to take overdose of the antidepressant drugs instead of

knowing all the problems related to him. As a result, diseases and death of person may occur. Accidental cases and suicidal cases are very common in comparison to homicidal cases. Such type of cases occurs due to mishandling of doctors especially in accidental cases. When the cases of antidepressant are investigated, the Forensic chemist asked for the answers of following questions:

- Which type of antidepressant drugs was given or taken by the subject?
- What was the dose given to the subject?
- At what time medicine was given.
- Is the patient was suffering from any other diseases or not etc.

After finding answers of all these questions the forensic chemist may conclude what was the mode of death and either the death was natural or the person was killed intentionally.

Forensic detection and identification of antidepressants

The separation of analyte of interest forms two basic approaches, the first one is the sample preparation step and the second is the detection of the compound of interest. A number of extraction techniques have been routinely used for the removal of as many as interfering compounds and pre concentration of the analytes. A variety of methods GC-MS, LC-MS have been given in literature for identification and determination of the amount of antidepressants in different biological matrices which can fabricate a broad investigating area in the field of therapeutics as well as forensics. These methods offer good precision and accuracy over the entire analytical range, allowing the development of very rapid and efficient methods.

Conclusion

In the present work a study has been made on antidepressant drugs by forensic scientist at crime scene from medicines of different trade names. This study helps to forensic scientist in identification of drugs at crime scene as well as in laboratory. A very small number of cases involving non-medical use of antidepressants have been reported over the past 30 years. Several cases of the misuse of amitriptyline alone or together with method one or in other drug dependent patients and of dothiepin with alcohol or in methadone patients have been reported. Therefore, we have concluded that with the help of these information a forensic scientist or police can easily identify the different types of antidepressant drugs from the crime scene and it also help in identification of persons who bought or misuse these drugs via intentionally or not.

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Review Article

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Mitochondrial DNA Typing for Forensic Identification

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Abstract

Mitochondrial DNA has more useful genetic information as compared to nucleic DNA because they are present in more number per cell. In decomposed or old biological samples nuclear material in the cell may not exist for a long period, so it is difficult to perform DNA analysis with the nuclear DNA from remains of biological samples. This high copy number in mtDNA increases the possibility of recovering sufficient DNA from compromised samples. For this reason, mtDNA can play an important role in the identification of missing person investigation, in mass disasters and other forensic investigations involving samples with limited biological material. Additionally, mtDNA is maternally inherited. Therefore, barring a mutation, an individual's mother, siblings, as well as all other maternally-related family members will have identical mtDNA sequences. As a result, forensic comparisons can be made using a reference sample from any maternal relative, even if the unknown and reference sample are separated by many generations. Anthropologically, mitochondrial DNA in the fossilised source is used to trace the human ancestry particularly of maternal lineage.

Keywords: Mitochondrial DNA; D-loop; Hyper variable region; PCR-RFLP; Sequencing; Hetroplasmy; Maternal lineage.

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Introduction

In Forensic case work analysis, Mitochondrial DNA (mtDNA) is generally used when evidence material contain insufficient amount of DNA. In decomposed or old samples nuclear material in the cell may be less or not exist for a long period, so it is difficult to perform DNA analysis with the nuclear DNA from remains of such biological samples. Mitochondrial DNA has useful genetic information and it degrades slower as compared to nucleic DNA. Mitochondria are present in more number per cell. This high copy number increases the possibility of recovering sufficient DNA from compromised samples, and for this reason, mtDNA can play an important role in the identification of missing persons investigation, in mass disasters, and other forensic investigations involving samples with limited biological material or old decomposed samples. Additionally, mtDNA is maternally inherited [1]. Therefore, barring a mutation, an individual's mother, siblings, as well as all other maternally-related family members will have identical mtDNA sequences. As a result, forensic comparisons can be made using a reference sample from any maternal relative, even if the unknown and reference sample are separated by many generations.

Mitochondrial DNA is transmitted maternally via the egg cell. The human mitochondrial DNA (mtDNA) is a circular double-stranded molecule, and it was first time sequenced by Anderson et al. in 1981 [2]. It has been reported that mtDNA is present in high copy number in human cells, with high mutation rate, the mutation rate is five to ten times higher than that of nuclear DNA. It has three highly variable regions HV1, HV2 and HV3 (non-coding region) which are differentiated by sequencing or by hotspot analysis in which SNPs alleles are identified within these and other regions. Mitochondrial DNA (mtDNA) is a small circular DNA molecule located in mitochondria. Mitochondrial DNA analysis is a valuable technique and its applications are relevant to many different fields [3]. The technique is likely be refined further to provide even more success in the future. In this paper we are reviewing the application possibilities of mtDNA typing in forensic practice.

Structure of Mitochondrial DNA

The mitochondrial genome is circular It has two strands that are differ significantly in their base composition. The heavy strand (H-strand) is purine rich, having a greater number of guanine nucleotides, whereas the light strand (L-strand) is pyrimidine rich and thus physically lighter. The sequence of the mtDNA genome codes for a total of 37 genes, all of which are essential for normal mitochondrial function. A total of 28 gene products are found on the H-strand and 9 on the L-strand. Of the 37 genes, 13 are for proteins which are necessary for cellular respiration, including the NADH dehydrogenase 6 enzyme, 22 are for mitochondrial transfer RNAs (tRNA) and the remaining two encode the 16S and 23S subunits of ribosomal RNA (rRNA) Fig 1 [4]. This types of RNA help to assemble protein building block (amino acid) into functioning proteins.

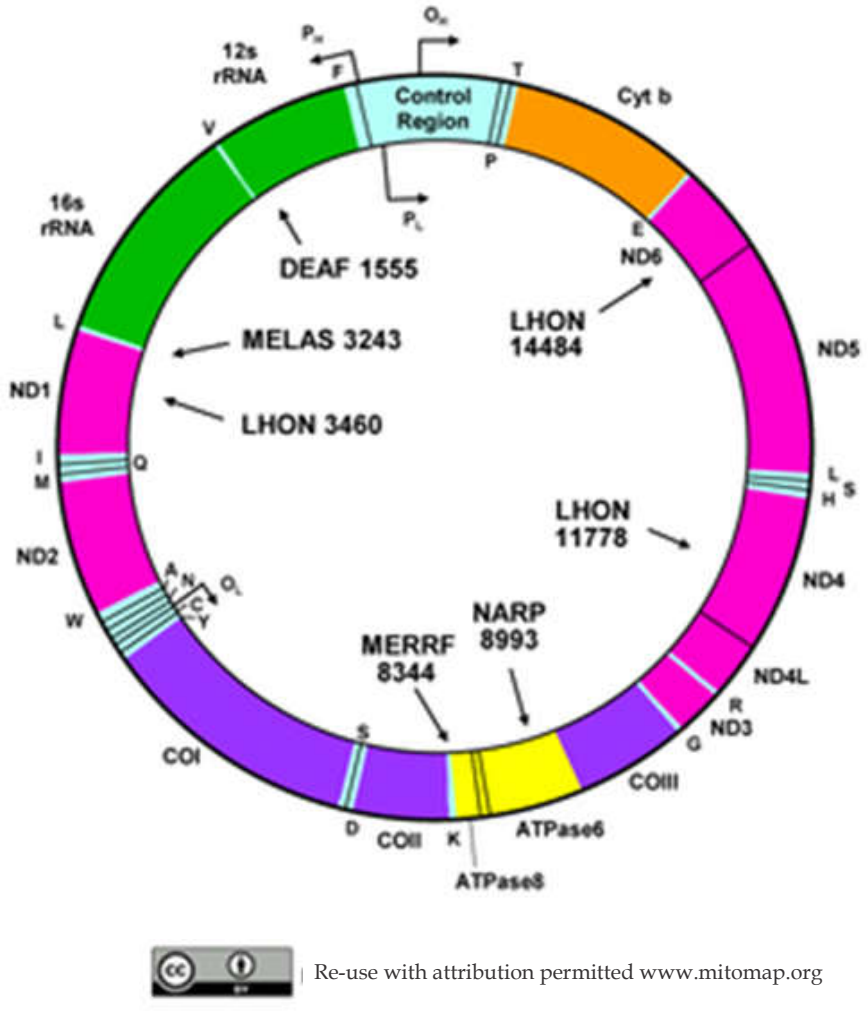

Fig. 1: Structure of Mitochondrial DNA

Configuration of Mitochondrial DNA

The mitochondrial genome is much more efficiently organized than the nuclear DNA genome, containing very little non-coding sequence (7%) compared to the nuclear genome where approximately 97% is not expressed. The 37 genes encoded by the mitochondrial genome are nearly contiguous with each other, lacking introns and only occasionally having one or two base non-coding sequences separating them. Because of this protein coding sequence, there are relatively few sites at which variations in sequence can be tolerated. Therefore, mtDNA is considerably less polymorphic than its nuclear counterpart, despite its higher rate of mutation. In fact, the only significant region of the genome that does not code for a gene product is the displacement loop region, also known as the D-loop. This 1.1 kb stretch of often triplex DNA, known as the "control region", contains essential regulatory functions, including the origin of replication for the generation of multigenic transcripts and DNA replication of the heavy strand. Despite the limited amount of sites for tolerated sequence variations, the mitochondrial genome has been shown to have a mutation rate within the control region up to ten times that of comparable nuclear DNA sequences. Forensic analysts are particularly interested in specific portions of this control region, designated hyper variable regions 1 (HV1) and 2 (HV2), because of their non-regulatory and non-protein-coding status and the dense array of sequence variability they exhibit within human populations [5].

HV1 and HV2 regions span roughly in positions 16024-16383 and 57-372, respectively, whereas the HV3 region spans positions in 438-574 (numbered corresponding to the rCRS (GenBank accession number NC_012920). There are two separate homopolymeric stretches of C nucleotides (poly-C repeats, or C-stretches) in both the HV1 and HV2 regions, therefore there may be possibility of more mutation instead of single nucleotide mutations between individuals. The degrees of polymorphism in the D- loop is so great that direct sequencing may be the most efficient method of typing mtDNA.

MITOMAP, an internet mitochondrial sequence database, maintains a list of published mtDNA polymorphisms found within the D-loop and forensically informative HV1 and HV2 regions (http://mitomap.org/bin/view.pl /MITOMAP/ Polymorphisms Control) [6,7]. Displacement loop or D-loop is a region in the mtDNA structure is consists of a stretch of 1123 base pair sequences. This region is close to the area of mtDNA replication and transcription. It bears two variable regions- HV1 at position 16024-16383 and HV2 at position 57-372.

HV1 Region at position 16023-16383

16023 gttctttcatggggaagcagatttgggtaccacccaagtattgactcacccatcaaca
16081 accgctatgtatttcgtacattactgccagccaccatgaatattgtacggtaccataaat
16141 acttgaccacctgtagtacataaaaacccaatccacatcaaaaccccctccccatgctta
16201 caagcaagtacagcaatcaaccctcaactatcacacatcaactgcaactccaaagccacc
16261 cctcacccactaggataccaacaaacctacccacccttaacagtacatagtacataaagc
16321 catttaccgtacatagcacattacagtcaaatcccttctcgtcccatggatgacccccc
16383 tca

HV2 at position 57-372

56 atttt
61 cgtctgggggggtatgcacgcgatagcattgcgagacgctggagccggagcaccctatgtc
121 gcagtatctgtctttgattcctgcctcatcctattatttatcgcacctacgttcaatatt
181 acaggcgaacatacttactaaagtgtgttaattaattaatgcttgtaggacataataata
241 acaattgaatgtctgcacagccactttccacacagacatcataacaaaaaatttccacca
301 aacccccctcccccgcttctggccacagcacttaaacacatctctgccaaaccccaaaa
374 acaaagaaccctaa

C-strech region: MtDNAhyper variable region (HV1) contains a C-continuous tract termed the C-stretch, which is located in 16184~16193 nt and is associated with sequence-length variations.This variation have been developed by slipping of the DNA polymerase during replication. The C-stretch evolves much faster than other regions of mtDNA, and variations in this region have been demonstrated widely among unrelated individuals. The mtDNA control region, especially the C-stretch, may be involved in the development of human diseases. It has been reported that C-stretch length heteroplasmy within HV1 and HV2 were observed and the rates of heteroplasmic length variation revealed significant differences among distinct populations. Heteroplasmy is the presence of more than one type of organelles genome within a cell or individual. It is an important factor in considering the severity of mitochondrial diseases because most of the eukaryotic cells contain many hundreds of mitochondria with multiple copies of mitochondrial DNA, it is common for mutation to affect only some mitochondria leaving most unaffected. An individual can exhibit two or more different C-stretch lengths in different tissues as well as in the same tissue, especially in hairs. C-stretch length heteroplasmy was demonstrated in different hair shafts and even in different parts of the same hair. As the 'out-of phase' nucleotide pattern, C-stretch is not easily detected when compared to other regions of mtDNA, and C-stretch length heteroplasmymay be difficult to interpretation of DNA sequencing. C-stretch is located in the middle of mtDNA HV1, and these sequence variations may

hinder the application of the mtDNA control region to forensic and population genetics. Therefore, the C-stretch might be highly significant for forensic identification and population genetic studies [8].

Aspects of Mitochondrial DNA for Forensic purpose

a. Higher Mutation Rate

Mitochondrial DNA has a number of characteristics which makes it an idealchoice for forensic use. It has been estimated that the mtDNA genome evolves at a rate that is up to ten times that of its chromosomal counterpart. Thisis an important factor when considering that data consistently show that unrelated individuals are extremely likely to have different mtDNA haplotypes thus making mtDNA useful for purposes of human identity testing. This higher mutation rate can be accounted for by such factors as DNA repair inefficiencies, oxidative damage, and the greater number of replicate cycles that mtDNA undergoes during cell growth. Evidence also suggests that in spite of such an elevated mutation rate, the majority of mtDNA molecules within a given individual will still be represented by asingle sequence (homoplasmy). Occasionally, however, a *de novo* mutation may occur and propagate, resulting in the phenomenon known as heteroplasmy. Heteroplasmy is a state in which two distinct mtDNA haplotypes coexist within a single individual. This is thought to be due to an mtDNA genome copy "bottleneck" during the early stages of oocyte development. The bottle neck theory purposes that the number of copies of mtDNA in each early oocyte is reduced to a small number of copies as compared to the mature oocyte. Thus, a small number of molecules are chosen as the founder population for all of the mtDNA molecules that are transmitted to the next generation [9]. This set of molecules could contain a homogenous population of mtDNA, orperhaps a heterogeneous mixture due to mutations. Sometimes, such heteroplasmy may increase the discriminatory power of mtDNA identification by providing an additional inclusionary tool for the mitotype, such as situations where an evidentiary sample and a reference sample both exhibit heteroplasmy at the same nucleotide. Other times, it can lead to confusion when comparing two sequences that are assumed to be concordant, as itmay be considered a mixture of mitotypes from more than one individual [10].

b. Inherited Maternally

Human mtDNA is thought to be almost completely maternally inherited. It has been reported that mtDNA is transfer via cytoplasmic inheritance which means via maternal inheritance. This can be explained by the nearly 100,000 copies of the mitochondrial genome residing in the oocyte, and the fact that the few (possibly only two or three) mitochondria present in the spermatozoa are concentrated in the mid-piece and tail region, which are lost following fertilization [11]. Additionally, if the sperm mitochondria do make it to the oocyte, they appear to be preferentially degraded. A group of scientists found a potential explanation. They observed that in *C. elegans* (a type of roundworm), paternal mitochondria are eliminated when the sperm and egg fuse or mt DNA of father (sperm) is eradicated during fertilization. If this process was disturbed, embryo survival rates decreased. This is the first time a study showed experimental evidence to suggest that maintaining paternal mtDNA may be harmful. For this reason it is potentially applicable in identification of maternal relationship. And cannot be used for paternal relationship [12]. Despite this maternal preference, some research has reported a few incidences known as "paternal leakage,"where some paternal inheritance of mtDNA and recombination has occurred. A single case of paternal co-inheritance of mtDNA in humans has been reported so far, in a male individual with a mitochondrial myopathy. In addition, such paternal inheritance of mtDNA has been reported in species ranging from mussels to sheep. Although paternal leakage may occur in rare instances, the normal detectable inheritance pattern of mtDNA is maternal. This maternal inheritance pattern, barring multiple mutations, allows for forensic identifications to be made using reference samples from within the entire maternal lineage, including those that may be separated by several generations, when those of close relatives are no longer obtainable.

c. High copy number

MtDNA is present in a high copy number within most cells. It is estimated that a single cell may contain hundreds of mtDNA genomes for every copy of nuclear DNA. Depending on the needs of the particular cell type, the actual copy number present per cell can vary greatly among different tissue types. For instance, there are more mitochondria in muscle and brain cells than in skin cells. The general abundance of mtDNA can prove vital in situations where the amount of sample may be limited or its quality may be degraded, which is often the case in forensic DNA analyses. Samples that are typical candidates for mtDNA

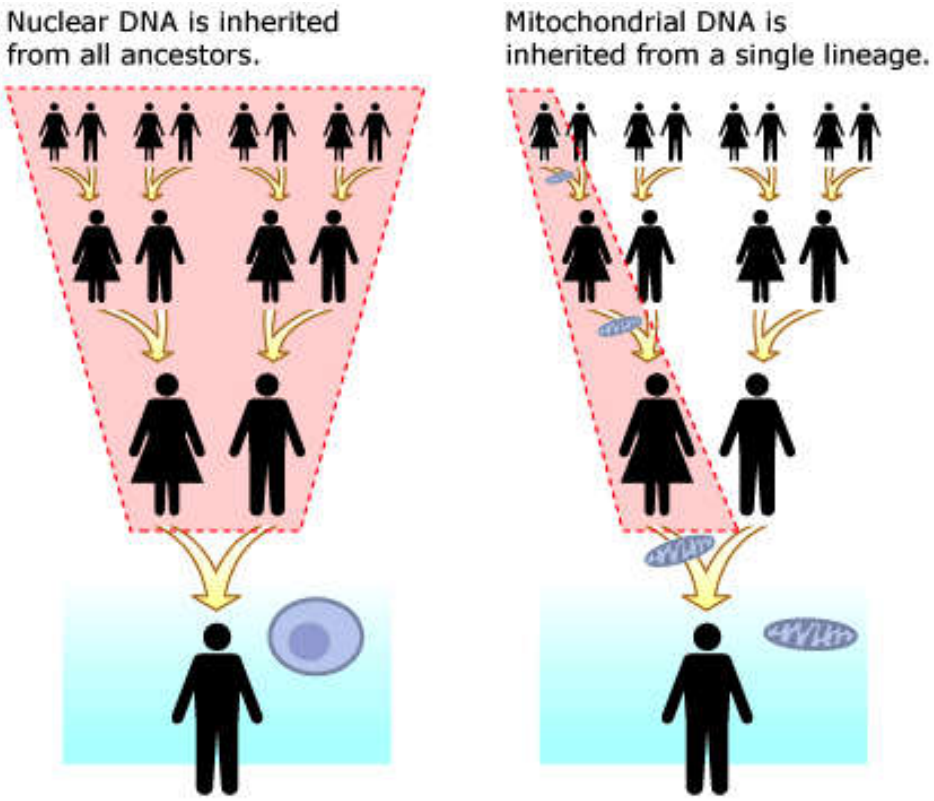

Fig. 2: Inheritance of Nuclear DNA (All Ancestors) and Mitochondrial DNA (Maternal lineage)

analysis include aged bloodstains, skeletal remains, fingernails, teeth, and hair shafts lacking root tissue. The use of mtDNA typing of skeletal remains is often essential in cases of missing persons or in events such as mass disasters where small bone fragments may be the only remaining source of DNA available. In addition, mtDNA testing of hair shafts is of particular importance because shed hairs are common sources of evidentiary material at crime scenes [13].

Molecular Methods for Mitochondrial DNA in Forensic Cases

PCR RFLP: PCR-restriction fragment length polymorphism (RFLP)-based analysis, also known as cleaved amplified polymorphic sequence. It is a popular technique for genetic analysis. It has been applied for the detection of intraspecies as well as interspecies variation. There exist several techniques that are related with PCR-RFLP and also involve gel electrophoresis including techniques for DNA fingerprinting and expression profiling.

PCR-restriction fragment length polymorphism (RFLP)-based analysis is a popular technique for genotyping. The technique exploits that SNPs, MNPs and microindels often are associated with the creation or abolishment of a restriction enzyme recognition site [14]. The first step in a PCR-RFLP analysis is amplification of a fragment containing the variation. This is followed by treatment of the amplified fragment with an appropriate restriction enzyme. Since the presence or absence of the restriction enzyme recognition site results in the formation of restriction fragments of different sizes, allele identification can be done by electrophoretic method in which fragments resolve as per size. Important advantages of the PCR-RFLP technique is that it is not expensive and does not require advanced instruments. The mtDNA D-loop region is useful for PCR RFLP that is amplified by PCR using primers and PCR products can be digested with restriction enzyme such as *Hae* III and *Alu* I. Greater polymorphic rate in the D-loop region has been reported and polymorphism patterns of this region are variable in human population [15]. Disadvantages include the requirement for specific endonucleases and difficulties in identifying the exact variation in the event that several SNPs affect the same restriction enzyme recognition site. Moreover, since PCR-RFLP consists of several steps including an electrophoretic separation i.e relatively time-consuming. This technique is not suitable for the simultaneous analysis of a large

number of different SNPs due to the requirement for a specific primer pair and restriction enzyme for each SNP. This limits its usability for high through put analysis which can be possible by sequencing approach.

Sequencing: In a forensic setting, human mtDNA is analyzed by direct comparison of DNA sequence data of the HV1 and HV2 regions to the rCRS. Standardizing alignments of sequences with the rCRS and following consistent nomenclature for sequence differences is critical to avoid unintentionally describing two sequences as different when in they are actually the same. In fact, several publications have dealt with the nomenclature of sequence data by establishing specific "rules" to follow when determining an mtDNA haplotype. Briefly, differences are reported using the nucleotide positions and the particular base mutation. For example, a sequence that is identical to the rCRS except for having a T instead of a C at position 16150 is designated as 16150T [16].

In the situation of length polymorphisms in the poly-C stretches, any extra Cs are added onto the end of the poly-C stretch. The variant is named using a decimal notation to indicate the number of nucleotides that were in addition to the poly-C repeat in the rCRS. For example, if a particular mtDNA sequence has an additional C compared to the rCRS following the C-stretch of positions 303-315, it would be designated as 315.1C. A similar nomenclature is used to describe insertions or deletions of nucleotides as compared to the rCRS. For instance, if an additional T was inserted following position 294, it would be designated as 294.1T. Finally, deletions are the result of nucleotides that are missing as compared to the rCRS; an mtDNA sequence that was missing nucleotide 325 would be named 325D.

The general rules for naming profiles are as follows as described by Wilson 2002:

- Profiles should be characterized so that the least number of differences from the reference sequence are present.
- If there is more than one way to maintain the same number of differences with respect to the reference sequence, differences should be prioritized as follows:
    1. Insertions/deletions (indels)
    2. Transitions (purine-to-purine or pyrimidine-to-pyrimidine changes)
    3. Trans versions (purine-to-pyrimidine or pyrimidine-to-purine changes)
- As all genes have a 5′ to 3′ direction of transcription and mtDNA genes are encoded on both the heavy and light strands of the closed circular molecule, so that insertions and deletions should be placed 3′ with respect to the light strand of human mtDNA.
- Insertions and deletions should be combined in situations where the same number of differences from the reference sequence is maintained [17].

In order to determine a person's mtDNA haplotype, total genomic DNA is extracted from the biological source material. The extracted DNA is then subjected to amplification of the HV1/ HV2 regions (total of 608bp) using four primer pairs (Table 1). For the HV1 region, two primer pairs, L15997/H16236 and L16159/H16391, are used to amplify overlapping 278 and 271 base pair fragments designated HV1A and HV1B, respectively. The HV2 region is amplified by primer pairs L048/H285 and L172/H408 which typically yields overlapping products of 278 and 277 base pairs designated HV2A and HV2B, respectively (Figure 3). The "L" and "H" designation refers to the light and heavy strand of the mtDNA genome from which the primer sequence is derived and the number indicates the corresponding position of the 3′ end of the primer with respect to the rCRS [18].

The D-loop region of the mitochondrial genome is divided into two main fragments (HV1 and HV2). For universal forensic amplification and sequencing, each fragment is divided into two smaller overlapping fragments (HV1A, HV1B, HV2A and HV2B).

Table 1: Human mtDNA Primers: primers used for control region amplification of human mtDNA.

Hyper variable region 1A (HV1) Primers	
(L15997) A1	5′CAC CAT TAG CAC CCA AAG CT 3′
(H16236) B2	5′ CTT TGG AGT TGC AGT TGA TG 3′
Hyper variable region 1B (HV1) Primers	
(L16159) A2	5′TAT TTG ACC ACC TGT AGT AC 3′
(H16391) B1	5′GAG GAT GGT GGT CAA GGG AC 3′
Hyper variable region 2A (HV2) Primers	
(L48) C1	5′ CTC ACG GGA GCT CTC CAT GC 3′
(H285) D2	5′ GGG GTT TGG TGG AA TTT TTT G 3′
Hyper variable region 2B (HV2) Primers	
(L1720) C2	5′ ATT ATT TAT CGC ACC TAC GT 3′
(H408) D1	5′ CTG TTA AAA GTG CAT ACC GCC A 3′

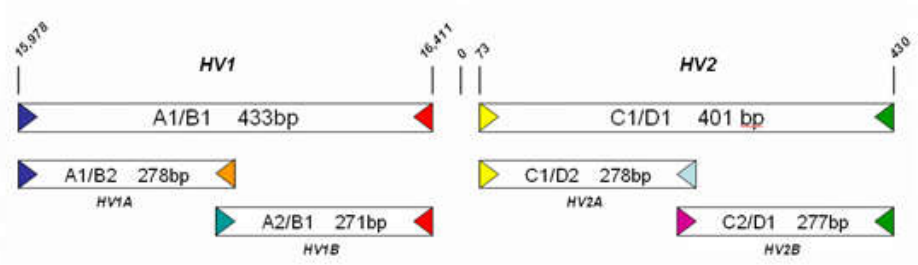

Fig. 3: HV1 and HV2 Primer Overlap Scheme

Sequence analysis

Once the overlapping products are amplified, they are sequenced using the dideoxy chain termination method, *i.e.*, the Sanger method. The Sanger method allows for differential fluorescent labelling of chain terminat or ddNTPs. This allows single reaction sequencing where each label emits fluorescence at a different wave length. In this method, DNA templates are denatured and new strands of DNA are synthesized by *Taq* polymerase. The in corporation of dideoxyribonucleotides creates populations of strands that are terminated with a fluorescent tag at all possible base positions along the template strand. This makes it possible to unambiguously identify the final base of each amplified mtDNA fragment. The resulting sequence product (*i.e.* pool of mtDNA fragments) is then fractionated by capillary electrophoresis (CE) using such commercial systems as the ABI Prism® 310, 3100, or 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA). In CE, the terminated DNA chains are subjected to an electric field that separates the amplified fragments based on their size. The amplified products must be separated in order to determine the specific order of incorporated nucleotides across a target sequence. A laser excites the fluorescent dye terminators as they pass a fixed transparent window in the capillary. Light emitted by the excited fluorophores is then detected by a CCD camera. The different bases are ultimately represented as colored peaks on an electropherogram. Next, the data from each individual sequence reaction are parsed to data analysis software, such as Sequencher® (Gene CodesCorp, Ann Arbor, MI) for sequence alignment and examination by an mtDNA analyst [19,20].

Mitochondrial DNA from forensic and clinical samples

Analyses of hyper variable segments of mitochondrial DNA (mtDNA) are used for forensic analysis, human molecular genetics, evolutionary biology, human migration studies and recovery operations in identifying deceased persons, both ancient and modern. mtDNA coding region increases the resolving power of mitochondrial DNA forensic typing.

H.S.P. Garritsen et al. in 2001 studied DNA sequencing to investigate polymorphisms present in two hypervariable segments such as HVR1 and HVR2 (non-coding region) of the mitochondrial genome among 100 platelet apheresis donors. Alignments were made with the Cambridge Reference Sequence (CRS) for human mitochondrial DNA (mtDNA). Combining the sequencing information of HV1 and HV2 they demonstrate that, of the 100 investigated mtDNA samples, none was identical to the CRS. They reported total of 2 ± 17 polymorphisms per donor in the investigated regions, most of them were base pair substitutions (563) and insertions (151). No deletions were found. Sixty-six of the 110 detected polymorphisms were detected in more than one sample. Seven polymorphisms are newly described and have not been published in the Mitomap database. Their results further demonstrated that polymerase chain reaction analysis of the many polymorphisms found in the hypervariable region of mitochondrial DNA represents a more informative target than previously described mitochondrial polymorphisms for discriminating donor ± recipient cells after transfusion or transplantation [21].

A Salas et al. in 2001 reported a forensic case where mtDNA analysis was performed to compare a blood sample obtained from a raped woman with a single hair shaft found in the suspect's car. Two different portions of a single hair shaft were extracted and sequenced for the two non-coding hypervariable segments (HV1 and HV2) of the control region. The results showed differences in sequence between different portions of the hair and the victim's sequence. These differences are related to various heteroplasmy events. The concordance between the hair sample and the potential source (victim) of this sample is questionable and the strength of the evidence depends on how the sequence information is interpreted by the expert. They suggested the necessity to evaluate heteroplasmic events in routine forensic work [22].

Lee H Y et al. 2008 analysed mitochondrial DNA (mtDNA) of control region sequences from highly degraded skeletal remains, they designed seven set of mini-primer, of which four set form HV1 and three set form HV2 region. These modified mini-primer set is less affected by nucleotide variability and was able to amplify the mtDNA sequences of 55-year-old skeletal remains with high efficiency, suggesting that it is a useful tool for analyzing mtDNA control region sequences from highly degraded forensic samples [23].

Mitchell M. Holland et al. 2011 have analysed hypervariable segment 1 (HV1) of the mtDNA control region from 30 individuals using the 454 GS Junior instrument. Mock mixtures were used to evaluate the system's ability to deconvolute mixtures and to reliably detect heteroplasmy, including heteroplasmic differences between 5 family members of the same maternal lineage. Amplicon sequencing was performed on polymerase chain reaction (PCR) products generated with primers that included multiplex identifiers (MID) and adaptors for pyrosequencing. Data analysis was performed using Next *GENe*R software. The analysis of an autosomal short tandem repeat (STR) locus (D18S51) and a Y-STR locus (DYS389 I/II) was simultaneously performed with a portion of HV1 to illustrate that multiplexing can encompass different markers of forensic interest [24].

Doosti A et al. in 2011 studied D-loop region polymorphism in Bakhtiarian population in southwest Iran on 168 healthy people by PCR -RFLP. mtDNA D-loop region was amplified by PCR using specific primers. Restriction fragment length polymorphism (RFLP) was analyzed by *HaeIII* and *AluI* restriction endonuclease. The results of study showed 5 restriction patterns for *HaeIII* enzyme (with 1 heteroplasmy) and 2 restriction patterns for *AluI* enzyme (with 2 heteroplasmies) in Bakhtiarian population. Their findings showed a low level of genetic polymorphism in D-loop region and it is related to high kinship marriages and low range of migration in Bakhtiarian population [25].

Sayed AM et al. in 2017 have conduced study on 36 bone samples from human remains for DNA profiling by Autosomal STRs and Mt DNA polymorphism of control region (D loop) by PCR amplification and sequencing. They reported that MtDNA was more efficient than autosomal STR profiling in discriminating among human bone samples especially those which have low and/or degraded DNA content [26].

Sayed AM Amer et al. 2017 have conducted STR and mitochondrial SNPs technologies for identification of old human bone remains and compared the efficiency of techniques in identifying the bone remains that exposed to severe burning and reported MtDNA was more efficient than autosomal STR profiling in discriminating among human bone samples especially those which have low and/or degraded DNA content [27].

Cai FF et al. in 2011 have studied mutational rate in Mt DNA at D loop region in breast cancer cases. The two hypervariable regions HVR1 and HVR2 in the D-Loop region were sequenced in ten paired tissue and plasma samples from breast cancer patients, they have reported MtDNA mutations were found in all patients' samples, suggesting a 100% detection rate. On examining germline mtDNA mutations, a total of 85 mutations in the D-loop region were found; 31 of these mutations were detected in both tissues and matched plasma samples, the other 54 germline mtDNA mutations were found only in the plasma samples. Regarding somatic mtDNA mutations, a total of 42 mutations in the D-loop region were found in breast cancer tissues. This study concluded that somatic mtDNA mutations in the D-loop region detected in breast cancer tissues were not matched in the plasma samples, suggesting that more sensitive methods will be needed for such detection to be of clinical utility. [28]

Warner JB et al. in 2006 conducted a study on DNA (mtDNA) polymorphisms to detect allogeneic transfused platelets at three hypervariable regions (HVR1, HVR2, and HVR3) within the displacement loop (D-loop) region of the mtDNA, for this PCR sequencing were carried out in 119 unrelated blood donors. The Polymorphic sites were found in all three regions: 66 in HVR1, 44 in HVR2, and 18 in HVR3. All sequence information of HVRs resulted in 105 different genotypes of which 95 were unique. This study showed discriminate between two randomly chosen individuals with a random match probability of 1.2 percent and concluded that D-loop region of mtDNA contains a wealth of informative molecular markers for chimerism and survival studies after transfusions of cellular blood components [29].

Chen F et al. 2009 have conducted study to investigate mitochondrial DNA (mtDNA) hypervariable segment-I (HVS-I) C-stretch variations and explore the significance of these variations in forensic and population genetics studies. The C-stretch sequence variation was studied in 919 unrelated individuals from 8 Chinese ethnic groups using both direct and clone sequencing approaches. Thirty eight C-stretch

haplotypes were identified, and some novel and population specific haplotypes were also detected. The C-stretch genetic diversity (*GD*) values were relatively high, and probability (*P*) values were low. Additionally, C-stretch length heteroplasmy was observed in approximately 9% of individuals studied. There was a significant correlation (r=−0.961, $p<0.01$) between the expansion of the cytosine sequence length in the C-stretch of HVS-I and a reduction in the number of upstream adenines. These results indicate that the C-stretch could be a useful genetic maker in forensic identification of Chinese populations. The results from the Fst and dA genetic distance matrix, neighbour-joining tree, and principal component map also suggest that C-stretch could be used as a reliable genetic marker in population genetics [30].

Senafi S et al. 2014 have conducted the study to amplify HV1 and HV2 regions of human mtDNA to determine individual geographic ancestry using human peripheral blood sample for maternal lineage. Twelve pairs of primers for HV region in human mt DNA were designed after PCR amplification and sequence of six DNA samples were analysed by Mitomap to determine possible haplogroups. Among the analysed samples (1 and 1a, 2 and 2a, 3 and 3a) three haplotypes shown same maternal lineage as they share the same set of mutation in the HV region, they demonstrated that the 12 primer which were designed can be useful for determining haplogroups for geographical ancestry [31].

Ramos A et al. 2013 to investigate the frequency and the mutational spectrum of heteroplasmy in the human mtDNA genome. To address this, a set of nine primer pairs designed to avoid co-amplification of nuclear DNA (nDNA) sequences of mitochondrial origin (NUMTs) was used to amplify the mitochondrial genome in 101 individuals. The analysed individuals represent a collection with a balanced representation of genders and mtDNA haplogroup distribution, similar to that of a Western European population. The results show that the frequency of heteroplasmic individuals exceeds 61%. The frequency of point heteroplasmy is 28.7%, with a wide spread distribution across the entire mtDNA. In addition, an excess of transitions in heteroplasmy were detected, suggesting that genetic driftand/ or selection may be acting to reduce its frequency at population level. In fact, heteroplasmy at highly stable positions might have a greater impact on the viability of mitochondria, suggesting that purifying selection must be operating to prevent their fixation within individuals. This study analyses the frequency of heteroplasmy in a healthy population, carrying out an evolutionary analysis of the detected changes and providing a new perspective with important consequences inmedical, evolutionary and forensic fields [32].

In Indian scenario there are study on simple method of isolation of mtDNA from biological, and a case report on forensic odontology where mtDNA were extracted from human dental tissue samples under prolonged formalin fixation and sequenced (D loop region) successfully. Another study of genetic diversity on non-tribal Indians was conducted to drive maternal lineage by HV1 region by Barnabas S and co-workers at Pune, Maharastra [33, 34, and 35].

Conclusion

The analysis of genetic variation in the nucleotide sequences of mitochondrial DNA has allowed to unravel evolutionary aspects concerning the origin of modern human populations and the clarification of ancient human migration patterns. The differences in mutation rate and heterogeneity between hyper variable regions are enough for individual identification and the maternal lineage of mtDNA. Mitochondrial DNA (mtDNA) analysis has been validated as a useful tool for forensic analysis. Mitochondrial DNA has useful genetic information as they are present in high copy number that increases the possibility of recovering sufficient DNA from compromised samples and for this reason mt DNA is a useful tool in investigation of missing person (unidentified body) by using mt DNA profiling when nuclear DNA STR profiling for Parentage test is not genetically analysed. There are several aspects of techniques for the analysis which need to be considered in order to evaluate the value of the evidence. PCR-RFLP and sequencing generally works well on samples that are unable to be analysed through numerous other techniques. It has been reported that mtDNA analysis may have some times disadvantages in few cases because of low discriminatory power (1:200) and heteroplasmy i.e. few single base pair difference might be there in different cells of the same individual unlike the nuclear DNA.

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Case Report

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Differential Decomposition: A Case Report

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Abstract

Estimation of Postmortem Interval (PI) or Time since Death (TSD) is an important objective of post-mortem examination which connects an accused to the particular moment of time to prove his guilt or innocence. This plays an essential role in investigation of medico legal cases. In India, the PI/TSD is estimated on the basis of naked eye changes after death and it is always mentioned in every post-mortem report done by the Forensic Pathologist. This estimation is always a challenge for Forensic pathologists due to various variable factors. The authors present a case of a person who died in unknown time duration and the external changes observed were differential in different parts of the body, a phenomenon rarely cited in standard literature. The authors recommend intensive studies to be initiated in Indian settings to determine a scientific data for the actual time taken for the process of decomposition.

Keywords: Time since Death; Differential decomposition; Forensic Pathologist; External and internal factors; Taphonomy.

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Introduction

Determination of Postmortem interval is an integral part of Postmortem examination. Estimation of Postmortem Interval (PI) connects an accused to that particular moment of time of an incident to prove his guilt or innocence and plays an important role in investigation in medicolegal cases. Its estimation continues to be a major challenge for Forensic pathologist particularly in tropical countries. In India, the autopsy surgeon usually considers the gross external changes in a dead body after death to opine about the time passed since death. The various gross changes in the body after death used for giving opinion about PI are the loss of corneal reflex and changes in eye, cooling of the body, post mortem hypostasis, rigor mortis, decomposition and other putrefactive changes [1-8]. The Forensic Medicine textbooks mentions a range of time duration in which each of these changes appear in a dead body [1-8]. The entire body is exposed to similar environmental conditions and the decomposition changes observed in one human body is expected to be similar throughout. However, there can be instances where different parts of the body show different grades of decomposition changes. The authors present one such instance involving differential decomposition changes at different parts of the same body, which is quite rare as per the standard literature.

Case Details

History

A 40 year old male Cab driver was reported to be missing since 22nd November, 2017. Two days prior to this, he had last contacted his family by his mobile phone. He remained missing and his body was not recovered for more than a month. On 2nd January, 2018, police was informed about a partly decomposed body found hanging from a tree near a jungle (Images 1 and 2). The police removed the body and from the personal belongings confirmed the dead body to be that of the missing cab driver. The dead body was brought to AIIMS and postmortem examination was conducted on the next day. The body was preserved in cold chamber overnight till autopsy.

Image 1: Scene of recovery

Image 2: Corpse hanging from tree

Autopsy Findings

The deceased was a male of moderate nutrition and average built. The deceased was wearing light blue coloured half sleeve shirt, a dark blue coloured trousers, black leather belt, blue underwear, but did not have any socks or shoes. The following significant external findings were observed (Figures 3-8).

Rigor mortis had passed off. There was blackish discoloration over upper limbs, entire abdomen & legs. Peeling of skin was present over forearms, hands, legs & abdomen at places. There were multiple maggots of varying length around head, neck and chest. A blue colored nylon rope with a single running knot was present in front of the neck encircling the neck in two loops. The neck was elongated and third and fourth cervical vertebrae were detached from each other and the head area was joined with the rest part of the body only by the posterior muscles of the neck. The soft tissues over face & forehead was missing, thus exposing underlying bones. Soft tissue of anterior aspect of neck was lost due to extensive maggot activity and decomposition. Phalanges of both lower limbs were absent and evidence of nibbling was present. However, the back muscles were mostly normal and spared from any decomposition changes. No external ante-mortem injury could be appreciated over the body.

Image 3: Head with Nylon rope in situ

Image 4: Blackened lower limbs

Image 5: Skin peeling and blackening

Image 6: Nibbling of toes

Image 7: Minimal decomposition of back muscle

Image 8: Partial skeletonization of face

All internal thoracic organs were blackened and shrunken into black mass and contained maggots (Images 9—11). The brain matter was softened, liquefied and found to be grey in color. The peritoneal cavity contained about 500 ml of orange-yellow colored paste like greasy material at places. All the intestinal organs including liver, spleen and kidneys were soft, flabby and pulpy. Small intestine, large intestine, mesentery and pancreas showed decomposition changes. The samples preserved in this case were: a portion of thigh muscle (for toxicological analysis), molar teeth (for DNA profiling). The opinion about time since death and the cause of death was kept pending, until the receipt of toxicological analysis reports.

Image 9: Thoracic cavity with maggots

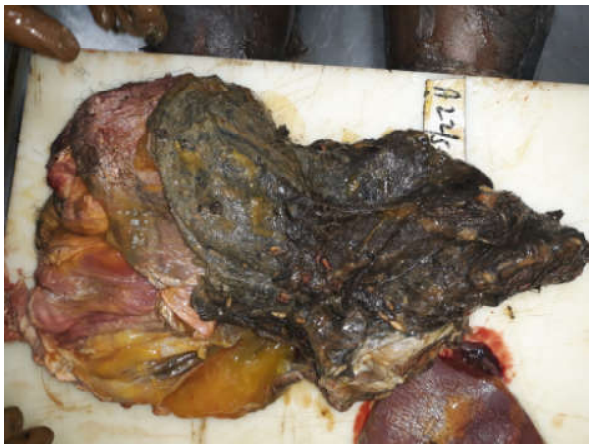

Image 10: Blackened & softened internal organs

Discussion

After death, the corpse undergoes putrefaction or decomposition, wherein the complex organic tissues get dissolved into gases, liquids and salts. Knight mentions that decomposition changes are initiated at a variable time after death and is expected to begin in an average temperate climate at about 3 days in unrefrigerated corpse [1]. Rigor mortis starts disappearing at the onset of decomposition [2]. The earliest sign of decomposition is greenish discoloration of the lower quadrants of the abdomen, visible in the first 24-36 hrs and this may appear in 12-18 hours in summer and in 1-2 days in winter [3]. The marbling of skin begins in 24 hours and is visible in 36-48 hours while slippage of skin is seen in about 60-72 hrs [4]. In the present case, there was evidence of skin peeling over the upper limbs, while there was blackening of skin in both lower limbs. In a hanging body, the postmortem lividity appears in a 'glove and stocking' pattern and the lower limbs being the most dependent portion leads to maximal pooling of peripheral blood [1-8]. This may have lead to blackening of skin only of the lower limbs, while the other portions of the body were relatively spared from this decomposition change. There was presence of gnawing activity on the toes, as the body was discovered in a forest area, where there could be presence of scavenger activity on the corpse. The environment temperature and moisture, particularly in Tropical Countries, influence the onset of putrefaction. Decomposition is hastened by obesity, heavy clothing, and sepsis, all of which keep the body warm [2]. The deceased was wearing a half sleeved shirt and a trouser and the back area was fully covered by the wearing apparel. This may be the probable reason behind the presence of lesser decomposition changes in the back muscles of the body, which remained covered from external environment due to the presence of clothes.

As is evident from the autopsy findings in this case, the decomposition changes were variable in different areas of the body. While the back muscles were completely spared from any putrefactive changes, the limbs showed blackening and peeling of skin, which can be seen in a cadaver 3—6 days after death. Also, the internal organs were mostly converted into a blackish softened pulpy material along with presence of maggots, both mature and immature, in different phases of their life cycles. There was also partial skeletonization of face and neck areas. These changes are evident usually 3—4 weeks after death in tropical climates. As per the circumstantial evidence, the person was last seen alive 42 days before his corpse was discovered by the police. The authors could not specify the postmortem interval in this case in the presence of differential decomposition changes in different areas of the cadaver. In this present case, the report of chemical analysis of the viscera for the common toxicants was negative, thereby ruling out the possibility of poisoning. The body was discovered in a hanging state from the branch of a tree, with a nylon rape present in situ encircling the neck. However, most of the soft tissues of the neck region were missing and the thyro-hyoid complex had completely disappeared due to extensive maggot activity. Thus, the authors could not comment, whether the person committed suicide by hanging himself or whether he was killed, and then postmortem suspension of the corpse was done, using the nylon rope as a ligature material. The final opinion as to cause of death and time since death were both opined as "undetermined", in the presence of such varied decomposition changes, and lack of any definite autopsy finding of specific asphyxia death.

Even after exhaustive searches about decomposition changes, the authors realized that no studies has been done in India in recent times related to estimation of PI/TSD from naked eye changes like rigor mortis and decomposition changes in the body after death. In India, the PI/TSD is mentioned almost in every postmortem report done by the Forensic Pathologist and this is given entirely on the basis of naked eye changes after death. In court proceedings, the Honorable Judges relies heavily on the Time since death given in the autopsy report to decide the culpability of an accused. This is a matter of concern, as there are no studies in Indian scenario to substantiate or refute the claims of International literature related to the

rate of decomposition changes in the cadaver. There are a few studies which have been done in the past in other countries [9-13]. According to Knight [1] and Dimaio [2] all the methods used to determine PI/TSD are unreliable and inaccurate and have a wide range of variability, making them of dubious scientific value. The circumstantial evidences are more accurate than the scientific methods as seen in most cases. Yadav et al. describes a case report, where the rate of decomposition changes observed in a cadaver were far too advanced, as compared to the probable time since death, which was obtained based on circumstantial evidence [14]. A single body may show different grades of decomposition changes, depending on posture, wearing apparel, external environment, etc. Thus a wrong interpretation may lead to a gross miscarriage of justice with an accused being exonerated or an innocent man being punished for criminality.

Conclusion

The authors describe a case with differential rates of decomposition changes in the same body, thereby questioning the prevailing theories about decomposition findings. The Investigating authorities and Judiciary should be made aware of the existing fallacies about estimation of time elapsed since death and the fact that it cannot be fixed by any method, and only an approximation can be made, that too with several considerable biological variations in individual cases. "Body Farms" should be developed in Indian settings for detailed Taphonomical studies so as to generate scientific data about the actual time taken for the process of decomposition to begin and get completed in tropical climate with seasonal variations. To conclude, in situations like these, circumstantial evidence is very important and often more precise in estimating time since death.

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Domestic Violence Ends Three Innocent Lives: A Case Series

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Abstract

Violence against women is any act of gender-based violence that results in, or is likely to result in, physical, sexual or mental harm or suffering to women, including threats of such acts, coercion or arbitrary deprivation of liberty, whether occurring in public or in private life. Domestic violence can be defined as a pattern of behavior in any relationship that is used to gain or maintain power and control over an intimate partner. Abuse can be physical, sexual, emotional, economic or psychological actions or threats of actions that influence another person. This includes any behaviors that frighten, intimidate, terrorize, manipulate, hurt, humiliate, blame, injure or wound someone. Domestic violence can happen to any couple irrespective of their race, age, sexual orientation, religion, gender, socio-economic backgrounds and education levels. The management of domestic violence essentially requires combined effort of law enforcement, social welfare and healthcare services. The current article throws light on three separate incidents of domestic violence, where the victim was forced to commit suicide, but employing a different method in each case e.g. hanging, burning & consuming poison.

Keywords: Domestic violence; Physical & emotional abuse; Socio-demographic background; Suicide; Heinous crime.

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Introduction

In a country where womanhood is praised through epics and their devotion to goddesses, it is very disturbing as well as discouraging that there are continuous acts of violence against women. The United Nations defines violence against women as "any act of gender-based violence that results in, or is likely to result in, physical, sexual or psychological harm or suffering to a woman, including threats of such acts, coercion or arbitrary deprivations of liberty, whether occurring in public or private life" [1]. The United Nation's special report on Violence Against Women identified different kinds of violence against women, such as:

(a) Physical, sexual and psychological violence occurring in the family, including battering, sexual abuse of female children in the household, dowry related violence, marital rape, female genital mutilation and other traditional practices harmful

to women, non-spousal violence and violence related to exploitation.

(b) Physical sexual and psychological violence occurring within the general community, including rape, sexual abuse, sexual harassment and intimidation at work, in educational institutions and elsewhere, trafficking in women and forced prostitution.

(c) Physical, sexual & psychological violence perpetrated or condoned by state, if it occurs [1].

Domestic violence can be defined as a pattern of behavior in any relationship that is used to gain or maintain power and control over an intimate partner [2, 3]. There are four main reasons for domestic violence to persist in India: Male dominated society, lack of awareness of Laws, laxity in implementation of the existing Acts, Bureaucracy & Fear [4,5]. Researchers suggest it is useful to think of three sources of Domestic Violence: Childhood socialization, previous experiences in couple relationships during adolescence, and levels of strain in a person's current life [6,7]. This article describes the incidence of domestic violence involving three different women, the existing law & incorporation of new act safeguarding them from domestic violence.

Case Details

Case 1

History: A 24 year old female was married in a Hindu nuclear family of urban residence from upper middle class status. It was an arranged marriage for 5 years, and she had no children. Her husband alleged that she was having an affair with another teacher, a Co-worker in the same school where she taught Bengali. One night, after one such fight with her husband, she was found at her living room by her mother-in-law, who discovered that the dead body was hanging from the roof by a rope from the ceiling, a chair was lying on the floor. She was removed from her hanging position & rushed to the nearby hospital by her relatives & neighbor, where she was declared brought dead. Her parents alleged that there were frequent episodes of physical violence against her by her husband, as he suspected her to be involved in an extramarital affair with her co-worker. Postmortem examination was done on the next day.

Autopsy Findings (Images 1 & 2)**:** Dried marks of salivary stains present over left angle of mouth & chin. External Injuries: a non-continuous ligature mark 40 cm by 1.5 cm placed obliquely, high up around neck with gap of 7 cm over right mastoid region. Upper margin of mark present 5 cm below right angle of mandible, 8 cm below chin, 10 cm above supra-sternal notch, 8 cm below left angle of mandible, 7 cm below tip of right mastoid process. Skin of ligature mark brownish, parchmentized, furrowed & abraded and on dissection, the subcutaneous tissue under ligature mark was whitish, hardened, condensed & glistening in appearance without any extravasation of blood. All injuries showed evidence of vital reactions. No other injuries except those noted were detected. All internal organs were congested. Opinion about Cause of Death was given as 'Death was due to asphyxia due to ante-mortem hanging'.

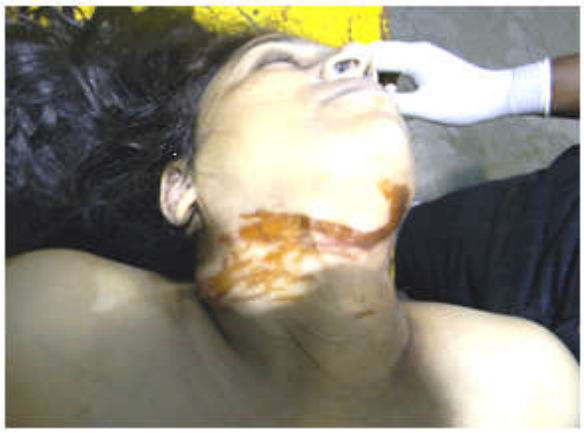

Image 1: Ligature Mark

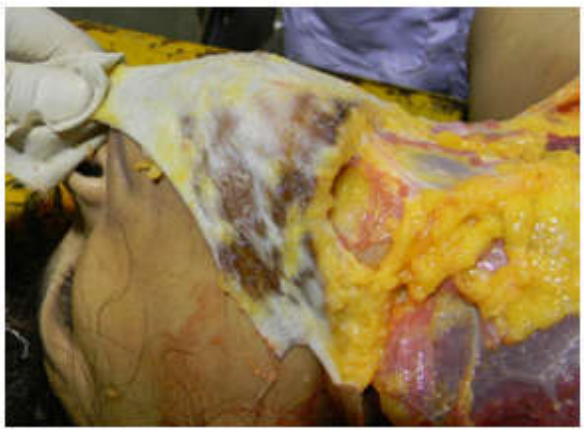

Image 2: Ligature mark after dissection

Case 2

History: A 22 year old illiterate female was married in a Muslim joint family of rural residence from lower middle class status. It was an Arranged marriage with a man who had smoking and drinking habits. They were married for 4 years, with 2 girl children. She was regularly tortured both physically & mentally by her husband & in-

laws demanding more dowry from her and also abusing her, as she did not give birth to a male child. After one such altercation with her husband, she allegedly poured kerosene oil & set herself on fire. She sustained severe burn injuries & was hospitalized for treatment. However, she expired on the next day and postmortem examination was done.

Autopsy Findings (Images 3—5)**:** Dead body of a moderately built female. Rigor mortis present all over, along with burning & singeing of hair. External Injuries—Dermo-epidermal burn injuries of varying depth present over entire face and neck all around, whole of both upper limbs, anterior & posterior chest and abdominal wall all around, whole of both lower limbs up to ankle joint, with no injury over both feet dorsum & sole. All injuries showed evidence of vital reaction. The margins & bases of injuries were congested. On internal dissection, all organs were congested. Histopathological examination of congested kidney and lung was done. Lung revealed interstitial oedema, while Kidney showed features of Acute Tubular Necrosis (Images 6 & 7). Opinion about Cause of Death was given as 'Death was due to the effects shock as a result of ante-mortem thermal flame burn injuries involving about 98% of total body surface area'.

Image 3: Deceased with External Burn Injuries

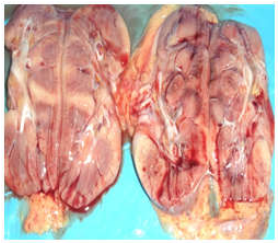

Image 4: Congested Kidney

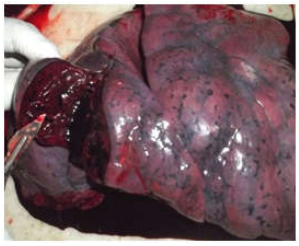

Image 5: Congested Lung

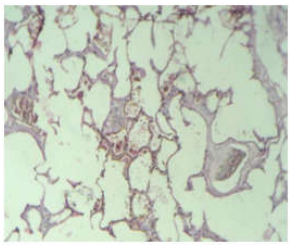

Image 6: HPE of Lung: Insterstitial Oedema

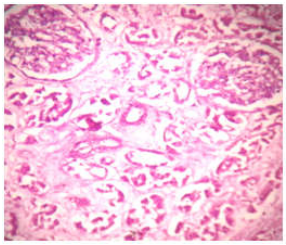

Image 7: HPE of Kidney: Acute Tubular Necrosis

Case 3

History: An 18 year old female had a love marriage into a Hindu Joint family of rural residence, belonging to Middle Class status, in March, 2018. She had completed her Higher Secondary examination and her husband owned a stationery shop. She had regular quarrels with her in-laws, who demanded dowry from her parents. In August, 2018, she had a major quarrel with her mother-in-law and allegedly consumed an unknown poison and fell ill; was rushed to the nearest hospital, where she was declared brought dead. Her parents gave history of her being tortured mentally by in-laws over dowry matters, & by her husband over petty matters but there was no history of any physical abuse by husband. Postmortem examination was done the next day.

Autopsy Findings (Image 8)**:** Dead body of a moderately built female. No external or internal injury could be detected. On dissection, Stomach contained 100 ml yellow mucoid fluid with unpleasant pungent smell. The mucous membrane was grossly congested and there were sub-mucosal hemorrhage at several places. Viscera were preserved and sent to FSL for chemical analysis. Opinion about Cause of Death was given as Death was due to the effects of poisoning, however the nature of poison is to be given after receipt of FSL report.

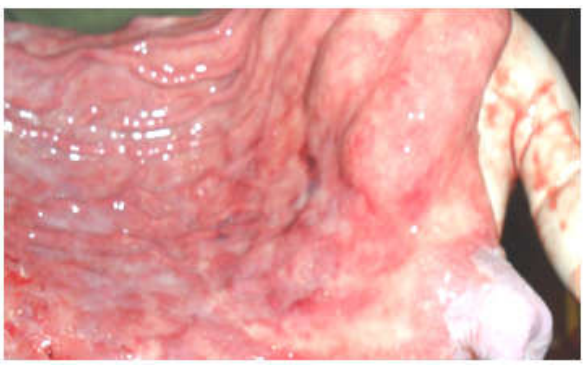

Image 8: Stomach with Submocosal Haemorrhage

Discussion

In the present article, the authors discuss about three separate incidents of suicide by married women in different socio-demo graphic backgrounds. They differed in their age, education, marital status, residence, the type of family, socio-economic status, husband's profession & habits. But they had one thing in common–all were subjected to domestic violence at their in-law's place regularly, which forced them to commit suicide. In the first case, the husband suspected her of having an extramarital affair and subjected her to emotional violence regularly. In the second case, the female was subjected to physical, emotional and economic torture by her husband and his family members, who demanded for more dowry and also wanted her to give birth to a male child. The female in the third case was tortured physically and emotionally on the grounds of trivial household matters and also for dowry demands.

There are many different theories as to the causes of domestic violence. These include psychological theories that consider personality traits and mental characteristics of the perpetrator, as well as social theories which consider external factors in the perpetrator's environment, such as family structure, stress, social learning [8].

As with many phenomena regarding human experience, no single approach appears to cover all cases and there can be three main sources of Domestic Violence [9]:

1. Childhood socialization
2. Previous experiences in couple relationships during adolescence, and
3. Levels of strain in a person's current life. People who observe their parents abusing each other, or who were themselves abused may incorporate abuse into their behaviour within relationships that they establish as adults.

Domestic violence can occur due to the following causes [10]:

1. *Psychological:* personality traits and mental characteristics of the offender.
2. *Jealousy:* when one partner is either suspected of being unfaithful.
3. *Social Stress:* Stress may be increased when a person is living in a family situation, with increased pressures due to inadequate finances or others which may further increase tensions.
4. *Mental Illness:* Many psychiatric disorders are risk factors for domestic violence, e.g. personality disorders: all Cluster BPDs, (esp antisocial), paranoid and passive-aggressive.

Domestic Violence may be of the following types[11]:

1. *Physical Abuse:* contact intended to cause feelings of intimidation, pain, injury, or other bodily harm which includes hitting, slapping, punching, choking, pushing, burning, etc.
2. *Sexual Abuse and Marital Rape:* any situation in which force or threat is used to obtain participation in unwanted sexual activity, which is an act of aggression and violence.
3. *Emotional Abuse:* humiliating the victim privately or publicly, controlling what the victim can and cannot do, deliberately doing something to make the victim feel diminished or embarrassed, isolating the victim from friends and family, implicitly blackmailing the victim by harming others when the victim expresses independence or happiness.
4. *Economic Abuse:* when one intimate partner has control over the other's economic access.

All these violence may have the following effects [12]:

1. *Physical:* Bruises, broken bones, head injuries, lacerations, and internal bleeding that require medical attention and hospitalization.
2. *Psychological:* Depression is also common, as victims are made to feel guilty for 'provoking' the abuse and are frequently subjected to intense criticism. The most commonly referenced psychological effect is Post-Traumatic Stress Disorder (PTSD).
3. *Financial:* Due to economic abuse and isolation, the victim usually has very little money of their own and few people on whom they can rely when seeking help.

The response to domestic violence is typically a combined effort between law enforcement, social services, and health care and these can be [13]:

1. *Medical Response:* Many cases of spousal abuse are handled solely by physicians and do not involve the police.
2. *Counseling for Person Affected:* counselors

and therapists should assess every client for domestic violence with each individual privately during the initial interview, in order to increase the victim's sense of safety in disclosing domestic violence in the relationship.

3. *Counseling for Offenders:* to minimize the offender's risk of future domestic violence and should emphasize minimizing risk to the victim, and should be modified depending on the offender's history, risk of reoffending, and criminological needs.
4. *Law Enforcement:* The Protection of Women from Domestic Violence Act 2005 (PWDVA) was enacted in India from October 26, 2006. [14] This Act has 5 chapters & 37 sections, out of which a few relevant section are being discussed here:

Section 3: Definition of domestic violence: For the purposes of this Act, any act, omission or commission or conduct of the respondent shall constitute domestic violence in case it:

a) harms or injures or endangers the health, safety, life, limb or well-being, whether mental or physical, of the aggrieved person or tends to do so and includes causing physical abuse, sexual abuse, verbal and emotional abuse and economic abuse; or

b) harasses, harms, injures or endangers the aggrieved person with a view to coerce her or any other person related to her to meet any unlawful demand for any dowry or other property or valuable security; or

c) has the effect of threatening the aggrieved person or any person related to her by any conduct mentioned in clause (a) or clause (b); or

d) Otherwise injures or causes harm, whether physical or mental, to the aggrieved person.

For the purpose of determining whether any act, omission, commission or conduct of the respondent constitutes 'domestic violence' under this section, the overall facts and circumstances of the case shall be taken into consideration.

Section 4: creates a social responsibility on members of the community at large who have knowledge of an impending or already committed act of domestic violence, to come forward to file complaint on behalf of the victim.

Section 5: social enactment that creates various legal, social, judicial, and administrative mechanisms to provide assistance to victims of domestic violence.

Section 6: clarifies that Shelter Homes are bound to provide shelter.

Section 7: person in charge of a medical facility shall provide medical aid to the aggrieved,

Section 8: Protection Officers should be women and should be appointed as full-time positions.

Section 9: defines the duties and functions of the Protection Officers.

Section 10: lays down the duties of Service Providers.

Section 11: lays down the various duties of the government to give the Act wide publicity through the media, to conduct periodic sensitization and awareness training of the state/central/police/ judicial officers, to coordinate different ministries/ departments, periodical reviews and to ensure that protocols for the various ministries concerned including courts are prepared and put in place.

Sections 12, 13, 14, 15 & 16: some provisions & procedures for obtaining orders or reliefs.

Section 17: every woman in a domestic relationship has the right to reside in the shared household whether or not she has any right, title or beneficial interest in it. The aggrieved person shall not be evicted or excluded from the shared household or parts save in accordance with the procedures established by law.

The purpose of PWDVA is to provide remedy under the civil law which is intended to protect the women from being victims of domestic violence & to prevent the occurrence of domestic violence in society. It is armed in providing support to woman facing domestic violence. Legal remedies pertain to civil relief such as injunction, compensation and monetary relief. There can be no arrest made on a complaint filed under this law [15].

Conclusion

Every couple possesses a unique set of problems & also need a different solution. There can be several factors for domestic violence. In spite of efforts made by various sections of society and the Government to curb the menace of domestic violence against women, there is a rise in domestic violence. If applied correctly the Protection of Women against Domestic Violence Act 2005 (PWDVA) is a powerful act. But, it will remain less fruitful if there is lack of awareness amongst the people of society, which could be brought about by educating every woman about their rights,

removing fear and coming out of the traditional set up of tolerating violence, counseling all family members along with the victim when the case is reported, creating awareness of social responsibility on members of society to report domestic violence, awareness produced through media sources, sensitization of the police to these issues and the power given to them, lawyers should connect criminal, community screening for domestic violence, providing adequate assistance to the victim by offering safe shelters, crisis intervention, advocacy, education & prevention programs and by provision of strict laws and punishment for offenders. Thus, in conclusion, it is essential that every strata of society must contribute to curb this heinous crime against women.

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Case Report

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Accidental Drowning of Child or Maternal Filicide by Strangulation: A Case Report

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Abstract

Introduction: Filicide, defined as the deliberate act of a parent killing their own child, is not uncommon in India and usually occurs if the child is female. Females are killed off even before they are born, as they are considered to be a burden to the parents and family. The risk of a child being killed is highest during the first year of life. The child may be killed by the perpetrator by using knives, blunt objects, manual strangulation, poisoning and drowning.

Case Details: A body of a seven months female was brought for autopsy with the alleged history of accidental fall in a bucket of water and subsequent drowning. On external examination multiple abraded dermal contusions were found on the neck along with one crescentic shaped abrasion. On dissection hematoma was present over the underlying muscles. Pulmonary findings were within normal limits. The mother declared that she was alone in the house with the child at the time of alleged drowning. Later after exhaustive police investigation, she confessed to murdering her daughter. She clarified that she was suffering from depression, marital discord and was apprehensive that the female child was only a financial burden to their family.

Conclusion: The article discusses about the reasons that prompt a mother to kill her own child and if a female child is that much a burden to the family that she does not deserve even an iota of chance to live. Further research is needed to improve identification of children and mothers at risk. Suggestions for prevention are made based on current literature and the authors' experience.

Keywords: Filicide; Depression; Manual Strangulation; Drowning.

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Introduction

Infanticide means murder of a child under the age of one year by his or her parent or parents. Filicide refers to murdering a child till the age of 18 years by his or her parents or guardians and stepparents. Mothers are more often found guilty of committing filicide than fathers. Often the perpetrator is suffering from some form of psychiatric dysfunction and depression. In new mothers postpartum or lactation depression could be a reason for such extreme step. The other reasons to end life of child could be disability, sex of the child, lack of finances, illegitimacy etc. Filicide occurs in all part of the world with variation in motives. More Cases of Filicide are reported in Western Countries as compared to India. The statistical data and motives behind such acts vary from country to country; like in South East Asian countries female child is often

victimized as they are considered financial burden [1-5]. This case report illustrates a case of maternal filicide where the female infant was killed by the mother having chronic depression.

Case History

Mother of the deceased infant alleged that while she was asleep the baby woke up, climbed down the bed, crawled to the attached washroom and accidentally fell into the bucket filled with water (Scene of incidence as shown in Fig 1 & 2). Infant was taken to hospital where she was declared brought dead. Dead body of the infant was brought to the Department of Forensic Medicine & Toxicology for postmortem. During postmortem examination, it was observed that Crown—heel length of the body was 65 cm, head circumference was 41 cm, chest circumference 41 cm and neck circumference 26 cm. There was a dermal abraded contusion of length 11 cm and breadth 8 cm over the antero-lateral aspect of neck, 4 cm below mentum and 3 cm above suprasternal notch along midline. Left lateral end of this injury was 3 cm in breadth, located 3 cm below left angle of jaw and 3 cm below left pinna. Right lateral end of the injury was 3 cm in breadth, located 3 cm below right angle of jaw and 3.5 cm below right pinna. The other injury comprised of a crescentic shaped abrasion

Fig. 1: Scene of Incidence

Fig. 2: Scene of Incidence

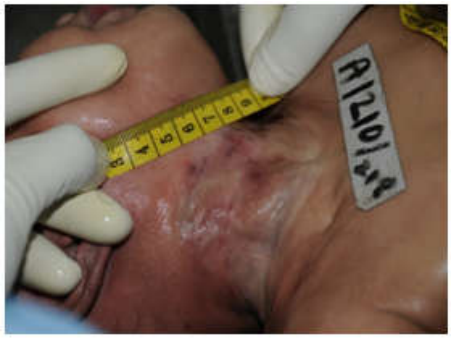

Fig. 3 & 4: External neck injury

Fig. 5: External neck injury

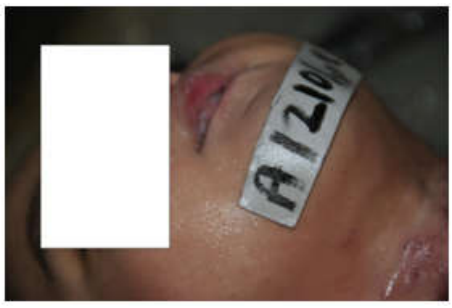

Fig. 6: Crescentic abrasion below chin

measuring 0.6 cm by 0.1 cm placed 3 cm below chin and 1.5 cm lateral from midline on right side of neck having downwards convexity (Figs. 3,4,5 & 6). On dissection of neck, a hematoma of size 1 cm by 0.5 cm was detected over left sternocleidomastoid muscle. Streak of hemorrhage was present within the fibers of sternothyroid and sternohyoid muscles.

On dissection of scalp, sub-scalp hematoma was present over right parieto-occipital region over right sub-occipitalis muscle, 2 cm lateral from midline. Skull did not show any fractures. Brain was congested with multiple petechial hemorrhages over the parenchyma. Both right and left lungs were congested and weighed 117 gm and 104 gm respectively. Heart was unremarkable and weighed 40 gm. Stomach was empty with congested mucosa. Liver, spleen and both kidneys were congested. No congenital anomalies were detected. On thorough postmortem examination no sign of drowning could be appreciated and the cause of death was opined as 'Asphyxia due to Manual Strangulation'. During further investigation by the police, biological mother confessed that she strangled the baby with a dupatta and later on framed the story of baby being drowned into a bucket filled with water. Mother was a patient of chronic depression, and believed that the baby had brought bad omen to the family.

Discussion

Resnick studied 131 cases of filicide and categorized motive of filicide into the following five: [6]

1. *Altruistic*—the parent kills the child as they perceive the world to be cruel or to safeguard them from disease and disability.
2. *Acutely psychotic*—the parent suffering from psychosis or in automatism due to seizure or in post-ictal state kills the child without any rational motive.
3. *Unwanted child*—an unwanted child considered as hindrance by the parent is killed and in rare cases to get insurance or to marry another person.
4. *Accidental*—the parent accidentally kills the child during physical assault, like in Munchausen syndrome.
5. *Spouse revenge*—to take revenge from a separated or unfaithful partner a parent may kill the child.

As per Resnick's study, the most common motive was altruism, accounting to 49% of all the cases reviewed and the least common motive was spousal revenge accounting to 2%. He also concluded that ratio of mother: father perpetrators were nearly 2:1 [6]. This comprehensive classification system can be applied to both female and male perpetrators. Pitt and Bale in their study conducted in 1995 identified females as the main perpetrator of filicide [7]. However, the study conducted by Jason et al. in 1983 showed that the fathers were most common perpetrator and male children were more victimized than female [8]. In another study conducted by Freidman et al. in 2005, 65% of the perpetrators were fathers and remaining were mothers; the median age of victim was 2.2 years and altruism was the commonest motive in association with depression among parents [9]. Most of the maternal filicides occur due to maternal psychiatric illness than due to maltreatment of the child. Mothers may kill a sobbing child who won't stop crying. A mother with battered childhood is likely to batter her own children as well [10,11]. A study conducted by Jennings et al. in 1999 compared depressed mothers with non-depressed mothers of children less than 3 years of age and concluded that 41% of depressed mothers had thought of harming their children as compared to 7% of non-depressed mothers [12]. Another study conducted by Levitzky S et al. on 23 mothers having colicky infants found that 70% of the mothers experienced aggression while 26% had ideation of infanticide; among these 23 mothers, 90% had marital tension and social disruption [13].

The studies mentioned above depict the international scenario of the filicide, but in India and other South Eastern countries, the categorization of filicide has an additional feature of being sex selective killing, often victimizing girl child. A prospective study conducted by Chandra PS et al. depicts Indian statistics of maternal aggression toward infants among hospital admitted cases of women with postpartum mental illness. 43% and 36% of women had infanticidal ideation and infanticidal behavior receptively, while 34% had both infanticidal ideation and behavior. Infanticidal ideas were associated with depression and psychosis whereas infanticidal behavior had another additional factor i.e. having a female infant [14]. A case of maternal filicide was reported by Sahu et al. in 2014 where a mother with depressive disorder, abusive marriage, alcoholic husband, belonging to low socio-economic status killed her daughter to get rid of the financial burden [15]. Another case report of suicide-filicide has been reported by Behara et al. where the mother hanged herself after hanging her children [16]. A case study of four maternal filicide cases conducted by Gowda et al. in 2018 provides

description of maternal filicide in India. The mean age of mothers in this study was 32.7 ± 4.1 years. All the risks factors of maternal filicide were found associated in these cases i.e. depression, low socio-economic status, nuclear families, unemployment and limited education [17].

In the present case report, the authors describe a case of maternal filicide, wherein a female child aged seven months was strangled by her mother, who was suffering from chronic depression. The mother belonged to a low socio economic status with limited education and history of chronic depression. She tried to present the death of her child as accidental, but postmortem examination revealed a completely different scenario. The presence of external injuries in the form of abraded contusion over neck and crescentic nail marks pointed towards application of manual pressure over the child's neck and corresponding internal hematoma further refuted her version of the incident. The features of asphyxia in both drowning and strangulation overlap each with presence of cyanosis over lips, nail beds, mucus membranes and a generalized congestion of organs. In infants the neck folds are often mistaken as ligature marks and an autopsy surgeon needs to very carefully differentiate such neck folds from pseudo strangulation. Since the skin of infants is very sensitive to injuries, a contused abrasion with dimension of 11 cm by 8 cm over the neck raised suspicion about the manner of death. Moreover the crescentic nail marks over the neck are typical features of manual strangulation. On internal examination, presence of neck hematoma, sub-scalp hematoma and also the absence of any specific findings of drowning e.g. oedematous lungs, presence of froth in nostrils etc. further confirmed the cause as well as manner of death. Considering the post mortem finding and circumstantial evidence like inquest papers and crime scene photographs, the final cause of death was opined as 'Asphyxia due to manual strangulation'. Final opinion of the postmortem report helped the investigating authorities to investigate about the actual sequence of events. During the investigations, it was brought into light that the mother was a known case of chronic depression and since birth of the infant considered her to have brought ill fate to the family. In many Western countries there is Infanticide Act which provides partial defense to women killing their infants, considering the disturbed mind by reason of not being fully recovered from the child birth or due to the effect of lactation. But in Indian context, there is no specific Infanticide Act and such offence is charged and punishable for murder.

Conclusion

Instances of maternal filicide in India are far less as compared to Western countries, but such cases are not as rare as presumed to be. Most of the mothers killing their children have some form of depressive disorder in association with low socio-economic status, abusive husband, marital discord, financial strains and low education. Mothers with suicidal ideation often kill their children with an altruistic motive. This case report also points out that depression among mothers' especially new mothers is a risk factor for commission of filicide. Enquiring about the proper history of the case, circumstantial evidences, meticulous autopsy as well as knowledge and skill of the autopsy surgeon can collectively help to determine the cause, manner and purpose behind death of filicide victims; and in turn also guides the investigating agencies to solve such mysterious deaths.

Conflict of interest: None declared.

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[9] National Statistics Online—Trends in suicide by method in England and Wales, 1979-2001. www. statistics.gov.uk/downloads/theme_health/HSQ 20.pdf (accessed Jan 24, 2005): 7-18. Only verified references against the original documents should be cited. Authors are responsible for the accuracy and completeness of their references and for correct text citation. The number of reference should be kept limited to 20 in case of major communications and 10 for short communications.

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