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Journal of Forensic Chemistry and Toxicology

January – June 2020, Volume 6 Number 1

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Original Article

Journal of Forensic Chemistry and Toxicology
Volume 6 Number 1, January–June 2020
DOI: http://dx.doi.org/10.21088/jfct.2454.9363.6120.1

Coping Strategies, After Death Misconceptions and Paranormal Phenomenon amongst Police Officers of Ernakulam, Kerala handling Deceased: A cross sectional study

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Abstract

Police officers help to maintain peace and security in any nation and checks whether people abide by law of the country and instrumental in bringing justice all over the world. But they go through thick and thin during their career. The aim of the present study is to find out the coping strategy, misconceptions of after-death and paranormal phenomena amongst police officers who handle dead bodies. Participants consisted of Police officers within the age group of 25–60 years. Total of 32 samples were collected from different police stations across Ernakulam District in state of Kerala. Method used was Interview and the data was qualitatively analyzed. The study explored that certain police officers had belief in paranormal phenomenon and misconceptions about after death, other reported that belief in rebirth was based on their religious background. Coping strategies varied in each police officer.

Keywords: Police officers; Paranormal phenomenon; Coping strategies; Misconceptions; After death.

How to cite this article:
Anu Achamma Varghese, Farhana Mutharintavida, Fathima Ubaida et al. Coping strategies, After Death Misconceptions and Paranormal Phenomenon amongst Police Officersof Ernakulam, keralahandling Deceased: A cross sectional study. J Forensic Chemistry Toxicol 2020;6(1):5–10

Introduction

Police officers help in bringing peace and security to the state by preventing and detecting crimes, ensuring law enforcement, protecting and assisting the public and maintaining the public order[1,2]. Unlike other professions police does not work on a predetermined work schedule rather is more focused on emergencies and crisis that occur each day[3]. In a single day they will have to work in totally different contexts, ie, the pattern of their work differs with the daily cases they encounter. Behind the ironed crisp uniforms and fighting the bad guys, which is usually portrayed in the movies and other media, these officers deal with a lot of stressful and emotionally draining situations. One such is handling body remains and death scenes. These tasks are often laden with emotional significance and are usually followed with unpleasant sights, smell, sound, and touch sensations that stick along with them for hours or even days. The gruesome accidents and deaths leave anundeniable impression on the officers. These experiences can make the officers dealing periodically with it to adapt new coping strategies. It is important that the officer's mental state is fit and healthy as much as the physical body. But most of the time the training and techniques are given to enhance physical health rather than the mental health. Along the years there has been a considerable amount of change in bringing attention to the mental health of people working in these areas.

Death scenes and handling dead bodies come with a lot of physical and emotional stress. Officers

who work with these often experience the brutal reality of death and loss of the closed ones during the initial phase of their career, but later form an emotional resistance. They get to see and work on dead bodies of different forms. There may be a body of a person died just few hours ago sleeping on the bed and sometimes it is of a person buried in soil and decayed for a long time. There might be even a body that is scattered all around the place from a train accident or similar dismembered one which involves collecting all the pieces and careful assembling. It is crucial they are utmost careful and concentrated in the work they handle as any tiny error could destroy evidences. This also means they cannot let any trace of emotional weakness. Depending on the death scene and the details of the body, the officers can have different traumas or difficulties in working with them. It also depends on the mental capacity of officers handling them. The environment around with which they work also play a significant role in determining how much affected they will. An officer working in a stressful police station or coming from a distorted family can find it more depressing than officers from a pleasant work place or family. There are many factors that affect their mental health. Their experience associated with death and after death will be unique when compared to lay person. Different culture and religions offer to pay various contributions after death. One of the most common beliefs is that after a death the physical body remains in the earth while a soul from the body is taken to the heaven or hell depending on the deeds of the person in his or her life time.

After-death is a subject that remains mystery for us as one can never be sure about the events that follows the death of a person. There has not been a clear indication of the after-death events except the knowledge of the decaying of the physical body. Many people like atheists don't believe in the existence of a soul or the concept of heaven and hell. Since the after-death involves so much mystery there are many speculations regarding it. One such is the presence of ghosts. In folklore, the ghost is described as the soul or spirit of a dead person appearing alive. Its description varies from an invisible presence to translucent to lifelike forms. Although the experiences and stories of ghosts date to ancient times, the overwhelming consensus of science is that ghosts do not exist. Despite centuries of investigation there has been no evidence regarding presence of ghosts or particularly haunted locations or people. People engage in different practices like Ouija board where it is believed that one could talk to spirit of a deceased person. Souls are called as burdened souls because most of the spirits wandering are believed to have died suffering some tragedy or injustice and is wandering to fulfill their revenge or gain justice so that they can finally rest in peace. Since police officers deal with crimes and such deaths, it is rumored to have the police officers suffer from such after-death misconceptions. Medias and movies can be seen often portraying constables or officers that have to work with dead bodies and guard them for a long time appearing as frightened or paranoid. It also shows about them experiencing paranormal phenomena. Paranormal events are purported phenomena described in popular culture, folk and other nonscientific bodies of knowledge, whose existence within these contexts is described as beyond normal experience or scientific explanation. Many groups and organizations, popularly in western countries, have established to record paranormal activities in haunted house. They have a heavy unit of microphones, cameras and other recording and taping devices installed to monitor such events.

Kerala Police officers undergo basic training at Kerala Police Academy, which includes crime scene analysis and basic forensic studies and lot more. Earlier studies have showed that police officers who were involved in handling dead bodies didn't show significant psychological distress or psychiatric morbidity.[4] Also, reported that low level of Post-Traumatic Stress Disorder (PTSD) was due to police officers resilience and managerial and organizational factors.[5]

Police officers had traumatic experiences early in their careers and remained in their memories as visual, tactile and olfactory sensations. The same study reported that talking about the event with their colleagues helped them to overcome the stress.[6] North C.S et al. (2005), suggested that firefighters had low rates of PTSD related to handling the case of Oklahoma city bombing.[7] It was reported that PTSD can be due to reduced job satisfaction, functional impairment or work load.[8] Whereas, another study suggested that Police officers, those encountered death related stressors had higher levels of PTSD.[9] Studies showed that when an individual is exposed to paranormal activities, the tendency of that individual to believe in paranormal phenomenon increases.[10] Another study found that there was no significant gender difference in PTSD symptoms among police officers.[11] Study by Sharps M.J et al. (2010) suggested that paranormal beliefs were due to depression, dissociation and Attention Deficit Hyperactivity Disorder (ADHD)

among eyewitnesses.[12] Study among Scottish police officers investigated the coping strategies. It found that in addition to healthy methods like exercise, they increase use of alcohol, smoking and eating to relieve stress.[13] Another study suggested that among police officers the resilience built in training program improved their family relationships, enhanced work performance and more effective communication.[14]

Need and Significance

Mental health is crucial for all professionals and particularly police officers attending deceased and handling dead bodies should be taken care beyond the physical fitness. Their mental health should be addressed as important as any aspect of the case. Research has indicated a high stress level for officers attending such cases. This is a study to investigate the stress and coping strategies of officers dealing with cadavers and dead bodies in Kerala and to see if they have certain misconceptions regarding after death and paranormal events. This study helps in educating and exploring areas that help in enhancing their mental health and to create awareness among people of the real hardships and misconceptions they face.

Objective

The aim of the study was to investigate the coping strategies, paranormal phenomenon and misconceptions about after-death among police officers who handle dead bodies.

Methodology

The subjects of the study were police officers from[9] different police stations in Ernakulam District. The police stations were selected randomly. The sample size was 32, which consisted of Sub Inspector (SI)- 9, Assistant Sub Inspector(ASI)-8, Senior Civil Police Officer (SCPO)-11 and Civil Police Officer (CPO)-4. Interview method was used with a self-prepared questionnaire. It was an open-end interview and verbatim recording was done. The purpose was to investigate the mental health of police officers who were involved in handling dead bodies.

The permission for data collection was taken from the Sub Inspector of Police from respective police stations. Thereafter, the interview was conducted with each police officer who waspart of handling the dead bodies. After the data collection, the samples were classified into 3 parts based on their designation, years of experience and number of dead bodies handled. They were then subdivided into other groups.

Result and Discussion

The study was done among the Kerala Police officers in Ernakulam District. In the interview method, they were asked whether they accept what was depicted in movies (such as stress, fear while handling dead bodies), witnessed any sort of shadows or apparition, belief in soul wandering and rebirth, and whether they had stress, discomfort or fear after handling dead bodies. These participants were divided into three different parameters and categories as below.

(A) PART- 1

When the participants were grouped on the basis of designations such as SI, ASI, SCPO and CPO; it was found that among these groups, stress/ discomfort was least experienced by CPO(25%) followed by ASI (63%), SI (78%) and SCPO (82%). Stress is higher in all the 3 groups which can be due to direct contact with the dead bodies. It was reported by all the 4 groups that they had never witnessed a shadow. In the case of Belief in soul wandering, ASIs have no such belief, whereas, SCPO (9%), SI(11%) and CPO (25%) had belief. The CPOs have this belief may be due to fact of having full responsibility of the dead body and are supposed to guard the dead body until it is released to the family.

When asked about their belief in Rebirth, ASI had the least belief in rebirth with 13%, followed by SI (22%), CPO(25%) and SCPO (27%). It is seen that only 9% of the entire population has belief in rebirth. This might be due to their bringing up, religious background rather than their work environment.

Fear was found only among SIs (33%), this might be because they're doing the inquest. It was not found among all the other 3 groups (ASI, SCPO and CPO).

Among SIs, the coping strategies used were; Engaged in other activities (44%), Not Applicable (22%), Shares experience with Others (11%) and Alcohol consumption (11%). In the case of ASIs, Not applicable (38%), Engage in other activities (25%), Part of duty (25%) and shares experience with others (13%). Whereas among SCPOs, Shares experience with others (25%), Engage in other activities(25%), Part of duty (25%), Smoking (8%) Prayer (8%) and N/A (8%). CPOs; Not applicable (50%), Prayer (25%) and Alcohol (25%).

(B) PART- 2

In the second grouping, the participants were grouped into 6 categories based on years of experience-I (5 to 9 years), II (10 to 14 years), III (15 to 19 years), IV (20 to 24 years), V (25 to 29 years) and VI (30 to 34 years). Stress was found to be highest among group I (100%) and least among group VI (50%). Reason might be, as experience increases, the ability to cope up with the stress also increases.

It is clear from all the groups that, none had witnessed shadow after handling cadavers. (0%)

Belief in soul wandering was found as 0% in group I, group III and group VI. Whereas it was 33%, 8% and 14% in group II, IV and V respectively. Lower groups highly accustomed, whereas higher groups after handling more dead bodies had feeling of haunting. In certain groups, such belief system was not seen.

In the case of belief in rebirth, least was found among group III(0%) and highest in group VI (50%). This might be, as age increases, spirituality increases. The same finding can be seen in part 3 grouping of belief in rebirth.

Fear, was found lowest among groups-II, III and IV. It was highest in group VI (50%) and 14% and 25% in group V and I respectively. Strategies among group I was found as; Engage in other activities (50%), Prayer (25%) and N/A (25%). In group II, Engaged in other activities (33%), Alcohol (33%) and N/A (33%). Among group III, Shares experience with others (25%), Engage in other activities (25%), Part of duty (25%) and N/A (25%). Group IV, Shares experience with others (38%), N/A(30%), Part of duty (15%), Smoking (8%) and Prayer (8%). In group V, Engage in other activities (38%), part of duty (38%), shares experience with others (13%), N/A (13%).

(C) PART- 3

The participants were categorized into 3 groups on the basis of number of dead bodies handled. Group A- 99 and below. Group B- Between 100 and 999. Group C- 1000 and above.

Stress, was found to be lesser in Group C (67%) followed by Group A(71%) and Group B (88%). Here, it is evident that all the 3 groups experience stress and there is only a small variation between the groups. Witnessing shadows, as earlier, is not found among all the 3 categories. Belief in soul-wandering, is found only in group A with 10%, experience is lower compared to other groups. The reason for no belief in soul wandering among Group B and C, can be as they have handled more dead bodies, they must have been desensitized. Whereas belief in Rebirth, the highest percentage is for group C(33%), then group B(25%) and A (19%). As mentioned earlier, the ones who had handled more dead bodies, might have more years of experience, thereby more belief in Rebirth.

Fear is found to be highest in group B (13%) followed by group A (10%) and C (0%). Group A and B have comparably equal percentage of fear. Handling the most number of dead bodies must have helped the group C members to overcome fear.

Strategies in group A; N/A (27%), Engage in other activities (23%), Shares experience with others, (18%), Part of duty (14%), Alcohol and smoking (9%) and Prayer (9%).

Among group B, Engage in other activities (38%), N/A (25%), Part of duty (25%) and alcohol (13%). In group C, Share experience with others (33%), Part of duty (33%) and N/A (33%)

Certain participants(28%), when asked about rebirth, did mention that they had belief in rebirth which was based on their religious beliefs. In the rational thought, some had told they had no belief in rebirth, but while sharing their experiences it was evident that their belief system has influence on them. It was found that, the belief on rebirth increases with age as spirituality increases. This indirectly indicates that their coping strategy was prayer. While 31% of the population did mention about the discomfort occur due to smell from the decayed dead bodies.

The police officers are obliged to work 24/7, which included law and order maintenance and crime investigation and are supposed to work in different atmospheres. Thereby, it makes their work more hectic. Though it was not asked directly, 28% mentioned about their work stress. They might have to handle dead bodies with AIDS, which increases the probability of having stress.

When all the strategies of the population was considered, it was seen that, Shares experience with others (15%), Engaged in other duties (26%), Part of duty (18%), Alcohol or smoking (8%), Prayer (5%) and N/A (26%). The participants who reported that they have no particular strategy might not be aware of the method they use to overcome or might not be able to verbalize it. Engaging in other activities has helped the police officers to enhance their mental wellbeing. As alcohol consumption during the duty time is against the law and order, such strategy was not mentioned by most of them.

Majority of the sample was emotionally affected and had memory impact after handling dead bodies especially, Children's cases (53%) and Run-over bodies (19%). Others did mention about decayed bodies and accident cases. Most number of cases reported by the police officers occurred during the initial stages of their career.

During the open end discussion, they shared several experiences, which included;

One case was reported as a young adult was found in a lodge room, at Ernakulam, when the police went for inspection, and couldn't find the body at first. They got broken beer bottle and poison from the scene. Later, they saw a decayed body lying under the bed. After the investigation, they found that the guy, attempted suicide with the beer bottle (cut throat injury) and had consumed poison.

Another case was such that, the police officers found an elderly lady dead in a well. During inquest they found evidence for a strangulation death. But Postmortem reported that it was a completely drowning incident. The police officer was quite stressed as he couldn't find the actual reason behind the incident.

Yet another case which dates back 20 years, 2 young females along with their parents reported to the police station that both were in love with each other and wanted to marry. The parents requested the police to help them in separating them. As the police officers had no prior experience in handling such cases, they did not take it seriously which emotionally affected the girls. Both of them stated that they will commit suicide if they pressurize them to get separated. Next day they were found dead in a pit nearby.

There were instances where the police officers were helpless and felt guilty. This shows how stressful their work is.Though, it is shown in movies that police officers has stress while handling dead bodies, it is true in the initial days. The fear they experience during the initial stages would have been overcome by repeated exposure in this field due to desensitization.

Limitations

As the data was qualitatively interpreted, there was much confusion regarding how to interpret the data even though inter-reliability was verified. Difficulty was faced during confirmatory analysis. As this is an exploratory study, further analytical comparison groups were not included.

Conclusion

The lower cadre is found to have higher rate of stress, thus effective stress management training should be given. Certain police officers reported that they use no particular strategy, and others reported they get engaged in other activities, but it is seen ineffective. Therefore, effective training must be implemented. Study proved that police officers had no paranormal phenomenon, but a small percentage had concepts of after death, which was based mainly on religious background. Theyall had different coping strategies to handle the stress/ discomfort and fear.

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Original Article

Journal of Forensic Chemistry and Toxicology
Volume 5 Number 2, July - December 2019
DOI: http://dx.doi.org/10.21088/jfct.2454.9363.5219.2

Gas Chromatography-Headspace (GC-HS) and its significance in Forensic Toxicology

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Abstract

Gas Chromatography (GC or GLC) is a commonly used analytic technique in any analytical laboratory for quality control as well as identification and quantitation of compounds in a mixture. High sensitivity, selectivity, resolution, speed, good accuracy and precision, wide dynamic concentration range, simple, economic and robust instrument design, and its ability to be interfaced with many established and emerging sampling and detection systems have made GC the instrument of choice in Forensic Science. A broad variety of organic samples can be separated and analysed as long as the compounds are sufficiently thermally stable and reasonably volatile. Gas chromatography can be used for the separation of gases, liquids and solids. Materials such as biological materials (tissues, blood, urine, etc), alcohol, fire debris, car paints, drugs, pesticides, plant material and fibres are the most common evidential materials analysed by forensic chemists/scientists and are characterized by a high degree of complexity. One of the major challenges is to ensure that the sample injected is truly the representative sample of the extract. Therefore, improvement in injection and sampling handling techniques has always been necessity. Inclusion of Headspace (HS) as a sample injection technique has improved and reduced the sampling error and sample loss during preparation. Headspace analysis is based on the principle that volatile components in avial, maintained at equilibrium, diffuse into the gas phase above the sample which is then extracted and introduced into the GC system for analysis. This ensures that the sample is the true representation of the test sample. In HS, sample injection volume can range anywhere between few µl to 1000µl. Some recent applications of GC-HS in Forensic Chemistry, including those in Forensic Toxicology, are presented in the article, which include alcohol estimation in drunken driving cases, estimation of drugs seized in bulk form, illicit drugs, estimation of pesticides in biological matrix and post-mortem volatiles in stored samples.

Keywords: Gas Chromatography Headspace; Applications; GC-HS; Forensic Toxicology etc.

How to cite this article:
Supriya Krishna, A K Jaiswal, Tisha Rosina Thomas et al, Gas Chromatography-Headspace (GC-HS) and its significance in Forensic Toxicology. J Forensic Chemistry Toxicol. 2020;6(1):11–36.

Introduction

Chromatography is a collective term for physicochemical techniques for separation of complex organic compounds in solid, liquid and gaseous state. Since it was first invented by Mikhail Tsvet in 1903, chromatography is being continuously modified and improved to meet the demands of ever expanding modern analytical chemistry[1,2]. Of the quasi-infinite possibilities in analytical chemistry, the technique finds its major applications in the field of Forensics for Alcohol analysis, Fire debris analysis, Metabolomics, Pharmaceutical industry, Polymer industry, Food industry for flavonoids, fragrances in perfumes and cosmetics[3–8].

In spite for various modifications in the instrumentation of chromatography the principle remains same. The technique is based on the principle of separation of an analyte as a consequence of partition between the mobile phase and stationary phase held on or inside a solid support[9–11]. The distribution of components between the two phases depends on the physicochemical properties of

adsorption, ionic interactions, diffusion, solubility or, in the case of affinity chromatography, specific interactions. Modifications in type of stationary phase, mobile phase and process of separation can divide chromatography into various techniques as illustrated in Fig. 1.

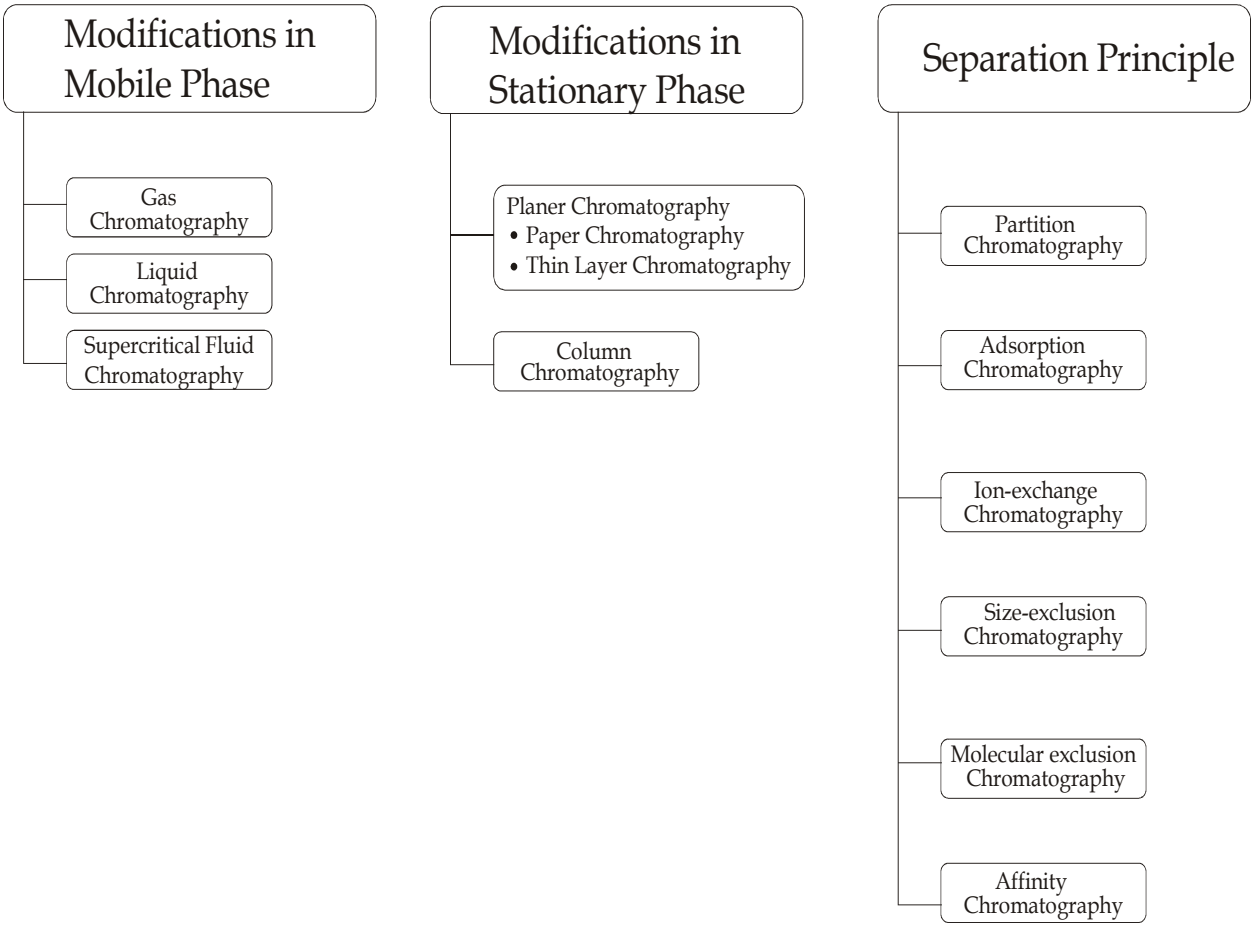

Fig. 1: Classification of chromatography according to modifications in stationary phase, mobile phase and process of separation.

Determination of volatile analytes in biological and non-biological samples is one of the most frequently done assay in a Forensic Toxicology Laboratories[12–14]. Routine analysis of volatiles has been done using titrimetric analysis, immunoassay, enzymatic reactions, chemical reactions, spectrophotometry and gas chromatography[15–19]. Due to various disadvantages in primitive techniques like lack of sensitivity, specificity, large reaction time, long sample preparation and improvement in the instrumentation of preexisting techniques. Sophisticated instruments like Gas Chromatography-Mass spectroscopy, NMR-Spectroscopy, Raman-spectroscopy, FTIR, Biosensors, Microdiffusion techniques have gained more acceptance nowadays[20–25].

Gas Chromatography is the most frequently used analytical technique for investigation of thermally stable organic volatile compounds or hydrocarbons within a matrix. GC is basically a separation technique in which the introduced volatile compounds get separated, fractionated by means of Selective Interaction (Partitioning) as a consequence of partition between a mobile gaseous phase and a stationary phase held inside a Stainless-Steel/ Quartz column. The components of a mixture possess different affinities for each phase, which causes the differential separation.

Samples in Forensic Toxicology can contain highly dense matrix with proteins, salts, fats, oils, drugs and other non-volatile material mixed with the target analyte that can remain in the GC system and result in poor analytical performance. Most samples need to be modified for the specific requirement of analysis of a particular analytical technique before injection. Therefore, laboratory analysts use extensive sample-preparation techniques to extract and concentrate the compounds of interest from this unwanted non-volatile matrix. Most of these cleanup procedures use some type of initial extraction procedure such as solvent extraction, solid-phase extraction, solid-phase micro extraction,

supercritical fluid extraction, distillation[26–28]. Such extraction and concentration techniques can become time consuming and costly depending on extend of extraction from sample. While the actual sample actual time to perform an analysis has been reduced with the improvement in instrumentation, sample preparation still is a time-consuming task. With the increase in sample load, more and more labs require automation, particularly in routine analysis.

Gas Chromatography-Headspace is the ideal choice for such operations with reduced cost and time of analysis. Static-Headspace sampling is an excellent technique for quantitative and qualitative analysis of volatile compounds that can be efficiently partitioned into the headspace gas volume from either solid, liquid or gaseous matrix. Direct Manual Injection and Headspace sampling are the chiefly used sample introduction techniques in GC but Headspace sampling has its advantage of automation, sensitivity, accuracy, less chances of alteration in sample preparation thereby reducing the sample error[29,30]. GC and GC-HS systems commercially available in many different models from different manufactures are illustrated in Fig. 2.

Fig. 2: GC/GC-HS from various manufactures A) PerkinElmer, B) Finnigan, C) Shimadzu, D) Chemito, E) Varian, F) ThermoFischer, G) Agilent, F) HP (Image source: Toxicology Lab, Deptt of FMT, AIIMS and Google images).

Principle of Gas Chromatography-Headspace (GC-HS)

Gas Chromatography-Headspace is a combination of two systems, a Headspace sampler for sample introduction and a Chromatography system for analyte separation and detection.

For analysis in GC, before getting injected in the inlet, the sample is changed to its volatile form. This is done by heating the sample in a sealed glass vial from the oven programmed at a stable temperature. At equilibrium this causes the volatiles from the sample matrix to get vaporized according to their boiling points and get concentrated in the neck region of the glass vial. The vapors are then extracted using an automated needle through the septa of the vial and introduced in the column for separation. Individual analytes then get separated according to the affinities for each phase in the column and get deferentially separated. Time taken within the sample injection and the emergence of individual peak is known as retention time, whereas the observed respective area/ height is propionate to the concentration. In order for correct separation and detection of the target analyte both the test and standard sample should be run under the same conditions of analysis.

Theory of Headspace

The term 'Headspace' is analytically associated with the vapour phase of a matrix, either solid or liquid sealed within a container. For analysis in gas chromatography, if the compound of interest is volatile mixed in solid or liquid matrix, the best way would be to examine the concentration of these analytes in the gas phase above the matrix in a closed container. This is done, either by taking the sample directly from the gas phase or trapping and concentrating the gas prior to analysis[31]. Headspace analysis reduces cost and time of analysis by directly sampling the volatile from the container in which the sample is placed to GC system.

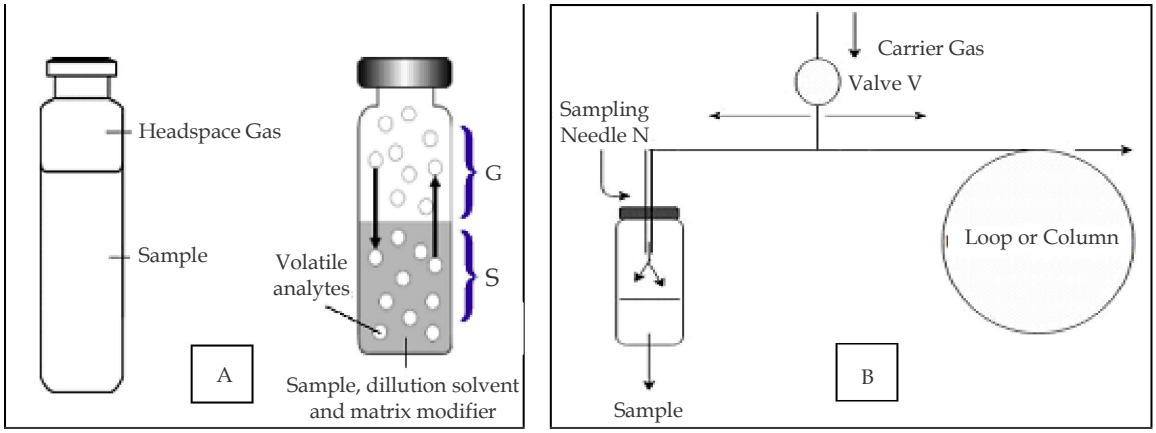

Fig. 3: A) Headspace vial and its components, B) Schematic diagram of Headspace analysis (Image Source: Analyticsshop.com).

The gas phase (G) is commonly referred to as the headspace and lies above the condensed sample phase in a sealed glass vial. The sample phase (S) contains the compound of interest (Fig. 3A). Once the sample phase is introduced into the vial and the vial is sealed, temperature provided to the sealed vial, diffuses the volatile components into the gas phase until the headspace has reached a state of equilibrium[32]. Inert carrier gas (Nitrogen/ Argon) enters the gas chromatograph through valve 'V' and branches before the column, part of the gas is directed to the sampling needle 'N' and rest to the column. When this sampling needle penetrates the septum, carrier gas flows into the vial and pressurizes it. Sample transfer is then done by closing this valve for a few seconds thus disconnecting the gas supply (Fig. 3B)[33]. The loop then disconnects from the valve to get connected to the 'Transfer line' from which the vapours within the vial are transferred to the column.The vial remains sealed inside the chamber until an aliquot is withdrawn for analysis, thus guaranteeing sample integrity.

Theory of Gas Chromatography

Gas chromatography is one of the most widely used techniques for analyzing hydrocarbon mixtures that can be vaporized without decomposition. It utilises a gaseous inert mobile phase to transport sample components through either packed columns or hollow capillary columns containing a polymeric liquid stationary phase. GC has

developed into a sophisticated technique since the pioneering work of Martin and James in 1951, and is capable of separating very complex mixtures of volatile analytes[10–15]. Some of the advantages of chromatography are the Dynamic range of measurement, the detection of a wide range of components in mixtures and standards, and the repeatability of the measurements. Like for all other column chromatographic techniques, a mobile and a stationary phase incorporated in a column are required for this technique. The mobile phase (carrier gas) is comprised of any of the inert gases i.e., Helium, Argon, or Nitrogen. The stationary phase consists of a packed column in which the packing or solid support itself acts as stationary phase, or is coated with the liquid stationary phase (high boiling polymer). Most analytical gas chromatographs use capillary columns, where the stationary phase coats the walls of a small-diameter tube directly (i.e. 0.25µm film in a 0.32mm tube)[34].

The separation of compounds is based on the different strengths of interaction of the compounds with the stationary phase and mobile phase. The stronger the interaction is, the longer the compound interacts with the stationary phase, and the more time it takes to migrate through the column or elute. The most common type of sample introduction injection port consists of a rubber septum through which a syringe needle is inserted to inject the sample (5 µl-25 µl). The injection port is maintained at a higher temperature than the boiling point of the least volatile component in the sample mixture to ensure that the whole sample will be vaporized. Since the partitioning behaviour is dependent on temperature as well as the different interaction of each component with the stationary phase coated on the column, the column is usually contained in a thermostat-controlled oven. Starting at a low oven temperature and increasing the temperature over time to elute the high-boiling point components accomplishes the separation of components with a wide range of boiling points. As the components exit the column they pass through a detector that generates a response that is registered as a deflection in the baseline in form of peaks[35]. Peak height and peak area are used to identify the compound quantitatively.

Instrumentation of Gas Chromatography-Headspace

Gas Chromatograph is a combination of carrier gas system, a sampling system, a separation system, a detection system and a data recording system (Fig. 4). These parts of a basic chromatograph have remained unchanged all through the years, with technological advancement only in design, material and methodologies.

Fig. 4: Parts of a basic Chromatographic system.

a. Carrier Gas System

The carrier gas system consists of carrier gas source, its purification panel and gas flow control. The purification panel of gases is composed of Hydrocarbon traps, Oxy traps and Moisture traps for trapping impurities in gas before introduction in the instrument as shown in Fig. 5. Helium, Nitrogen, and Argon are inert gases and frequently used as mobile phase in Gas Chromatography[36]. Use of these carrier gas in a methodology, is dependent upon type of detector used, for example detectors like Discharge Ionization Detection (DID) requires Helium as an carrier gas[37].

When analysing a volatile or gaseous sample, matrix is purged with these inert gases as they do not show in the response of detector. Nature of gas used is also significant in type of analytes, for example an analysis with poor resolution of peaks would be better analysed with a slow velocity gas like Helium, compared to Nitrogen. Also, safety and availability of gases are another factor deciding the use of gas, for example Argon and Helium are more costly than Nitrogen.

The flow rates of carrier gas effect the separation of analytes same ways as temperature. Higherflow rate increases the run time but decreases the resolution of analytes. Selection of flow rate is therefore dependent upon the level of separation and the length of analysis in the column[38].

b. Sampling System

Sampling and sample preparation majorly impact the integrity of GC analysis of forensic samples, especially when dealing with trace and ultra- trace levels of the target analyte(s) present in various complex matrices (e.g., biological, environmental,

Fig. 5: Gas traps for introduction of Nitrogen, Zero Air and Hydrogen in GC System (Image Source: Toxicology Lab, Deptt of FMT, AIIMS).

fire debris, and explosive residues). In addition, in the majority of cases, the volume of available samples to the forensic investigators is limited. Therefore, a valid sampling and sample preparation strategy should be adopted prior to beginning the analytical process in order to ensure that there is minimum sample loss and theanalysed samples are truly representative of the evidence matrix. Due to the complex and incompatible nature of the sample matrix where the analyte(s) of interest are present, most often, forensic samples cannot be introduced directly into the GC inlet. This incompatibility stems from two factors. First, the complex sample matrix, if introduced directly into the GC inlet without employing any sample treatment/cleanup procedure, may exert a detrimental impact on the performance of the GC by contaminating the inlet with residue, as well as by compromising the sensitive stationary phase of the GC column. Second, if the concentration of the target analyte in the sample matrix is very low so that it may fall below the detection limit of the GC, no usable chromatographic data would be generated (noise). Since every forensic case is unique, standardization of the sampling and sample preparation techniques for the forensic samples is necessary and often dependent upon the knowledge, experience, published literature and judgment of the chemist.

Sample preparation techniques frequently employed in processing forensic samples prior to GC analysis include Solvent Extraction, Solid-Phase Extraction (SPE), Purge and Trap, Liquid-Liquid Extraction (LLE), Supercritical Fluid Extraction (SFE), Steam Distillation, Accelerated Solvent Extraction (ASE), Microwave-Assisted Extraction (MAE), Solid-Phase Micro Extraction (SPME), Liquid-Phase Micro Extraction (LPME), Stir Bar Sorptive Extraction (SBSE), Solid-Phase Dynamic Extraction (SPDE), etc.[26,27,39].

Samples to be run in Gas Chromatography can be changed to gaseous or liquid state by dissolving them in appropriate volatile solvent. These can then be introduced in the inlet by a microliter volume syringe needle through a self-sealing septum consisting of thermally stable silicon rubber. The discrepancies associated with manual sampling in GC has led to higher sample injection techniques like Auto Liquid Sampling and Headspace Sampling as shown in Fig. 6.

Manual Injection

Manual injection is the biggest cause in variation of quantification when multiple injections and operators are compared. For manual injection, liquid sample is aspirated into the syringe by

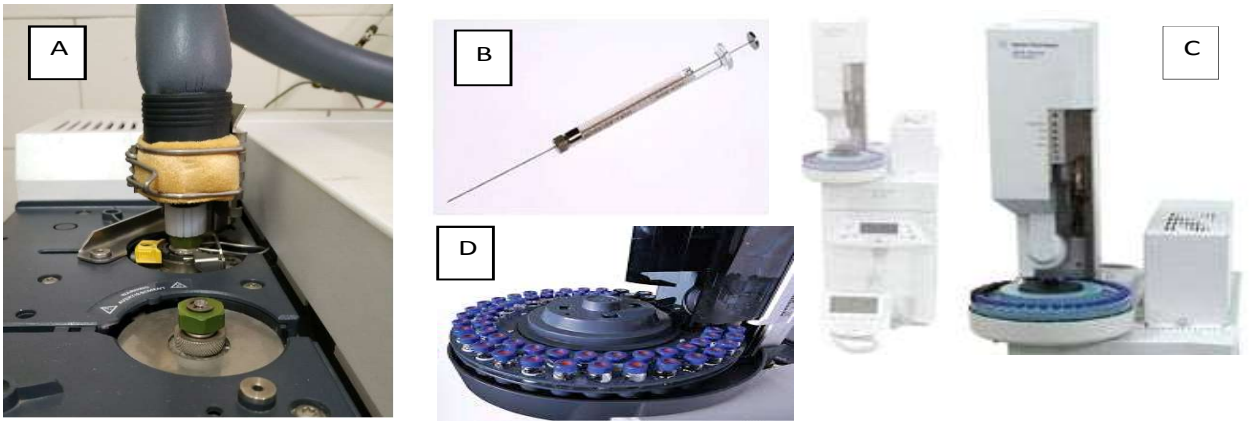

Fig. 6: Types of injection mode in GC systems A) Manual and Transfer line, B) 25µl Microliter Syringe, C) Auto Liquid Sampler, D) Carousel for autosampler vials (Image source: Toxicology Lab, Deptt of FMT, AIIMS and Google images).

withdrawing the plunger and ensuring no air bubbles in the cavity. Needle is immediately inserted in the inlet and plunger is pushed to its full depth. Needle is withdrawn after few seconds of injection.

Syringe injections are inherent to a number of problems causing reduced sample repeatability. Vaporisation of sample in needle before plunger is depressed, is the major problem in manual injection. Disproportionation of sample injection can occur when plunger is not fully depressed and some volume of sample is retained in the cavity of syringe[40]. This may cause considerable error in quantification. Syringe handling should be consistent in sample requiring low volumes of analysis or higher level of precision.

Autosampler

The autosampler provides the means to introduce a fixed volume sample automatically into the inlets, this technique is more effective and more reliable when compared by doing by hand. Automatic insertion provides better reproducibility and time-optimization. Different kinds of autosamplers exist. Autosamplers can be used anywhere when attached with GC system like forensics, environmental science, clinical setup, pharmaceutical and food and beverage industry.

Headspace Sampler

Allows introduction of volatile compounds from virtually any matrix directly into GC or GC/MS instrument. This is done by heating the sample in a sealed glass vial stored at equilibrium from the oven programmed at a stable temperature. At equilibrium this causes the volatiles from the sample matrix to get vaporized according to their boiling points and get concentrated in the neck region of the glass vial. The vapors are then extracted using an automated needle through the septa of the vial and introduced in the column for separation. Individual analytes then get separated according to the affinities for each phase in the column and get deferentially separated.

Methods of Headspace Sampling

Following three types of sample injection (Syringe injection, balanced pressure, and pressurized loop) are commonly used in Static Headspace.

1. Syringe Injection

It is the most commonly used and reproducible sample injection method of headspace sampling. Here the syringe is heated and agitated in oven for a predefined period of time. The heated syringe then removes an aliquot of the headspace and directly injects it directly into the GC (Fig. 7). The syringe

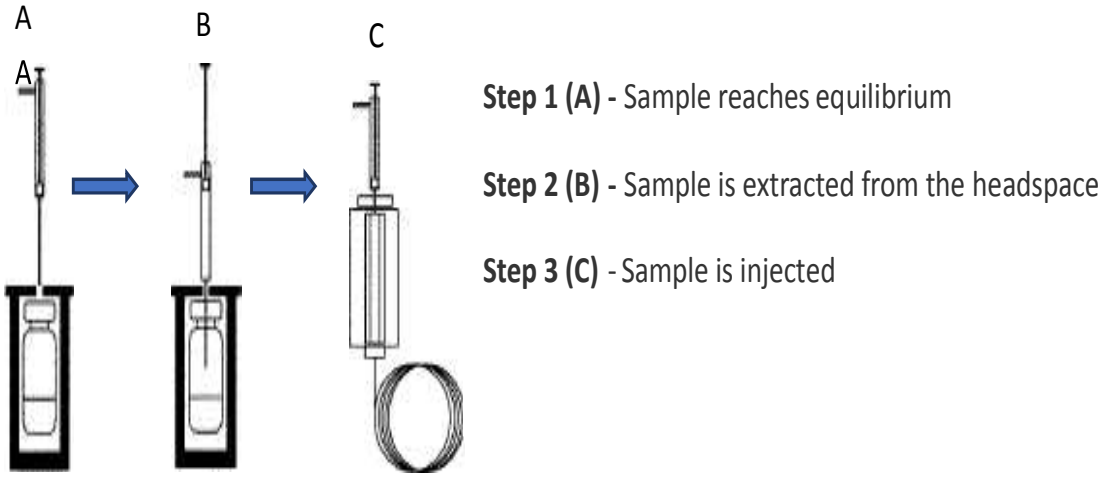

Fig. 7: Syringe Injection Technique (Image source: Analyticsshop.com).

must be heated few degrees above the temperature of the oven to avoid the risk of condensation and hence carry-over from one sample to the next. After injection, the syringe is flushed with nitrogen or carrier gas. This type of system having following advantages

1. Very high level of reproducibility
2. Low carry-over
3. Fast transfer of sample to GC/GLC
4. Precise control of sample syringe for sample size and injection speed
5. Easy to clean syringe
6. GC injection port is always free for manual samples
7. Many syringe auto samplers can be retrofitted onto existing GC systems.

2. Balanced Pressure

This technique uses a seamless injection directly from the vial into the carrier gas stream without moving parts other than a valve and a needle (Fig. 8). The balanced pressure technique, like other techniques, uses an incubation oven to thermostat the vial so the sample reaches equilibrium in a closed environment. During these initial steps, a needle is inserted into the vial and is then pressurized with a carried gas. After the vial is pressurized and equilibrium has been reached, the valve is switched on for a specific amount of time to redirect the sample into the transfer line and onto the column. In this technique the absolute volume of the sample injected is unknown. This technique can be quite accurate but can also suffer from disadvantages such as:

1. These negative aspects include sample carry-over
2. The injection port is always occupied and therefore not available for manual use.
3. Quantification becomes difficult.

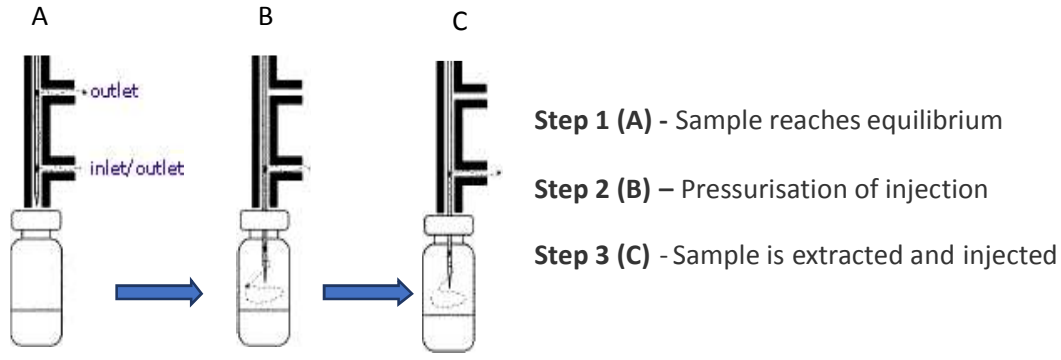

Fig. 8: Balanced Pressure Technique (Image source: Analticsshop.com).

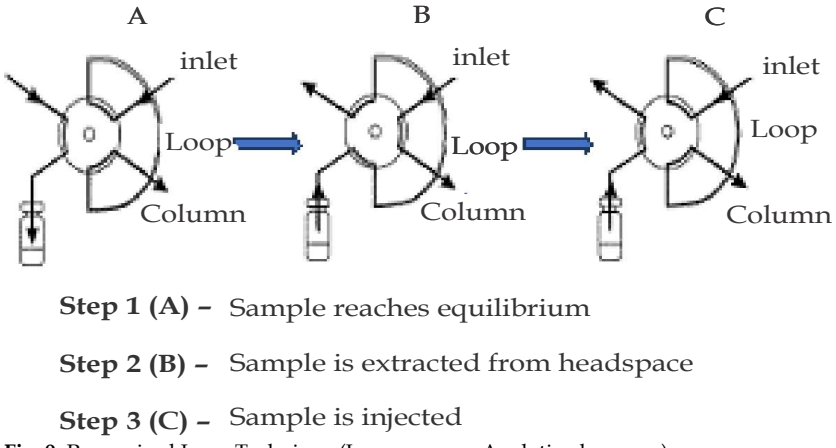

Fig. 9: Pressurised Loop Technique (Image source: Analyticsshop.com).

3. Pressurized loop technique

The pressurized loop system uses a known amount of sample. This technique typically uses a six-port valve, which thermostats and pressurizes the vial as in the previously described sample injection techniques. After pressurization, the valve is turnedand the loop is filled with the sample. Once the loop has been filled, the valve is turned again to redirect the gas flow and flush the sample into the transfer line leading to the column (Fig. 9). This type of system allows high temperatures to be used but it also suffers from the same disadvantages as the balanced pressure system such as sample carry over and the injection port is always occupied.

c. Column

A column is the heart of Gas Chromatography, because the components of the mixture are separated in it by the virtue of different interaction with the column packing. The column is chosen according to the polarity of the sample for maximum separation. The rate at which compounds move through the column depends on the nature and strength of the interaction between the analyte and the stationary phase. The column contains the stationary phase coated on an inert solid support. GC columns are of two types-Packed and Capillary[34].

Packed Column

Packed columns are usually made of Stainless Steel or Copper Tubing. Diameter of glass tube or metal is ¼" or 0.25 in with lengths ranging from 5-50feet. Short length columns are straight and installed vertically in the thermostat whereas longer columns are U-shaped but columns with over 1meter length are coiled.

Capillary Column

Capillary columns are also known as Open Tubular Column which are generally fabricated form Stainless Steel or Quartz. Its diameter is 1/16 inches or less with length ranging up to 200-300 mts.

As the analytes are carried to the column from the injection port they interact with the stationary phase and are retained. Components that interact more strongly with the stationary phase spend proportionally less time in the mobile phase and therefore move through the column more slowly. Other variables that affect Retention time are column temperature and carrier gas flow rate. Long elution times in experimentation should be avoided as they not only waste valuable resources but broadening of the peaks and loss of resolution will become evident when the elution times are too long. Thus the optimum conditions are those that result in complete separation of the peaks in the shortest possible time[41].

d. Detector

After separation analytes elute from the column, they interact with the detector. The detector converts this interaction into an electronic signal that is sent to the data system for representation in a readable form. The magnitude of the signal (mA) is plotted versus time (min), from the time of injection and a chromatogram is generated. Some detectors respond to any analyte eluting from the column while others respond only to analytes with specific structures, functional groups or atoms. Detectors that exhibit enhanced response to specific types of analytes are called Selective Detectors.

Variety of detectors may be employed for the detection, quantification, and/or identification of the analyte(s) which include Flame Ionization Detector (FID), Nitrogen Phosphorus Detector (NPD), Sulfur and Nitrogen Chemiluminescence Detector, Flame Photometric Detector (FPD), Atomic Emission Detector (AED), Thermal Energy Analyzer (TEA), Electron Capture Detector (ECD), Ion Mobility Mass Spectrometry (IMMS), Time-of-Flight Mass Spectrometry (TOFMS), and Isotope Ratio Mass Spectrometry (IRMS). However, the most popular is the Mass Spectrometer (MS) attached to GC as it offers both identification and quantification of an unknown substance with high confidence. In some cases, MS in tandem with another MS is also used as the detector.

General requirements of any detector are: high sensitivity; physically suitable; capable of operating up to maximum column temperature; ease of operation; no response to undesirable compounds; linear response exceeding to high concentrations. Based on these physical properties there are several detectors available[33,42,43].

1. Flame Ionisation Detector (FID)

FID is the most commonly used detector with main use for the detection of hydrocarbons or carbon containing compounds. It uses an air/ hydrogen flame to pyrolyze the effluent sample. The pyrolysis of the carbon containing compounds in the flame creates ions. A voltage is applied across the flame and the resulting flow of ions is detected

as a current (mA). Sensitivity of this type detector can range between 0.1-01ng.

2. Electron Capture Detector (ECD)

ECD detector are more suited for polyhalogenated organic compounds. It uses a beta emitter such as radioactive Tritium or Nickel and uses it to ionise the carrier gas. Fast beta particles generated by the radioactive source collide with the molecules of the carrier gas. Electronegative compounds capture electrons generated resulting in a reduction in the current. The amount of current loss is indirectly measured and a signal generated is displayed. Sensitivity of this type of detector can range between 0.1-10pg for halogenated compounds, 1-100pg for nitrates and 0.1-1ng for carbonyls.

3. Thermal Conductivity Detector (TCD)

TCD detectors are based on change in the thermal conductivity of the gas stream. It is universal detector with the detection of air, hydrogen, carbon monoxide, nitrogen, sulphur dioxide, inorganic gases and many other compounds. The difference in the thermal conductivity between the column effluent flow and the reference flow of the carrier gasproduces a voltage signal proportional to this difference. The signal is proportionate to the concentration of the sample components. The sensitivity of this detector can range from 5-20ng.

4. Flame Photometric Detector (FPD)

FPD detectors are based upon the luminous emission from a hydrogen rich flame in the presence of compounds containing either Sulphur (394nm) or Phosphorus (526nm). It consists of a hydrogen air burner and a photomultiplier. The detector is very specific with the detection based on type of optical filter used. The sensitivity of the detector can range from 10-100pg for Sulphur containing compounds and 1-10pg for Phosphorus containing compounds.

5. Photo Ionisation Detector (PID)

Typical photoionization detectors measure organic volatiles and other gases. Organic compounds eluting into a cell are bombarded with high energy photons emitted from a lamp. Compounds with ionization potentials below the photon energy are ionized. The resulting ions are attracted to an electrode, measured, and a signal is generated. The PID is used mostly to detect VOCs in soil, sediment, air and water. It is often used to detect contaminants in ambient air and soil during drilling activities and during spills to identify potential problems.

6. Electrolytic Conductivity Detector (ELCD)

ELCD Detectors are selective to Halogens, Sulphur and Nitrogen containing compounds. Compounds are mixed with a reaction gas (Hydrogen) and passed through a high temperature reaction tube. Specific reaction products are created which mix with a solvent and pass through an electrolytic conductivity cell. The change in the electrolytic conductivity of the solvent is measured and a signal is generated. Reaction tube temperature and solvent determine which types of compounds are detected. The sensitivity of the detectors can range from 5-10pg for halogens, 10-20pg for sulphur containing compounds and 10-20pg for nitrogen containing compounds.

7. Helium Ionisation Detector (HID)[44]

HID Detectors are a universal detector responding to all molecules except Neon. Detector uses Helium as both carrier gas and the ionization gas. HID uses ion detector which uses a radioactive source, typically β-emitters to create metastable helium species. The metastable Helium species have an energy of up to 19.8eV. These metastable helium species can then ionize all compounds with the exception of neon which has a bigger ionization potential of 21.56eV. As components elute from the column they collide with the metastable helium ions, which then ionize the individual components. The ions produce an electric current, which is the signal output of the detector. The greater the concentration of the component, the more ions are produced, and the greater the current. The drawback to HIDs are that they contain a radioactive source. Discharge ionization detectors have generally supplanted them.

8. Argon Ionisation Detector (AI)[43,45]

AI Detectors are based upon the production of metastable Argon atoms which are used to ionise the sample, which is held in a carrier gas. The produced electrons are focused towards the collector electrode and produced current is measured.

9. Mass Spectrophotometer (MS)

Mass Spectrophotometer can be used to detect the compound even in the absence of a certified standard. Complex mixtures can be separated

using an LC or GC system attached or simply injected in the inlet of MS. Ionised samples are bombarded with electrons (EI) or gas molecules (CI). Compounds fragment into characteristic charged ions or fragments. The resulting ions are focused and accelerated into a mass filter. The mass filter selectively allows all ions of a specific mass, as decided by the software, to pass through to the electron multiplier. All of the ions of the specific mass are detected. The mass filter then allows the next mass to pass through while excluding all others. The mass filter scans stepwise through the designated range of masses several times per second. The total number of ions are counted for each scan. The abundance or number of ions per scan is plotted versus time to obtain the chromatogram. A mass spectrum is obtained for each scan which plots the various ion masses versus their abundance or number. The detector is maintained under constant vacuum. The detector can be sensitive within a arrange of 1-10ng for full scan, 1-10pg for selective scan.

Different Accessories used in Operation of GC-HS

a. Headspace Vials

It is a small container, typically cylindrical and made of glass, headspace vials are available in 6, 10, 20 and 22 ml sizes (Fig. 10A).

b. Crimp

It is a kind of metallic cap used to provide consistently secure seals to the sample vials, along with the septa (Fig. 10B).

c. Septa

It is a circular membrane, generally white in colour, used to cap the sample vial to prevent any form of leakage (Fig. 10C). Most commonly used material for septa is Polytetrafluoroethylene (PTFE).

d. Crimper

A crimper is a tool to conjoin septa and crimp, using compressive force to constrict the edges around the neck of vial. This helps in proper sealing of the

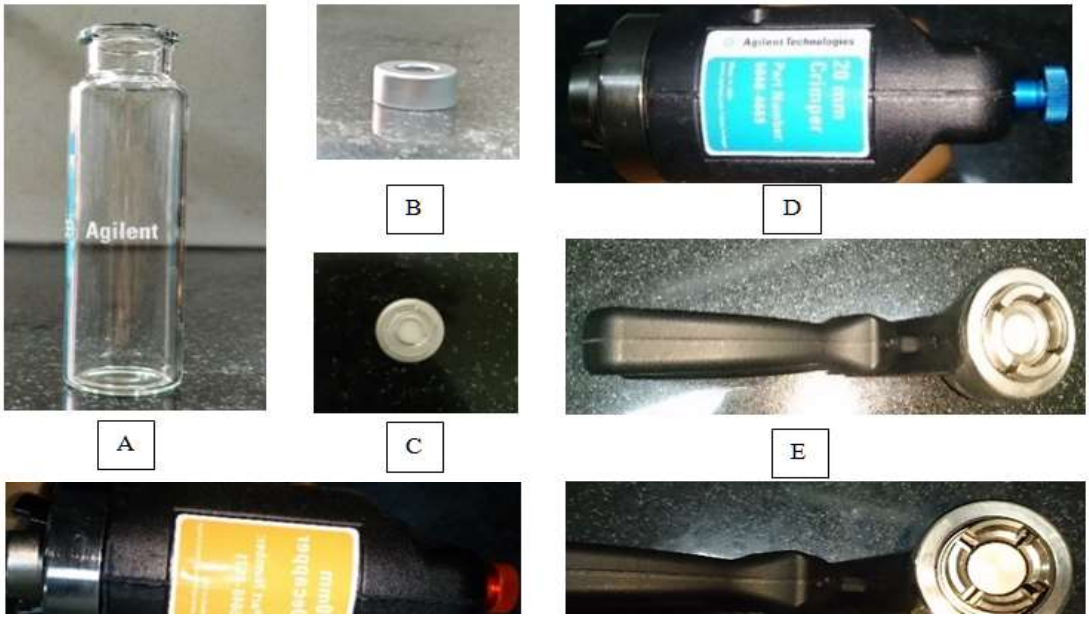

Fig. 10: Accessories of Gas Chromatography Headspace: A) 20ml GC-HS Vial, B) Metallic Crimp, C) PTFE Septa, D) Top View Crimper, E) Bottom view Crimper, F) Top View Decrimper, G) Bottom view Decrimper (Image source: Toxicology Lab, Deptt of FMT, AIIMS).

crimp on the sample vial (Figure 10D, E). They are generally colour coded with blue knobs and label.

e. Gas Traps

- Gas traps are external devices attached to the GC systems to remove the detrimental impurities from the carrier and the detector gases.
- Gas Clean Filter System or gas traps delivers clean gases, reducing the risks of column damage, sensitivity loss, and instrument downtime.
- Inserting a Gas Clean Filter System in the gas line immediately before the instrument inlet greatly reduces the level of impurities, thus improving trace analysis.
- The most commonly used traps in the GC systems are Moisture, Oxygen, and Hydrocarbon traps; however sometimes nitrogen and hydrogen traps are also used.
- Reducing impurity level can prolong column life and may improve sensitivity. The effectiveness of the traps depends on the initial quality of the gas.
- Little enhancement by traps to the GC system is obtained by traps when using very high purity gases (e.g., ultra-high purity or similar grades) while obvious improvement is obtained with lower grades of gas. Traps may provide some protection if there is a leak at or around the gas cylinder.

Different Consumables used for mantainence of GC-HS

a. FID Jet

The most common detector used in Gas Chromatography is the Flame Ionisation Detector (FID) is connected through FID jet. It measures the concentration of organic substances passing through the gas stream (Fig. 11A). After certain analysis it should be replaced by new one.

b. Column Inlet Nut

Column inlet nut is a very tiny installation part of the GC column, which should be very precisely placed for accurate and reproducible results. Manufacturers offers a selection of capillary column nuts for GC fittings to facilitate good column installation (Fig. 11B). Column nuts help prevent leakage as it strongly tightens the inlet and outlet portions with the column.

c. Autosampler Syringe (if injection is done without HS)

1 mL, 2.5 mL and 5 mL syringes are available for headspace. Syringes are consumables and need to be replaced frequently (Fig. 11C). In a gold standard autosampler syringe, the upper portion of the tapered needle offers the strength of a 23-gauge, while the lower portion at 26 s-gauge enables use with split/splitless or on-column injections with 0.53 mm id columns.

d. Inlet Septa

The general-purpose inlet septa are made from an enhanced injection-moulded silicone rubber material (Fig. 11D). The septa material, dark red or grey in colour, is specified to withstand over 200 automatic injections at an injection port temperature of 350°C.

e. Capillary Column

Capillary columns (Fig. 11E) show a higher degree of resolution because of its longer column, about 80-100 feet, and a narrow width of about 250 µm. Capillary columns are made of purified silicate glass, and have the inner surface coated with the stationary phase. Sufficient number of capillary columns should be in hand for different applications

f. Ferrule

Ferrule is a part of the GC column, which maintains a leak-free connection between the column and the injector. It is available in a wide range of materials and configurations (Fig. 11F). For instance, graphite ferrules can withstand temperatures as high as 450°C.

g. GC Inlet Liner

An injection port liner is used to make the connection between sample introduction and the GC column (Fig. 11G). Four primary injection techniques are used in GC; split, splitless, direct, and on-column.

Fig. 11: Different consumables used formentainence of GC-HS: A) FID Jet, B) Column Inlet Nut, C) Inlet Septa, D) Inlet Liner, E) Capillary Column, F) Ferrule (Image source: Google images).

Inlet liners are selected based on the injection technique being used to ensure optimal sample transfer to the column.

f. O-Ring

O–rings are used to seal the liners in the inlet (Fig. 11H). They are easy to use and remove, and help in eliminating out-gassing of contaminants. Graphite O-rings can be used when operating inlet temperatures exceed 350°C.

System Optimization for Gas Chromatography-Headspace Analysis

Chromatographic performance of a sample is greatly influenced by how it is introduced into the analytical column. Variables that affect sample preparation and transfer of sample from the headspace unit to the analytical column must be optimized to obtain reproducible and efficient separations. Different steps involved for system optimization are as follows.

a. Preparation of Sample

Samples for HS-GC must be prepared in such a manner as to maximize the concentration of the volatile sample components in the headspace while minimizing the unwanted co-extracts from other compounds in the sample matrix. Few points about sample preparation are as follows:

- Water vapor from the sample matrix also can cause problems by condensing in the transfer line. Incomplete or inefficient transfer of high molecular weight compounds or water vapor from sample matrices can deteriorate the column by producing adsorptive areas in the transfer line or injection port that can lead to split peaks, or irreproducible responses or retention time.
- To minimize matrix problems and prevent water condensation from aqueous samples, use a higher transfer line temperature (~125°C–150°C).
- High concentration can produce ghost peaks in subsequent analyses due to carryover of sample from previous injections.

- Sample carryover can be minimized by using higher transfer line and injection port temperatures, but some samples may need to be diluted and reanalyzed to obtain reliable results.

b. Selection of Sample Vial

Sample vial should be selected to match the type and size of the sample being analyzed. Few points about selection of sample vial are:

- Vials that are not properly cleaned prior to packaging or that absorb contaminants during shipping can produce unknown chromatographic peaks or ghost peaks. Ghost peak that are result of vial contamination can be identified by running method blanks and zero standards during the system calibration sequence.
- The septa used to seal the headspace of the vials also can be the source of the contaminants, which can bleed into the headspace of the vials during equilibration.
- Vials for sample and standard should be same.

c. Sample Vial Heater and Mixer

Once the sample is placed inside a clean, dry, sterile vial and the vial is sealed with septa, volatile compounds from the sample will partition into the headspace until the state of equilibrium is reached. Few points about sample vial heater and mixer are:

- Temperature, time, and mixing can improve the transfer of volatile analytes from the sample into the headspace of the vial.
- Sufficient time must be built into the sample cycle in order to achieve a constant state of equilibrium

d. Sampling

There are several techniques used to transfer samples from the vial to GC. Few points about sampling are:

- When using a gas-tight syringe for sampling, heat the syringe to a temperature comparable to the sample vial temperature. This minimizes pressure differences and condensation problems. Ensure the septum is well maintained to decrease the possibility of leak.
- For balanced-pressure sampling instruments, analyst should consider the inertness and efficiency of the components that make the sample pathway inside the auto sampler. If sensitive compounds are being analyzed, an inert pathway should pathway should be used to decrease possible adsorption.
- Analyst should ensure that balanced-pressure instruments are leak free and operate with the least amount of dead volume in the sample flow path. This will help optimal peak shape and sensitivity.
- When using pressure-loop sampling instrument, inert sample pathways and low dead volume systems will yield the best chromatographic performance.
- If low response or broad peaks are observed, it may be necessary to increase the sample vial pressure to ensure that the sample loop is completely filled with the headspace sample.
- If there are extraneous peaks present due to carryover of matrix contaminants, increase the sample valve temperature to prevent sample carryover,condensation and contamination.

e. Transfer Line

After the headspace sample is withdrawn from the vial, it is ready to be transferred to the GC/GLC. In balanced-pressure and pressure loop systems a short piece of tubing called transfer line is used to transfer the sample from the autosampler to the GC/GLC.

- The internal diameter of the transfer line should be chosen depending upon the internal diameter of the analytical column, the column flow rate and the flow rate delivered from the autosampler.
- Transfer line should be set depending on the analyst of interest and the sample matrix.
- A typical transfer line temperature ranges from 80°C to 125°C. To minimize matrix problems and prevent water condensation from aqueous samples, use a higher transfer line temperature (~125°C–150°C).

f. Injection Port Interface

The quality of the connection of the transfer line to the analytical column greatly affects the analyte separation. In most cases, the transfer line has a smaller internal diameter than the injection port liner and the vaporized headspace sample carrying the compounds of interest will be diluted into a larger volume of carrier gas when the sample elutes from the transfer line into the inlet liner. This can lead to broader peaks, tailing peaks, lower

sensitivity, and loss of resolution. Few points about injection port selection are:

- Using injection port liners that have smaller internal diameters and lower buffer volumes will help maintain a narrow bandwidth as samples move from the end of the transfer line to the head of the analytical column.
- If the band-broadening due to excess dead volume in the system is still a problem, peak shape may be improved by refocusing sample analytes at the analytical column head.
- Highly volatile compounds can be trapped at the column head and refocused into a narrow bandwidth by reducing the initial oven temperature below the boiling point of the compound of interest.

g. Derivatization Technique

Derivatization is another technique that can be used to increase sensitivity, chromatographic performance and enable detection for specific noncompatible compounds. Few points about derivatization technique are:

- Compounds such as acids, alcohols and amines are difficult to analyze because of the presence of reactive hydrogen. When attempting to analyze these types of compounds, they can react with the surface of the injection port or the analytical column and result in reduced detector performance in shape of tailing peaks and low response. In addition, they may be highly soluble in the sample phase, causing very poor partitioning into the headspace and low response. Derivatization of these compounds can improve their volatility, as well as reduce the potential for surface adsorption once they enter the GC system.
- Common derivatization techniques used in reaction headspace/GC are esterification, acetylation, silylation, and alkylation.
- Derivatization reagents, as well as the by-products from derivatization reactions, may be volatile and can partition into the headspace along with derivatization compounds. These extra volatile compounds may pose problems by eluting with similar retention times as the compounds of interest, causing either partial or complete co-elutions.
- Derivatization reactions are typically run at elevated temperatures than usual vial heating. Pressures inside the sample vial then may exceed the pressure handling capabilities of the vial or the septa. Specially designed caps are available that allow excess pressure to be vented during derivatization reactions. Use of the correct and compatible vial, cap and septa is important.

Different Factors which Affect the Sensitivity of GC-HS

a. Influence of the Sample and Temperature on Headspace Sensitivity

- The sample volume (Vs) is included in the phase ratio (β) but its influence on the headspace sensitivity is not independent of the partition coefficient (K).
- The latter can vary widely from practically zero in the case of gas sample up to several thousands, where the applicability of HS-GC ends.
- The phase ratio (β) and thus the influence of the sample volume does not generally span such a wide range. For example, 1mL sample in a 10mL vial has a phase ratio of 9, while with a sample volume of 5mL the phase ratio decreases to 1.
- This causes an increase in the resulting gas concentration, and thus on the resulting peak area, depends mainly on the partition coefficient (K>100) e.g. ethanol in water a change in the phase ratio from 1 to 5 will barely influence the headspace sensitivity in contrast where the partition coefficient is very small the sensitivity increases in proportion to the sample volume .
- The vapor pressure of a compound increases exponentially with temperature.
- However, there is a dependence on the partition coefficient. In case of non-volatile compound ($K \rightarrow \infty$) a higher temperature will not alter its non-volatility.
- In the case of a highly volatile compound ($K \rightarrow 0$ at room temperature) the temperature will not affect the headspace sensitivity either, because in this case nearly all the compounds are already present in the gaseous phase.

b. Sensitivity enhancement by Matrix modification

The partition coefficient can be altered by modifying the sample matrix.

- A common technique is the use of the salting-out effect. For aqueous samples with high

partition coefficient (ethanol in water) the addition of salt may enhance the sensitivity by up to a factor of 10.

- The result depends upon the value of the partition coefficient.
- In the case of a highly volatile compound (K→0) where nearly all of the analyte is already present in the gas phase, the sensitivity will not improve.
- A similar effect is achieved with a sample containing a non-polar volatile compound dissolved in water miscible organic solvent such as dimethylacetamide, dimethylformamide, etc.
- If water is added to this solution, the solubility of the non-polar compound will decrease and its volatility will increase.
- Common salts such as Sodium Sulphate, Sodium chloride, Sodium citrate, Potassium carbonate, Ammonium Sulphate, and Ammonium chloride decreases matrix effect.

c. Sensitivity Enhancement by modifying the volatile analyte

- Polar compounds particularly those with active hydrogen such as alcohols, phenols, acids, amines etc. usually have low volatility as a result of intermolecular interaction with the polar matrix through hydrogen bond formation.
- However, the reactivity of the active hydrogen can be used to prepare less polar derivatives with better volatility and lower solubility.
- Simple derivatization (such as esterification, transesterification, acetylation, etc.) reactions are preferred which are carried out in the headspace vial during the equilibrium time.
- An advantage of GC-HS is that the reaction products are less polar and more volatile thus shifting the equilibrium of the chemical reaction towards completeness.
- Sensitivity is increased when partition coefficient (K) is minimized.
- Sensitivity is increased when phase ratio (β) is minimized.
- Lower K and β results in higher concentration of volatile compounds in gas phase and better sensitivity

d. Headspace Sample

- In addition to working with the partition coefficient, phase ratio, derivatization techniques sensitivity can also be improved by simply increasing the size of the headspace sample that is withdrawn from the sample vial and transferred to the GC.
- Increasing the sample size also means that the amount of time it takes to transfer the sample to the column will increase in proportion to the column volumetric flow rate
- Sample size can be increased only to the point that increases in peak width, caused by longer sample transfer times, will not affect chromatographic separations.
- Larger sample sizes and longer transfer times can be offset by using cryogenic cooling and sample refocusing at the head of the column.

Do's and Don'ts while operating GC-HS

a. *Do's*

- Before starting the instrument, verify the entire gas cylinder. If the primary gas pressure is less than 3psi, replace it.
- Purity of carrier gas should be maintained to prevent degradation of chromatographic hardware.
- Once the system power is on, leave for 1hour to stabilize the baseline if the system is left off for 2 days.
- To minimize matrix problems and prevent water condensation from aqueous samples, use a higher transfer line temperature (~125°C–150°C).
- Inject standards and samples in order from low to high concentrations to help minimize carryover.
- For high concentration samples in a sequence of samples, run a blank after the suspected samples to reduce carryover contamination.
- Handle standards and method blanks the same way samples are handled to make any vial or sample preparation problems easier to identify.
- Always use pre-cleaned vials for sample preparation and storage.
- Septa with a PTFE face should be used to seal the headspace vial to eliminate bleed from the rubber portion of the septa.

- Built sufficient time into the sample cycle to achieve constant state of equilibrium.
- Adjust the temperature of the sample to change the solubility of the analyte as well as to drive the equilibrium in towards the gaseous phase.
- Shaking or vibrating the vial containing high viscosity sample matrices during heating can assist in achieving equilibrium faster.
- Heat the syringe to a temperature comparable to the sample vial temperature to minimise pressure differences, when using gas tight syringes.
- Flush the syringe after each injection to prevent carryover from the inside of the syringe.
- Ensure the septum of the GC injection port is well maintained to decrease the possibility of a leak.
- Increase the oven temperature after the samples are completely transferred to the column to increase the movement of compounds inside the column.
- Use injection port liners of small internal diameters and lower buffer volumes to maintain a narrow bandwidth.
- Use indicating traps closest to the GC to determine when to change the traps that are upstream.
- Indicating traps are not intended to be the primary oxygen removal trap, but should be used in conjunction with a high capacity non-indicating oxygen trap.
- Immediately change the expired oxygen traps to avoid gas contamination, in addition to failing to remove oxygen.
- During cartridge replacement, check valves and close off the system to the atmosphere, further minimizing the entry of contaminants.
- Replace split vent traps approximately every six months.
- GC instrument maintenance should include checking fittings and connections with a gas leak detector.
- Use an on-column syringe when injecting into an on-column inlet so that the injector, syringe and column are not damaged.
- To prevent stationary phase decomposition, the oven and inlet should be at room temperature when not in use and when changing the septum.
- After running the samples, condition the system before shut down.
- Before shutting down the system the inlet, detector and oven should be cooled and temperature condition to 500°C.

b. Don'ts

- Constant exposure of capillary columns to oxygen and moisture should be avoided especially at high temperatures as it may produce rapid and severe column damage.
- Improper handling or installation of plumbing should be avoided as moisture introduced by this can be a common cause of column stationary phase degradation.
- Do not use sample matrices containing high molecular weight compounds to avoid incomplete or inefficient transfer into the GC injection port.
- Transfer line temperature should not be kept low as water from the sample matrix can cause problems by recondensing in the transfer line.
- Avoid using high-concentrated samples lest they produce ghost peaks in subsequent analyses due to carryover of sample from previous injections.
- Do not use unclean or unpacked vials.
- Septa with PTFE face should not be reused.
- Do not use transfer line having smaller internal diameter than the injection port liner to avoid broader peaks, tailing peaks, lower sensitivityand loss of resolution.
- No need to use a large buffer volume in the liner to allow for sample expansion because headspace samples are already in the gaseous state.
- Excess sample analytes should not be used to avoid band-broadening.
- It is not recommended that regulator materials and choice of tubing be interchanged.
- Non-metallic types of tubing such as polyethylene and Teflon are not recommended for GC applications due to their gas permeability and difficulty in cleaning.
- Unclean or improperly cleaned tubing can lead to contamination of the system with disastrous results.
- Never open the GC door if oven temperature is more than 100°C or during running conditions.

- Do not inject air into the vials to prevent the vacuum. This often damages the cap seal.
- Avoid cleaning agents that are alkaline, contain phosphatesor are strongly acidic for syringes.

Application of Gas Chromatography-Headspace in Forensic Toxicology

Forensic science defines scientific principles, tools, and methodologies to resolve legal issues and disputes. Forensic chemists analyse a wide variety of forensic samples, extracting and interpreting information from the chemical and analytical data that may potentially have to withstand rigorous challenge when presented in court. As such, it is imperative that any analytical methodology developed for solving forensic problems should meet, at a bare minimum, the required standard set forth by the scientific community of uniformity and conformity.

Among all analytical instruments currently being used in routine forensic analyses as well as in forensic research, Gas Chromatography-Headspace (GC-HS) is one of the most widely used analytical tool. High sensitivity, selectivity, resolution, speed,accuracy and precision, wide dynamic concentration range, simple and robust instrument design, online and offline monitoring of the equipment and its ability to be interfaced with many established and emerging detection systems have made GC the instrument of choice in many facets of forensic science. As such, new GC instruments (hardware) along with their operating systems (software) are so simple and user friendly that even a novice operator with proper training and induction can operate it with confidence.

Due to the inherent advantages of GC-HS, applications of this reliable analytical instrument in forensic science isa necessary presence. Major application areas in forensic science include bulk seized drug analysis, drug screening from biological specimens, alcohol quantification in drunken driving cases, methanol estimation in illicit alcohol, postmortem toxicology, trace evidence analysis, explosive analysis, analysis of ignitable liquid residues from fire debris, noxious gases analysis in postmortem samples etc.

a. Analysis of Ethyl Alcohol in Driving Under Influence cases from Biological matrix

Ethanol in alcoholic drinks is one of the most widely abused licit drug all over the world. The age limit fixed by the government for the legal consumption of alcohol in India is 21 years. The statutory limit is 30mg% of alcohol in blood under the Motor Vehicle Act, (1988, amended) Section-185, if found driving under influence[46]. Alcohol intoxication associates mainly with road traffic accidents, unruly behaviour, Drug Facilitated Sexual Assault, homicides and suicides[47–50]. Therefore, the estimation of alcohol in blood is very important in medicolegal cases and GC-HS provides a sophisticated system for qualitative and quantitative analysis of alcohol[48,51–53]. In forensic laboratories Static Gas Chromatography-Headspace has become the instrument of choice for this purpose. GC-HS combines both qualitative and quantitative analysis based on detector response of peak height and peak area. Use of Headspace is preferred over manual injection over the convenience of minimised matrix

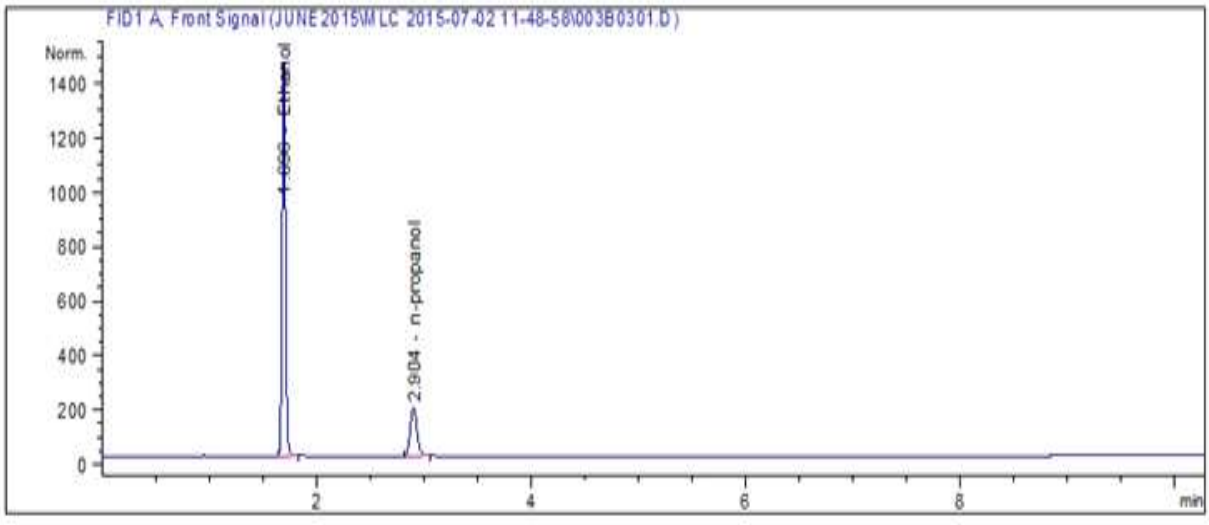

Fig. 12: Chromatogram showing ethanol and internal standard n-propanol in the matrix (Image source: Toxicology lab, Deptt of FMT, AIIMS).

artefacts, contaminants and minimal sample preparation to avoid loss of volatile analyte. Samples such as Blood, Serum, Vitreous Humour, Bile, Muscle, Gastric Content, cerebrospinal fluid, brain tissue can be easily and routinely analysed without any major sample preparation[17,54–56].

Analysis by one such method, developed and validated in the department laboratory is shown in Fig. 12. The methodology is valid for identification of ethanol in biological matrices like blood, vitreous humour with addition of n-propanol as an Internal Standard for quantification[57].

b. Analysis of Methyl Alcohol from Biological and Non-Biological Matrix

Methanol is commonly known as Wood alcohol, wood-naphtha, carbinol, or methylated spirit. It is widely encountered in day-to-day life as a solvent for extraction, antifreeze, fuel, and denaturant to render alcohol (industrial ethanol) unfit for consumption. Methanol is an alcohol, chemically, which is toxic to humans on consumption. Cases related to methanol analysis can be received in an forensic laboratory relating to illicit alcohol, poisoning due to consumption. Majority of cases of poisoning are accidental in nature which happen due to unintentional drinking of industrial ethanol, drinking illicit alcohol or malicious intent of poisoning[58–59]. Blood alcohol testing is one of the most accurate methods for measuring alcohol toxicity in clinical and forensic setting both, as the testing presents the physiopathological chemistry of compound and its metabolites in body. The analysis of blood and other body fluids for alcohol is most commonly performed using "Headspace-Gas Chromatography" due to its simplicity in operation and the number of matrices that can be analysed with one instrument with one method[13,14,30].

Analysis by one such method, developed and validated in the department laboratory is shown in Fig. 13. The methodology is valid for identification of methanol in whole blood with addition of Acetonitrile as an Internal Standard for quantification[60].

c. Analysis of Inhalants

In India, surprisingly a large number of children, young adults and adolescents partake in substance abuse[61,62]. Items such as paints, glues, correction fluid, thinners, nail polish removers, laboratory

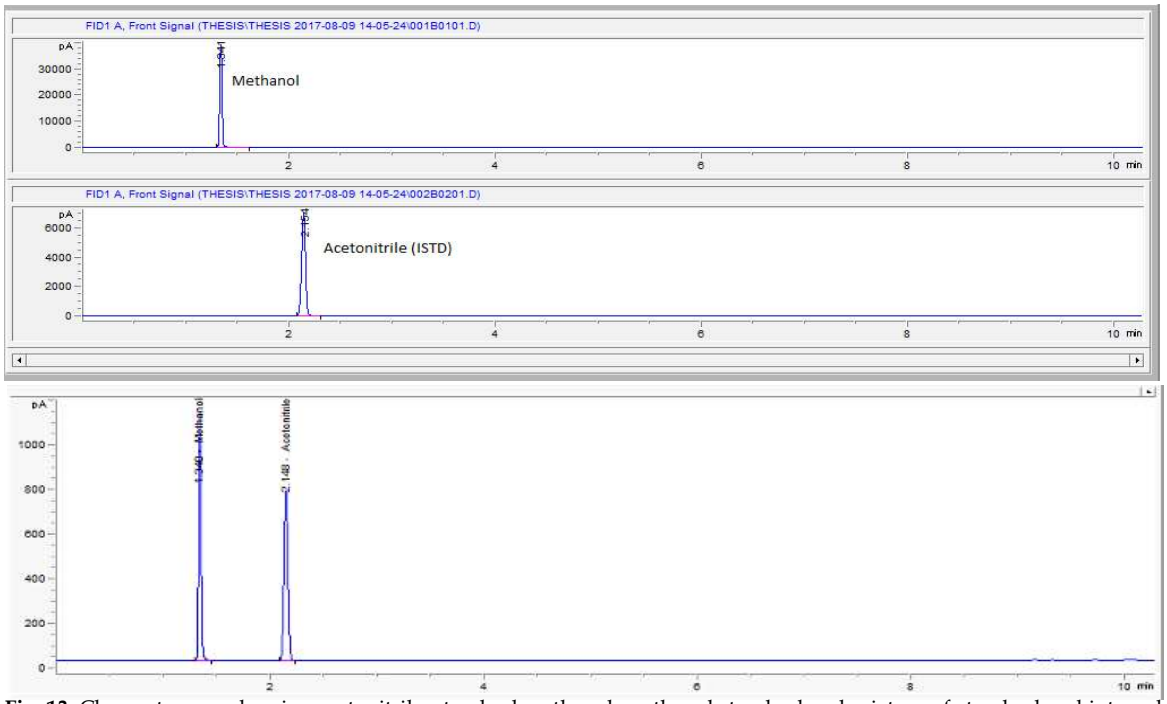

Fig. 13: Chromatogram showing acetonitrile, standard methanol, methanol standard and mixture of standard and internal standard in the matrix (Image source: Toxicology lab, Deptt of FMT, AIIMS).

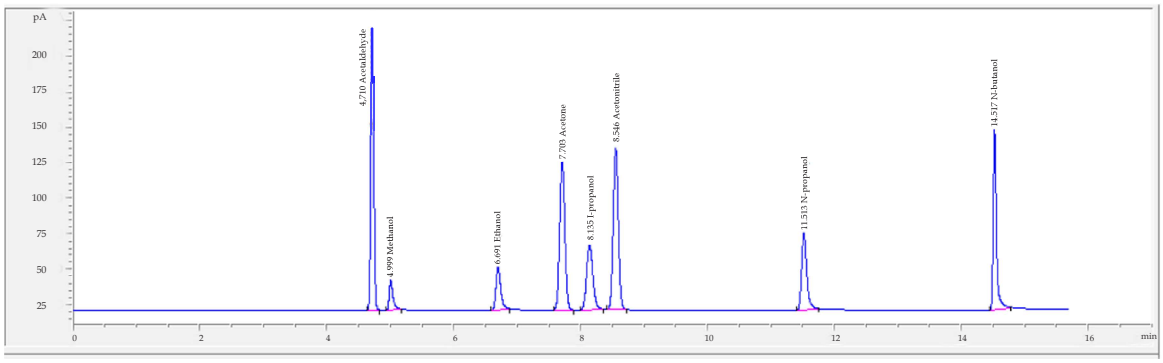

Fig. 14: Chromatogram showing Acetaldehyde, Methanol, Ethanol, Acetone, N-propanol, Acetonitrile, I-propanol and N-butanol separated in the matrix, (Image source: Toxicology lab, Deptt of FMT, AIIMS).

solvents, polish, varnish are the most frequently reported substances of abuse in this age group[63–65]. The common term for this type of substances is 'Inhalants'. Some of the main reasons, for such a large-scale addiction of inhalants is the cheap and easy availability in the near surroundings; legal alternative to other products of abuse and easy concealment as a routine product. Administration of inhalants can be achieved through numerous methods, such as direct inhalation of compressed air duster products through bags or breathing through solvent-soaked rags/handkerchief, and may be referred as huffing, sniffing, snorting, bagging, or spraying depending on the method of administration.

Chemically these compounds are Volatile Organic Compound(s), falling into several chemical groups such as Hydrocarbons, Oxygenated compounds and Halogenated compounds. These chemicals possess low to moderate molecular weights and low boiling points, allowing them to be vaporized and inhaled in their gaseous state at room temperature. The abuse of inhalants produces euphoric and psychoactive effects occasionally resulting in severe toxicity or death[66]. Although these types of cases are not frequently encountered in forensic toxicology casework yet seems like a limited but persistent case type.

Analysis of abuse by inhalants can be detected by direct detection of the parent compound in blood (antemortem) or tissues (postmortem), such as the heart, liver, kidney, and brain, but also on the detection of metabolites, particularly in urine. VOCs in biological matrices can beeasily determined by Gas Chromatography with FID/ ECD after extracting the compounds with static and dynamic Headspace techniques, or even with pulse-heating and solvent extraction methods[67,68].

d. Analysis of Pesticides from Poisoning and Residue cases

Analysis of pesticide in parent and in residue form in a routine analysis depending on the location and types of cases received in forensics laboratories. Out of all categories of pesticides, Organophosphorus pesticides are still widely used and reported. Following metabolism in the organism these compounds cause many cases of acute accidental or suicidal poisonings by the inhibition of cholinesterase activity via phosphorylation by the oxygen analogue. A rapid identification of the causal pesticide would provide very useful information to clinicians for making treatment decisions in emergencies.

Poisoning by organophosphorus pesticides can occur in various situations, India being an agrarian culture its use is widely reported in commercial and domestic agriculture which makes it the most commonly encountered poison in suicide and homicidal cases. Conventional methods for the determination of such compounds require special sample cleanup such as liquid–liquid extraction, Solid Phase Extraction[69,70]. These methods although traditional and most preferred in the routine analysis are labor-intensive, time-consuming, need large amounts of organic solvents, results in less recovery[26–39]. Even with the most careful cleanup it is nearly impossible to achieve absolute clean samples, thus often impurity peaks are found in chromatograms that can sometimes cover the real peaks leading to loos of information in qualitative and quantitative analysis. With the development of methodologies for Headspace-SPME method no matrix compounds are carried onto the GC column, thus limiting sample errors[71,72].

Literature review of the techniques chiefly being used for the analysis of some organophosphorus pesticides in urine, blood, plasma and other biological matrices have reported methodologies like Gas Chromatography-Flame Photometric Detector (GC-FPD); Gas Chromatography- Flame Ionization Detector (GC-FID); GC-Nitrogen Phosphorus Detector (GC-NPD); and GC-Mass Spectrometry (GC-MS)[72–74].

e. Analysis of Volatile Organic Compounds in Stored Biological Matrix

The decomposition process in human body begins immediately after death and may continue for years depending upon the environmental conditions and surroundings in which the body is placed. Formation of volatile organic compounds (VOCs) is an integral part of the decomposition process. In cases of postmortem sampling of biological fluids, in absence of a preservative and cordial temperature conditions, the samples remain in the state of continues putrefaction or decomposition[75,76]. During the course of decomposition, different macromolecules (proteins, carbohydrates, and lipids) breakdown to produce a variety of VOCs. For example, carbohydrates produce oxygen-rich compounds, including alcohols, aldehydes, ketones, acids, esters, and ethers; proteins yield nitrogen, sulphur, and phosphorous containing compounds; lipids break down into hydrocarbons, nitrogen, phosphorous, and oxygenated containing compounds. Estimation of these volatiles became important in scenarios where quantity of these volatiles prior to death is significant.

Quantification of low carbon chain volatiles in samples stored for ethanol estimation is one such condition[77–80]. Estimation of volatile profile during storage can prove to be an storage artefact and misinterpret the results. GC-HS/FID is the most frequently used technique for estimation of volatiles in stored samples for ethanol estimation[48,51-81].

Analysis by one such method, developed and validated in the department laboratory is shown in Fig. 14. The methodology is valid for identification of volatiles like, ethanol, methanol, acetone, acetaldehyde, n-propanol, iso-propanol, n-butanol in biological matrices ranging from blood, vitreous humour, urine, cerebrospinal fluid with addition of Acetonitrile as an Internal Standard for quantification[82].

f. Analysis of Carbon Monoxide Gas from Biological Matrix

Carbon Monoxide (CO) is a deadly, colorless, odorless, nonirritating and tastelessgas that is a product of the incomplete combustionof carbon containing materials. When inhaled, CO is readily absorbed from the alveoli in the lungs into the bloodstream to form a reversible complex with hemoglobin known as Carboxyhemoglobin (COHb) due to affinity of CO being 220 times greater than oxygen. Presence of COHb in place of Oxyhemoglobin in blood reduces the oxygen carrying capacity to the tissues, causing tissue hypoxia. Levels as high as >40% of COHb in blood have been reported to be fatal, with levels between 10%-40% indicative of exposure. Common sources of Carbon Monoxide poisoning include house fires, furnaces or heaters in enclosed spaces, wood-burning stoves, motor vehicleexhaust, and propane-fueled equipment[83].

Although clinical and forensic laboratories predominantly use aspectrophotometric method and colour test for the determination of carboxyhemoglobin (COHb) in blood. Use of GC is being preferred due to its inherent capacity to differentiate between Carbon Monoxide and other putrefactive byproducts like methemoglobin and sulfhemoglobin in postmortem samples. The analysis by GC-HS requires blood sample to be mixed with a cell lyser such as acid or ferrocyanide potassium in a glass vial. This releases carbon monoxide from cells, the gas thus released can be analyzed as a routine procedure in lab[7–84]. The commonest procedure is after headspace injection and gas chromatographic separation, the CO is reduced by a nickel catalyst to methane, which can then detected by using FID[85,86]. Although use of GC-HS for determination of carbon monoxide seems like a specific method with high accuracy and sensitivity problems such as time-consuming sample and standard preparation, expensive has prevented routine use of GC-HS for analysis.

g. Analysis of Cyanide from Biological Matrix in Postmortem Cases

Cyanide is a powerful chemical poison exhibiting cellular asphyxiation following administration. Cases can be received in a forensic laboratory following voluntary ingestion (suicide) of salt of cyanide (KCN, NaCN) or by involuntary inhalation (fire, accidental exposure) of Hydrogen Cyanide.

Cyanide ingestion causes asphyxiation by bonding to the cytochrome C oxidase, a mitochondrial enzyme responsible for respiration and the oxygen carrying hemoglobin forming cyanohemoglobin (CNHb). Blood concentration upto 2-3µg/ml of blood are considered lethal.

Laboratories employ traditional methods of colour tests and spectrophotometric detection preceded by distillation or microdiffusion pretreatment[87]. Technique of Gas Chromatography with headspace sampling provides a faster analysis, higher sensitivity, specific detection and higher rate of recovery[12–88,89]. The procedure of analysis involves sealing a sample in headspace glass vial and addition of internal standard and an acid through the septum, this releases cyanide in the gas phase where detection can then be done using highly specific detectors such as Nitrogen Phosphorus Detector (NPD)[90]. A method describing the determination of cyanide inblood by HS-GC with Electron Capture Detector (ECD) has also been reported. This method involves transformation of cyanide into cyanogen chloride by reacting the hydrogen cyanide with chloramine-T on a stick of filter paper in the space above the blood in the headspace vial[91,92].

h. Analysis of Phosphine from Biological Matrix in Postmortem Cases

Aluminium Phosphide is an inorganic poison if ingested and routinely used otherwise as a rodenticide, insecticide and fumigant for stored cereal grains. When in contact with moisture, water or acid, it releases a colorless, lethal phosphine (PH_3) gas. India has reported phosphine as one of the major agricultural poison. Phosphine can be administered in body by two major routes of administration, first, from oral route by direct injection of tablets or powders containing Aluminium Phosphide and second, by inhaling vapors of phosphine gas in a closed environment[93]. Cases received in forensic laboratories can range from suicidal (ingestion of tablets), homicidal (mainly children) and accidental (inhalation of fumes)[94,95]. Phosphine on absorption in body is rapidly metabolized into phosphite and hypophosphite. Its high lethality is attributed to inhibition of cytochrome C oxidase and oxidative phosphorylation and its lack of specific antidote making it one of the potent poisons.

Phosphine poisoning can be easily detected qualitatively by simple chemical and calorimetric tests in biological (viscera, gastric lavage, urine) and non-biological (liquid, tablets, food material, water) samples[96,97]. In biological samples stored for a certain duration, loss of volatile by evaporation or breakdown by-products can lead to false negative or positive results. Papers as early as 1983, have reported use of Gas Chromatography-Headspace with Nitrogen Phosphorus Detector (NPD), for detection of phosphine from postmortem collected samples[98]. Authors have coupled GC systems with Mass Spectrophotometry (MS) for confirmatory analysis. Sampling has been improved by immediately collecting biological samples like blood, urine, kidney, adrenals, brain and heart directly into the headspace glass vial and analyzing the sample immediately or storing then in HS vials for later analysis. For sample preparation, acid is added to the mixture through septa of the vial, sample is vortexed and vial used in Headspace[99–101].

Conclusion

In analytical chemistry, Gas Chromatography is one of the most frequently used and the most indispensable technique available for testing of thermally stable compounds in vapour form without degradation. Analysis if variety of organic compounds can be facilitated by knowledge about the chemistry of sample introduction, column and capacity of detector. The instrument can analyse single compounds as well as separate and quantify mixtures. Using the variety of detectors available specific to a specific category of compounds, the instrument can simultaneously detect and quantify the analyte. For fingerprint identification of a compound, simply the standard and sample need to be need in tandem against the same conditions of analysis, appearance of peak on same Rt can opion the unknown against known standard.

Ever since Gas Chromatography was commercially available, its application has gradually increased by embracing new growths and directions. In addition to its numerous advantageous features, the basic principle and the theory of GC has been well studied and understood since its inception more than half a century ago. Its use can be readily understood from breadth of its applications. The range of material which can be analysed by chromatographic methods is essentially unlimited with applications found in varied fields of Forensic Science, Food and Agriculture,

Pharmaceuticals, Biological and Clinical chemistry, Environmental toxicology, Polymer industry and many others.

Forensic science, like any other discipline of analytical chemistry, is heavily dependent on Gas Chromatography, a glimpse of its vide application as discussed in the paper. A continuous influx of new column chemistries, sample injection methods, sample extraction assemblies, development of highprecision thermal and pneumatic controlling systems, advancement in control electronics, and a large variety of detection systems have positioned gas chromatography as a formidable foe to other competing analytical instruments. Although the applications of Gas Chromatography are limited to volatile and semi volatile organic compounds, rapid development in derivatization chemistry and sample extraction has allowed for new organic compounds to be analysed by GC, extending its horizon.

Use of Static Headspace-Gas Chromatography as a persistent and mature technique of choice in forensic laboratories and can be demonstrated by the presence in majority of laboratories worldwide. Along the various applications, sample preparation with minimum modification, linear calibration curve, repeatable results, excellent validation parameters, no carryover and minimum handling of hazardous samples/standards/extracts are some of the unanimous reasons of worldwide acceptance of Headspace sampling technique. Capabilities of Headspace has been improved with the use of SPME for sample extraction, leading to application within a wide variety of compounds. The introduction of two-dimensional Gas Chromatography, Gas Chromatography-Isotope Ratio Mass Spectrometry, and Fast Gas Chromatography has contributed significantly to forensic applications by extracting additional information from the collected sample that can aid forensic scientists. It can be speculated with high confidence that Gas Chromatography-Headspace will remain a strong attribute in analytics in the forensic field and will continue to offer new and unique attributes to solve more challenging forensic problems with the growth in advancement of instruments and the samples to be tested.

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Original Article

Journal of Forensic Chemistry and Toxicology
Volume 6 Number 1, January–June 2020
DOI: http://dx.doi.org/10.21088/jfct.2454.9363.6120.3

Characteristics of Homicide followed by Suicide Incidence of our Experience

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Abstract

Background: Homicide-suicides are a distinct group of violent deaths that separate them from murders and suicides. Although such incidents are uncommon, they have devastating effects on families and societies, and typically attract a lot of mainstream media coverage. *Aims:* present study aims to incidents of homicide followed by suicides that occurred within our experience. *Materials and methods:* The observational data obtained from records of post mortem from the department of Forensic from 2017 to 2019. *Results:* Out of the total 82 cases of homicides there were 11 cases murder–suicides involving 11 victims and 5 assailants. It is about 13.4% of all homicides and 3.4% of 2384 medico legal autopsies conducted during the period. female victims were distributed in a wide range of age . Most commonly involved age group ranges between 0-14 years and 15-24 years with4 cases (36.4%), married subjects 6 cases (54%) of rural areas with five cases (63.7%). Drowning or burns was the method of choice in murder–suicide deaths. Most of the cases have a relationship to perpetrator is spouse , boyfriend or girlfriend 5 cases(45.5%) and most of the patients have mental illness in study of 6 cases 54.5%. *Conclusions:* A risk assessment should include determining the chance of involvement not only in suicide and homicide, but also in murder-suicide as a perpetrator or a victim.

Keywords: Homicide-Suicides; Victims; Assailants; Perpetrator.

How to cite this article:

P. Vijaya Sagar, Characteristics Of Homicide Followed By Suicide Incidence Of Our Experience. J Forensic Chemistry Toxicol. 2020;6(1):37–41

Introduction

Homicide–suicides, also referred to as dyadic deaths, involve homicide committed against one or more victims followed shortly thereafter by the suicide. Homicidesuicide denotes a perpetratormurder case, followed by his suicide almost immediately or soon after the homicide.[1] They are fairly uncommon, and responsible influences, and the mode of involvement varies from region to region.[2] Multifactorial factors such as extramarital sex, mental illness, tension at work, financial difficulties, domestic conflicts etc.[3] More than 95 percent of the offenders were identified to the victims of past murder / suicide studies.[4]

The attacker was most frequently a former or current husband or other intimate partner with the crime taking place at the victim's home.

Some experts consider the homicides to be simply a side effect of the suicide, wherein the specific decision to kill oneself precipitates a perceived necessity to kill others. Other experts say that murder-suicide cannot be categorized with either homicides or suicides but is actually a distinct behavior. Although there are some common risk factors among perpetrators of homicide, suicide, and murder-suicide, the latter behavior has some distinct characteristics. Although murdersuicide is a uncommon occurrence, it is widely reported in the media, and therefore such occurrences can appear to increase in frequency. This analysis looks at how murder-suicide rates are increasing.[5]

Some studies find the killings merely a side effect of the suicide, in which the particular decision to kill oneself precipitates a perceived need to kill others. Many experts claim murder-suicide can not be classified as either homicide or suicide, but it is a distinct activity in fact. While certain common risk factors exist among homicide , suicide and murder perpetrators

The attention given to murder-suicides in the past several years could make it seem that the incidence is increasing, but the trend has not been well-studied. This article incidents of homicide followed by sucides that occurred withinour experience.

Materials and Methods

The observational data obtained from records of post mortem from the department of Forensic from 2017 to 2019. We reviewed 2384 cases of medico legal autopsies conducted and found 82 cases of homicidal deaths. Out of these 179 homicide cases, there were 11 cases of 'murder–suicide'. Homicide and suicide incidents were identified by the manner of death recorded from the death certificates. Homicide-suicide incidents were defined as suicide incidents where the perpetrator committed at least one homicide within 1 day prior to his or her suicide death.

We ruled out cases of dyadic suicides and other cases where the person unintentionally died while attempting to save a person's suicide. For this case, the assailants' suicide took place at the same time as the accidents, and there was no need to draw a defined time frame for the assailant's suicide. Specific information of these incidents have been gathered from the post mortem report, police investigation, available history from the relatives or eye witness, etc., and in few cases from hospital case papers.

Table 1: Gender wise distribution of victims.

Gender	Number of cases	Percentages
Males	3	27
Females	8	63
Total	11	100

Sex wise, female victims outnumbered the male victims with a male female sex ratio of 0.4:1 female victims were distributed in a wide range of age.

Results

Out of the total 82 cases of homicides there were 11 cases murder–suicides involving 11 victims and 5 assailants. It is about 13.4% of all homicides and 3.4% of 2384 medico legal autopsies conducted during the period.

Most of the cases are of married subjects 6 cases (54%) followed by single 2 cases (18%).

Table 2: Age wise distribution of victims in study.

Age of victim	Number of cases	Percentages
0-14 years	4	36.4
15-24 years	4	36.4
25-34 years	2	18.2
>35 years	1	9
Total	11	100

Most commonly involved age group ranges between 0-14 years AND 15-24 years with4 cases (36.4%) followed by 25-34 years 2 cases(18.2%).

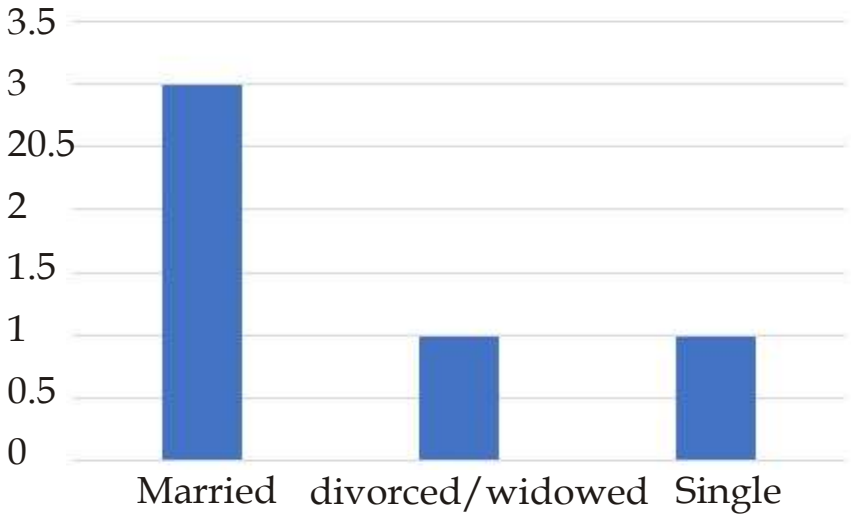

Fig. 1: Incidence according to marital status in prepetrators.

Table 3: Cases distribution of place of incidence.

Place of incidence	Number of cases	Percentages
Rural	7	63.7
Urban	4	36.3
Total	11	100

Majority of the cases were reported from the rural areas with five cases (63.7%) and urban cases are 4(36.3%).

Table 4: Cases distribution according to method of killing of victims in present study.

Method of killing	Number of cases	Percentages
Drowning	3	27.3
Burns	4	36.4
Poisioning	2	18.2
Stabbing	1	9.1
Others	1	9.1
Total	11	100

Drowning or burns was the method of choice in murder–suicide deaths. The assailants also adopted the same method to commit suicide asmajority by burns 4 cases 36% followed by drowning 3 cases(27%) .

Table 5: Relationship to perpetrator.

Relationship	Number of cases	Percentages
Spouse, boyfriend or girlfriend	5	45.5
Parent	2	18.2
Stepchild	3	27.3
Other known to victim	1	9.1

Most of the cases have a relationship to perpetrator is spouse , boyfriend or girlfriend 5 cases(45.5%) followed by stepchild 3 cases (27%).

Table 6: Characteristics of suicide following homicide in present study.

Characteristics	Number of cases	Percentages
Mental illness	6	54.5
Legal problems	4	36.4
Other relationship problems	5	45.5
Drug abuse	2	18.2
History of previous suicide attempts	4	36.4

Most of the patients have mental illness in study of 6 cases 54.5%.

Discussion

In present study total 82 cases of homicides there were 11 cases murder–suicides involving 11 victims and 5 assailants. It is about 13.4% of all homicides and 3.4% of 2384 medico legal autopsies conducted . it is 0.8 per. 10000 persons in study which is in coincidence with

Cohen et al.6 reported very high rate from 0.3 to 0.7 per 100,000 persons to 0.4 to 0.9 per 100,000 persons in their two groups involving elderly persons. According to them overall incidence in the United States and other countries was also ranged from 0.2 to 0.3 per 100,000 persons. Campanelli and Gilson7 who reported the incidence to be 0.26 per 100,000 persons; Hanzlick and Koponen8 reported the incidence to be 0.46 per 100,000 persons; while Hannah et al. 9in their two cohorts reported the incidence to be 0.34/100,000 and 0.38/100,000.

Sex wise, female victims outnumbered the male victims with a male female sex ratio of 0.4:1 . female victims were distributed in a wide range of age . Other studies have found that overcrowding increases the risk of violence, generally, and the risk of violence against women, in particular.10Other studies have found that overcrowding increases the risk of violence, generally, and the risk of violence against women, in particular,we also found that having a history of intimate partner conflict was highly common among most homicide-suicide perpetrators, even those who did not victimize their intimate partners in the homicide-suicide incident (e.g., extrafamilial homicide-suicide and filicide-suicide incidents). Our findings also suggest that homicide-suicide perpetration, particularly by males, is mostly preceded by intimate partner conflicts/violence as opposed to other determinants of suicidal behavior.

In present study most commonly involved age group ranges between 0-14 years and 15-24 years with 4 cases (36.4%) followed by 25-34 years 2 cases(18.2%) . literature reports indicated that the average age of offenders was 40 to 50 years. Moreover, the age ranges included almost all of the decades. The age range in Travis et al. ,16 research was 19 to 86 years. Friedman et al.11 stated in a recent paper on filicide suicide that fathers who killed their children, and then themselves, were typically older (mean age, 38.2 years) than mothers (mean age, 31.8 years). Salari5 concluded that older men (> 60 years) who are suicidal . Cohen et al.6 found that 83 percent of murder-suicides in the older age groups (over 55 years) were of the spousal/consortial type. In the younger groups the percentage was almost the same, at 79 percent. They found some distinct differences, however, between murder-suicides committed by younger and older people.

In present study predominantly married subjects of 54% followed by single 18% . Hannah et al.9 found

that the precipitating co-factor in 48 to 73 percent of the cases was impending divorce or separation. In the New Hampshire study, Campanelli and Gilson 7 found that only 31 percent of the pairs involved occupied the same home at the time of the murder-suicide. Palermo et al.12 found that 31.7 percent had filed for divorce or separated. Comstock et al.13found in Oklahoma that 30.1 percent had a current divorce or estrangement. Cohen et al.6reported that more than half of the younger couples involved in murder-suicides had separated.

In present study drowning or burns was the method of choice in murder–suicide deaths. The assailants also adopted the same method to commit suicide as majority by burns 4 cases 36% followed by drowning 3 cases(27%) . studies by different authors as Oklahoma study,13 97.3 percent of the murder-suicides were carried out with firearms. In their study of older people in Florida, Malphurs and Cohen 14 reported that 100 percent of the incidents involved firearms. Friedman et al.11 noted that in filicide-suicides, firearms were used 73 percent of the time. In England and Wales, Travis et al.15 sought to determine whether tougher gun laws had decreased the rate of murder-suicides

In present study more of the cases have a relationship to perpetrator is spouse , boyfriend or girlfriend 45.5% followed by stepchild in 27%. Malphurs and Cohen16 found that 70.5 percent of all murder-suicides were spousal/consortial. They also found that 10.5 percent were infanticides, 8.7 percent were extrafamilial, and 6.5 percent were familicides (destruction of the entire family). Saleva et al.17 studied homicide-suicide in Finland for one year and found that 90 percent of the victims were spouses and/or children, and 100 percent were family members. Bossarte et al.18 found that homicide-suicides with victims younger than 15 were primarily perpetrated by parents (73.9%).

In this study54.5 % had mental illness . Palermo et al.12 noted that depression often follows a breakup and then triggers the murder-suicide event. Campanelli and Gilson7 found in New Hampshire that 38 percent of those who committed murder-suicide were depressed. Rosenbaum19 found that most murder-suicide perpetrators had depression, whereas none of the homicide-only sample in his study did. Guileyardo et al.20 also mention drug abuse and alcohol as one of the subtypes of filicide. But in the present study there was no history of any drug abuse or alcohol consumption of the perpetrator mothers. Due to culture and other social restrictions.

Conclusions

Although homicide/suicide is a rare incident, each incident results in at least two deaths and frequently includes the death of a child. There are certain clinical presentations that should alert mental health professionals to be suspicious of the risk of possible murder-suicide: a middle-aged man who is recently separated or facing pending estrangement from his intimate partner and who is depressed ; or an older male who is the primary caregiver for a spouse who is ill or debilitated, where there is a recent onset of new illness in the male, depression. A risk assessment should include determining the chance of involvement not only in suicide and homicide, but also in murder-suicide as a perpetrator or a victim.

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Original Article

Journal of Forensic Chemistry and Toxicology
Volume 6 Number 1, January-June 2020
DOI: http://dx.doi.org/10.21088/jfct.2454.9363.6120.4

Pattern of Injuries in Two Wheeler Accidental Deaths in our Experience

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Abstract

Background: Deaths from injuries from motorcycle accidents have over the years remained a significant public health concern in India. *Aims*: Our aim is to determine the distribution of the victims' age and gender and to classify the cause of death and the anatomical pattern of injuries observed. *Materials and methods*: It is a 2-year autopsy based analysis of all fatalities from motorcycle accidents seen from our Teaching Hospital experience. The data were extracted from autopsy records, from police journal excerpts from hospital case notes and analyzed using SPSS version 20. *Results*: For Male there were 30 (85.7 percent) males and 5 (14.3 percent) females: 6:1 female ratio. The highest age group was 31-40 years . Majority of the victims 18 (51%) were Motorcycle riders, followed by passengers on back seat who accounted for 10 (29%), while the remaining 7 (20%) of the victims were pedestrians. Commonest injury in the cases are head injuries constituting 77% followed by thorax 57% and abdominal injuries 42.8%.Causes of death in study are mostly due to crainiocerebralinjuires 51.4% followed by multiple injuires in 40% and sever hemmorage 5.7% and septicaemia 2.8%.*Conclusion*: This study found that males are the main victims of death from motorcycle accidents during the fourth decade of life. Most of the victims were a motorcycle driver. Many died from craniocerebral damage.

Keywords: Injuries; Two Wheeler; Accidental Deaths.

How to cite this article:

P. Vijaya Sagar, Pattern Of Injuries In Two Wheeler Accidental Deaths In Our Experience. J Forensic Chemistry Toxicol. 2020;6(1):43–46.

Introduction

Globally, traffic deaths were the first cause of death for people 15-29 years of age For every person who died in a motorway At least 20 people suffered non-fatal injury in the accident. Such accidents could affect life considerably. Performance, yet also with substantial economic costs. Two- wheeled vehicles are rising in number around the world , especially in developed countries, as motorcycles are fairly cheap to own and run in comparison with other vehicles. The strength, pace and ease of circumventing road traffic holds ups, and its ability to navigate through challenging terrain has made the motorcycle a common means of transportation in major cities and remote areas of India.[1,2] The poor condition of The country's roads and the inefficiency of the public transport network, as well as the worsening of traffic congestion and increased unemployment are major reasons for the booming motorcycle industry.[3]

Head and neck accidents are the leading cause of death from motorcycle collisions, with many deaths taking place despite good use of the available and advanced rehabilitation interventions. Around the same time, there is ample evidence that motorcycle safety helmets are effective in minimizing head injury incidence and severity due to motorcycle accidents. According to the statistics, death is the result of just 1% of motorcyclists injured too seriously to seek medical attention while non-use of motorcycle helmets leads to a change in the continuum of accidents, not just to more deaths but also to more serious non-fatal accidents.

The aim of this paper is to examine the epidemiology of motorcycle accidents and the forensic examination of injury rates in fatal

motorcycle accidents.

Materials and Methods

This study is a 2-year longitudinal, systematic post-mortem analysis of casualties of all 35 fatal motorcycle incidents reported from December 2017 to November 2019 at the Teaching Hospital. Accident fatalities are reportable and victims' remains are stored in the morgue, the key center allocated.

Vehicles involved in the collision are carefully checked to collect evidence on deformation of the structure and protection devices as helmet . In fact, retrospective examination of the location of the accident is carried out on each event to classify . Related precrash activities such as manoeuvres, driving behavior and potential obstructions to the line of sight. A spot to stay. Accident illustration designed to proportion showing the actual location of the vehicle concerned, skid lines, Likewise debris, impact location, and trajectory are registered.

Crash speeds are computed from the vehicle Deformation, skid marks and witness accounts, and then validated using specific software (e.g. Virtual Crash). Crash site data are collected and matched with clinical injuries. All injuries are assessed release from the Emergency Department of a tertiary trauma center. The team shall provide information on injury typologies and severity coded with theAbbreviated Injury Scale (AIS)[4] by total body CT scans, Xray and MRI. A biomechanical correlation among injuries and causes is conducted for each case by a panel of physicians and engineers. The pattern of injuries was categorized according to the different anatomical regions, namely: head , thorax, abdomen, upper limbs, lower limbs, pelvic and spine.

The collected data was analyzed using version 20 of the Statistical Package for Social Sciences (SPSS), and the findings were presented in percentages, graphs , pie charts and bar chart.

Discussion

Nations like India that are experiencing quick financial and social change are encountering an epidemiological mosaic. In addition to the burden of communicable diseases such as malaria, tuberculosis, and HIV / AIDS, increasingly emerging countries have to deal with increasing rates of non-communicable diseases like road traffic accidents.

Table 1: Distribution of cases according to age and gender.

Parameter	Number of cases	
Gender		
Males	30	85.7
Females	5	14.3
Age in years		
1-10	1	2.8
11-20	2	5.7
21-30	11	31.4
31-40	12	34.3
41-50	6	17.1
Above 51	3	8.6
Total	35	100

For Male there were 30 (85.7 percent) males and 5 (14.3 percent) females: 6:1 female ratio.
The highest age group was 31-40 years with 12 cases (34.3 per cent) followed by 21-30 years with 11 cases (31.4 per cent) followed. Among the extremes of life only 3 cases and 3 cases have been seen.

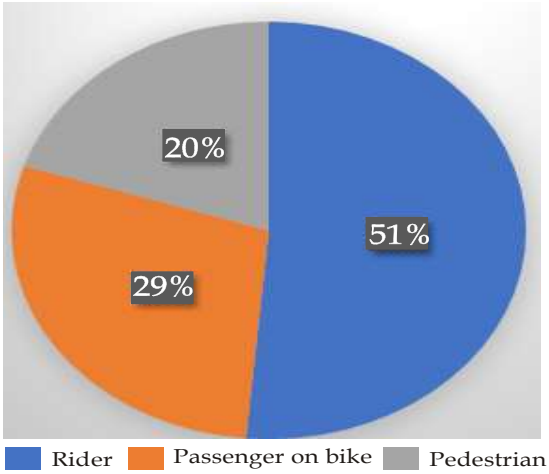

Fig. 1: Pie chart showing status of motorcycle accidents in victims.
Majority of the victims 18 (51%) were Motorcycle riders, followed by passengers on back seat who accounted for 10 (29%), while the remaining 7 (20%) of the victims were pedestrians.

Table 2: Crash configuration of two wheeler in accident.

Crash configuration	Number of cases	Percentage
Head on side	16	45.7
Head on	7	20
Sideswipe collision	5	14.3
Head on rear	4	11.4
Side on head	2	5.7
Rear end	1	2.8
Visibility condition		
Daylight	20	57.2
Night light	15	42.8
Road ways		0
Side Road intersection	16	45.7
Crossroad (four way)	19	54.3

Out of 35 cases more frequently observed crash configurations were: head-on-side (45.7%), headon (20.0%) and sideswipe collisions (14.3%). The majority of crashes occurred in daylight (57.2%) and night light (42,8%), The most frequent road intersection (19/35) was the crossroad (four-way) (54.3%).

Table 3: Region of injuries in the cases.

Injury region	Number of cases	Percentages
Head	27	77
Thorax	20	57
Abdomen	15	42.8
Upper limb	3	8.6
Lower limb	13	37
Pelvis	6	17
spine	1	2.8

Commonest injury in the cases are head injuries constituting 77% followed by thorax 57% and abdominal injuries 42.8%.

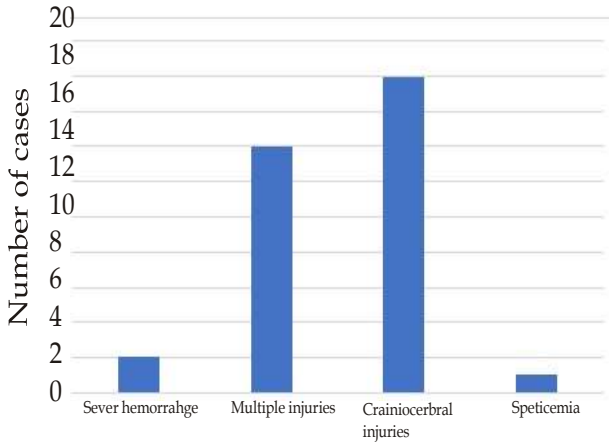

Fig. 2: Bar diagram showing cause of death.
Causes of death in study are mostly due to craniocerebralinjuries 51.4% followed by multiple injuries in 40% and sever haemorrhage 5.7% and septicaemia 2.8%.

Out among all non-communicable diseases, road travel accidents receive the least attention from health officials and clinical experts, as demonstrated by the paucity among accident registries and hospital-based studies on incidents, which are necessary to set up an injury monitoring system. This may be attributed to the assumption that determinants of road traffic collisions are non-medical, alongside the idea that collisions are, 'accidental' and not 'preventable'. The most famous analogy to injurious incidents, 'accidents,' evokes a sense of luck, tragedy, and helplessness. Hence, the term 'accident' should be omitted when addressing injury prevention, and rather, the emphasis should be on exposures

The results in the present research about the age and sex distribution of RTI cases as male there were 30 (85.7 percent) males and 5 (14.3 percent)females: 6:1 female ratio. The highest age group was 3140 years with 12 cases (34.3 per cent) are close to those of other researchers. Results on road traffic accidents in India has brought out that a majority of the casualties are young adults, with a maleto female ratio of 4: 1 to 5: 1.[4] Similarly, the same study has brought out that pedestrians and two wheeler / pillion riders are the most prone category in road traffic accidents.Jirojwang et al.[5] and Singh et al.[6] both stated that the major victim categories in nonfatal road traffic accidents were young adult males, and twowheeler riders and pedestrians were more at risk. An record 82,700 people died and 4,04,800 were injured in road traffic accidents in India in the year 2002, of which, 60 percent were pedestrians and motorized two-wheeler riders (Government of India, 2002). It has been recorded that one person dies in less than every five minutes in India, due to vehicular accidents and the accident rate i.e., number of accidental deaths per 100,000 population is 24.3 .[7,8]

In present study more frequently observed crash configurations were: head-on-side (45.7%) and majority of crashes occurred in daylight (57.2%) at crossroad (four-way) (54.3%).

Commonest injury in the cases are head injuries constituting 77% followed by thorax 57% and abdominal injuries 42.8%.This finding is like that of Heydari et al.[9] in an investigation in the Fars region in Iran saw that the head was the most every now and again harmed site (87.8%). Nwadiaro et al.[2] who detailed that head injury established 40.1% of the wounds in a clinical-based investigation . Studies in Ghana by Kudebong et al. in Uganda by Kigera and Naddumba likewise demonstrated that head injury was the commonest kind of injury, representing 32.2% and 20.0% individually.[10] The littler figure announced in Ghana and Uganda when contrasted with this investigation might be because of a more elevated level of head protector use in those nations.They opined that protective cap use among bike tenants was low here and that may promptly clarify this elevated level of head injury found in that review.

While there have been recent improvements in access to advanced emergency treatment services and in the rehabilitation of head injuries, much of the morbidity and mortality of head injuries continues through effective use of these medical advancements.[11] Injury prevention efforts are, thus, vital to decrease the impact of head injuries. Helmets have been found to minimize the risk of the likelihood of head injury, the seriousness of head injuries when they occur, and the risk of death in both bicycle and motorcycle accidents .In out of 35 cases Causes of death in study are mostly due to craniocerebralinjuries 51.4%. This is consistent with

the results of some research from within Nigeria and from other areas of the world with frequency varying from 33.3 percent to 87.8 percent [21-23]. While head injuries were seen in the majority of the cases, making it the most prevalent trend reported, it only accounted for death in around half of the subjects. The head is probably the most susceptible location of fatal injury in the event of a motorcycle crash, particularly in the environment of poor compliance with helmet use. Nzegwu et al. in Benin City found that none of the deceased victims in their analysis wore a crash helmet at the time of the incident .[12]

The discoveries in this examination bring to the front, the way that cruiser mishap injury passing's are a genuine general medical problem, and the socioeconomics of the casualties additionally calls for worry as these are for the most part people that make up most of the country's workforce. The way that dominant part of casualties bite the dust of head wounds additionally flags the requirement for more exploration endeavours designed towards head insurance for bike riders and travellers. We likewise suggest that the administration ought to make walkways to lessen the demise by people on foot.

Conclusion

New regulations and strategies aim to reduce the occurrence and severity of motorcycle brain injuries. Notwithstanding improved use of the helmet, however, many too many motorized Two-wheeler riders are now suffering extreme and serious head injuries due to the considerable danger of head hitting. Furthermore, twowheeler motorized riders continue to die of damage to critical thoracic and intestinal organs. More efficient helmets and equipment to avoid fatal chest injury and abdominal injury, on the one hand, and strict enforcement of protective gear regulation, on the other, are required to reduce deaths from motorized two-wheelers incidents, In the developed world in particular, where motorized two-wheelers are one of the most important forms of travel and not recreation. High speed motorized two-wheelers can be restricted to athletic activities and not to may road conditions. Technological advances, such as speed tolerance technologies, safe brake systems and well-maintained roads etc. will go a long way towards avoiding motorized two-wheeler accidents.

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Original Article

Journal of Forensic Chemistry and Toxicology
Volume 6 Number 1, January–June 2020
DOI: http://dx.doi.org/10.21088/jfct.2454.9363.6120.5

Liver Cirrhosis According to Forensic Autopsies in Moscow

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Abstract

Introduction: There has been an increase in the number of liver cirrhosis worldwide. Liver cirrhosis in forensic practice is common enough. Medical experts diagnose liver cirrhosis according to morphological data. At the same time this problem in medicolegal analytical reports and publications are still small. The purpose of our research consisted in characteristic of liver cirrhoses by forensic medical examinations for the 12-year period from 2007 to 2018 in the city of Moscow. *Materials and methods.* We studied 3.371 case of death from liver cirrhosis in proportion to the number of non-violent deaths. We analyzed the acts of forensic autopsies and histological studies performed at the Bureau of Forensic Medicine of the Department of Health of Moscow during 2007-2018. We calculated the frequency of occurrence, gender and age, annual changes and the overall trend of mortality from liver cirrhosis. we determined the liver cirrhosis form and liver failure stage.We determined the liver cirrhosis form and liver failure stage by the results of the section and histologic morphological signs. *Results.* We found that forensic examination of corpses diagnosed a significant number of liver cirrhosis. Their annual number averaged 1.15% of total non-violent deaths. During the studied period, the annual incidence of liver cirrhosis changed significantly: it increased in 2009, 2015, 2017, 2018 and decreased in 2013 and 2016. Change difference reached 20.5%. We determined that the highest number of liver cirrhosis were diagnosed between 2009 and 2012, the lowest in 2016. In the last 2 years, the number of liver cirrhosis cases tends to increase. Over a 12-year liver cirrhosis study period we were a steady trend in the decline established. We found that liver cirrhosis during forensic examination of corpses in Moscow were more likely in young and middle-aged men detected. The morphological structure was dominated by small and mixed morphological forms. We found that in histological forensic examination the terminal (48.9%) and decompensated (43.4%) stages of hepatic failure was most often diagnosed. *Conclusion:* According to traditional perceptions liver cirrhosis can rarely be the cause of sudden death and is therefore more likely to be the subject of an autopsy by anatomists rather than forensic experts. On the one hand we established that high (1/100) incidence of liver cirrhosis correlates with the prevalence of this disease among the population of the city of Moscow and on the other indicates a significant number of cases of non-violent death in the total volume of forensic autopsies. We have calculated that the number of annually diagnosed deaths from liver cirrhosis were proportional to the total number of non-violent deaths. During the period 2007-2018 a marked dynamics of decline in the frequency of forensic detection of liver cirrhosis, which is consistent with the general trend of reduction of cirrhosis incidence in Moscow. However, in the last 2 years there was some increase in the number of the liver cirrhosis. The young and middle-aged men in mixed and small-node morphological form at the terminal and decompensated stage of the disease were diagnosed.

Keywords: Liver Cirrhosis; Medicolegal Autopsies; Histologic Researches; Hepatic Acinus; Liver Failure.

How to cite this article:
Yu E. Morozov, P.K. Chattopadhyay, Liver Cirrhosis According to Forensic Autopsies in Moscow . J Forensic Chemistry Toxicol. 2020;6(1):47–51

Introduction

Hidden diseases can cause sudden death. Nonviolent sudden death accounts for up to 60 percent of forensic autopsies and is the subject of scientific study of forensic practice. Liver cirrhosis (LC) represents one type of nonviolent death[1]. In economically developed countries LC is among six main reasons for death at the age of 35–60 years meeting from 14 to 30 cases at 100.000 population. According to literature of five-year prescription the increasing indicators of incidence and mortality from LC are noted.[2, 3] In Russia the incidence of LC is one of the first places. In 2010 it increased by 73.8%, and in St. Petersburg reached 124.4 cases on 100 thousand people that is 3 times higher, than figures for Russia on average. This problem is linked to the prevalence of drug addiction and chaotic sexual encounters.[4]

Although LC is often found in forensic practice, analytical materials and scientific publications on this subject are nevertheless very limited.[5] Since the annual reports of forensic institutions only take into account the types of deaths and the mortality from the LC is combined into a general group of diseases of the digestive system, it is therefore impossible to obtain data on the mortality from LC from these documents. Exhaustive data on the LC are available in primary acts of forensic section and histologic researches. Medicolegal diagnosis of the LC is based, mainly, on results of autopsies and histologic liver researches. Therefore, only forensic documents contain important and exclusive information about the frequency of sudden death from the LC. This information can be used in conjunction with clinical and instrumental laboratory data to substantiate liver failure.

Materials and methods

In this work we studied acts of the forensic autopsies and histologic researches performed at the Bureau of Forensic medical examination of the Department of Health of the Moscow. These documents containing data on death from the LC for the 12-year period of 2007-2018. We calculated the frequency of LC detection in relative sizes a percentage of total non-violent death autopsies for each calendar year. The LC frequency was calculated according to the formula: $R=n/N \times 100$ where R - Index of frequency LC detection (in %); N–total number of forensic researches of corpses at nonviolent death in a year; n – the number of autopsies with the established medico-legal diagnosis of the LC. The direction (trend) of changes (growth or decline) was determined by the values of R-index of the frequency of LC detection.

Also the age and sex of the dead from the LC in groups of comparison were studied. By age, all cases were divided into: 1. Young-aged (1.1. 20-29 years; 1.2. 30-39 years); 2. Middle-aged (2.1. 40-49 years; 2.2. 50-59 years); 3. Old-aged (3.1. 60-69 years; 3.2. 70-79 years). From the documents (records) found out the reasons for the development of LC, specified the presence of comorbid diseases. The results of immune blotting obtained from the Moscow City Center for AIDS Prevention and Control were considered to justify HIV infection. The Acts of histological studies were analyzed, in which, when describing the liver, the Knodell index confirming chronic alcoholic intoxication was determined [6, 7]. The incidence of LC complications, which were the cause of death, was recorded. Among the studied observations, the indicators of occurrence of alcohol-viral; alcoholic and viral; tumor and medicinal causes were revealed.

According to the standard classification we have recorded the following morphological forms of LC [8, 9]: 1. The Small Nodular LC (Small Nod LC) with a diameter of knots in limits of 1-3 mm; 2. The Large Nodular LC (Large Nod LC) with knots diameter more than 3 mm; 3. The Mixed Nodular LC (Mix Nod LC) with both small and large knots. To establish liver failure in LC, the microscopic structure of liver parenchyma was examined. Histological changes were determined in the structural-functional departments of the hepatic acinus: portal triads; hepatic beams; central veins. Depending on prevalence of destructive and dystrophic changes in departments of a hepatic acinus allocated three stages of a liver failure [6]:

1. The compensated stage, when initial destructive and dystrophic changes of a hepatic parenchyma only in portal triads departments of acinus were found;
2. The decompensated stage when destructive and dystrophic changes of hepatocytes in both portal triads and hepatic beams departments of acinus were expressed;
3. A terminal stage of a liver failure when destructive and dystrophic changes in hepatocytes in all three departments of hepatic acinus - portal triads, hepatic beams and central veins areas were astablised.

Results of a research

During the 12-year period 2007-2018 in Moscow,

forensic experts diagnosed 3.371 cases of death from LC. The annual LC count was between tens and hundreds of cases, and the average annual frequency of LC detection was 1.15% of the total of all forensic autopsies in nonviolent death. In different years of the studied period the number of the diagnosed LC's differed significantly. Therefore, for each calendar year, we calculated the R-index of the frequency of LC detection relative to the total number of autopsies in non-violent death. We have found that R-index of the frequency of LC detection have increased in 2009, 2015, 2017, 2018 and decrease in 2013 and 2016. In 2009 the R-index of the frequency of LC detection compared to 2008 is increased by 3.77 times. Throughout 4 years (2009-2012) the sizes of R-index of the frequency of LC detection were high and ranged from 33.98% to 27.03%. Then we have revealed that the highest R-index of the frequency of LC detection in 2009 and the lowest in 2016 were installed. From 2013 to 2018, the dynamics of the number of detected cases of LC changed in a wave-like manner (Chart 1).

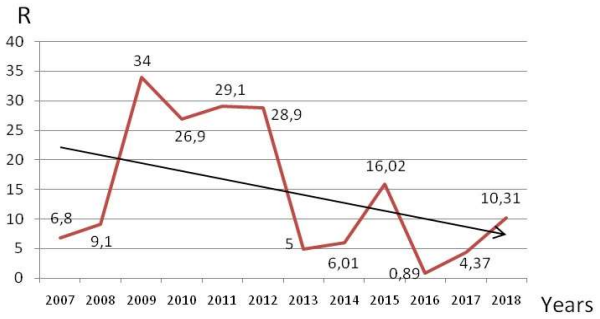

Chart 1. Frequency of occurrence of LC during 2007-2018
R – Index of frequency LC detection (concerning at non-violent death, in %)Trend (direction of LC frequency dynamics).

The largest distinctions R-index of the frequency of LC detection (falling by 17.9 times) between 16.01% (2015) to 0.89% (2016) were established. We found that R-index of the frequency of LC detection annual changes were proportional to the number of non-violent deaths. We revealed that for the 12-year period baseline trend shows steady dynamics decrease frequency of LC occurrence. The last 2 years R-index of the frequency of LC detection have increased by 79.6 and 57.7% respectively.

We have established a percentage distribution of the age groups studied relative to the total number of installed LC: 20-29 years - 3.1%; 30-39 years – 44.6%; 40-49 years – 28.9%; 50-59 years – 9.7%; 60-69 years – 7.3%; 70-79 years – 6.4%. It turned out that men's death from LC prevailed over women. The male was assigned in 78.6%. We have identified the causes of the LC: Virus-Alcoholic (41.2%); Alcoholic (35.0%): Tumoral (12.0%); Virus (8.9%); Medicinal (2.9%). In all years the quantity of mixed and small nodular forms LC were prevailed. However, since 2012 the quantity the large nodular LC forms got a tendency to increase The frequency of morphological forms of LC is presented on chart 2.

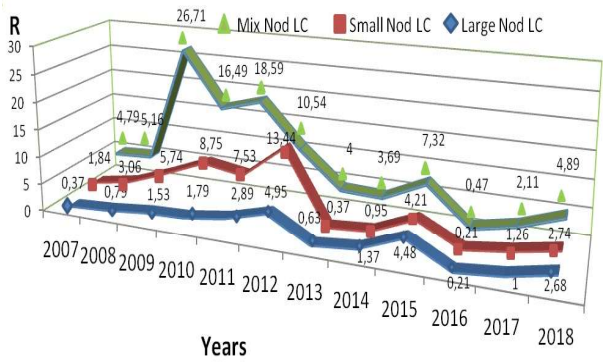

Chart 2. Morphological forms of LC.
R–Index of frequency of detection of morphological forms of LC (relative to the number of non-violent deaths).

Years	Stades of a Liver Failure Frequency, concerning LC autopsies, in %		
	Compensated	**Decompensated**	**Terminal**
2007	1.6	43.6	54,8
2008	0.0	56.4	43,6
2009	1.9	52.4	45,7
2010	4.6	49.5	45,9
2011	1.9	44.9	53,2
2012	2.3	44.2	53,5
2013	2.6	26.3	71,1
2014	8.8	35.3	55,9
2015	3.4	43.2	53,4
2016	0.0	46.2	53,8
2017	7.7	38.5	53,8
2018	3.4	40.7	55,9

We established that by histological forensic examination the terminal (48.9%) and decompensated (43.4%) stages of hepatic failure was most often detected.

Discussion of the results

It is thought that LC can rarely be the cause of sudden death, because in the case of severe decompensated LC patients are usually treated in clinics. Autopsies about death from LC are performed, as a rule, by doctors pathologists, rather than forensic experts. We have found that in Moscow LC occupies a significant place. In the total number of forensic autopsies in 2007-2018 LC with an average frequency of 1/100 from cases of non-violent deaths were detected. According to literary data a marked increase in the number of deaths from LC in Russia was observed between 2009 and 2012, which was associated with excessive use of alcohol and drugs [10]. The highest LC mortality rates characterized by an increase in drug addiction

occurred in 2009. Since 2013 there has been a decrease in the number of deaths from this disease.

Our research supports these literary data. We established that in 2009 the number of deaths from LC in Moscow were increased by 3.77 times as compared to 2008 and remained so high until 2012. From 2013 to 2018 the LC level was reduced and then changed in a wave-like manner. During the whole studied period from 2007 to 2018 in Moscow a steady trend of decrease of mortality from LC were revealed. We have established a marked dynamics of decline in the frequency of forensic detection of LC, which is consistent with the general trend of reduction of cirrhosis incidence in Moscow. Annual changes of LC were proportional to the number of non-violent deaths. The reduction of LC proportional to non-violent death is new information not reflected in recent publications.[11]

The prevalence of alcohol-viral LC in recent years is mentioned in available sources of literature.[5] Our research has confirmed that alcohol-viral (41.2%) and alcohol (35.0%) LC were most often detected by their etiological affiliation. Tumor (12.0%), viral (8.9%) and medicinal (2.9%) LC etiology were less common. In men (78%) LC more often than in women were detected. The majority (73.5%) young and medium age were deceased, which is consistent with the data previously obtained.[12]

Hepatocyte necrosis at the LC due to the direct toxic action of ethanol is an important feature of alcoholic genesis. LC formation occurs for many months or years in combination with autoimmune processes. During this time, the gene apparatus changes and new generations of pathologically altered liver cells appear [6]. In order to evaluate morphological changes of liver parenchyma at LC used a new approach to analyse the structural-functional departments of hepatic acinus are histologically examined. The prevalence of destructive-dystrophic hepatocyte changes is a morphological component of hepatic insufficiency. It is considered that in LC destructive changes of hepatocytes first appear in portal triads, then in beams and in the area of central veins, characterizing successive stages of severity of hepatic insufficiency [7]. Destructive hepatocyte changes found in all three structural regions of the acinar tract indicate the onset of the terminal stage of hepatic insufficiency. [12]

We executed histological examination of liver parenchyma changes to study LC pathogenesis. A new approach for evaluating hepatocytes in the structural functional departments of hepatic acinus was used. We have found that when dying from LC, decompensated and terminal stages of hepatic failure are recorded more frequently. We found that in cases of death from LC decompensated and terminal stages of hepatic failure were more often recorded.

It is noteworthy that in the terminal stage of hepatic insufficiency we were revealed the patho-morphological changes and separate clinical-laboratory indicators from different sides characterize the same condition - the liver coma. [12] Between 2013 and 2018, there was a significant decrease, an average of 5.93 times (by 20.5%) histologically confirmed cases of hepatic failure. These changes require further reflection to clarify their causes. It is possible that at this time the falling in the number of detected cases of liver failure is due to the decrease in the frequency of alcoholic excesses accompanying sudden death. [1,3] The main scientific significance of the study is the possibility to estimate the dynamics of mortality and pathogenesis of LC by morphological characteristics of developing liver failure.

Conclusions

Mortality from liver cirrhosis in Moscow according to forensic autopsy data for 2007-2018, averaged 1.15% of non-violent deaths. The highest figure was in 2009. After 2013, the number of liver cirrhosis began to decrease. The lowest frequency of detection of liver cirrhosis was established in 2016. Over a 12-year period, there was a steady trend towards a decrease in liver cirrhosis mortality. The number of diagnosed liver cirrhosis was proportional to the number of autopsies in nonviolent death.

Among the causes of liver cirrhosis were viral-alcoholic (41.2%) and alcoholic (35.0%) etiological forms. Mixed and small-node morphological forms of the disease were more common in young and middle-aged men. We found that histological forensic examination was more likely to detect terminal (48.9%) and decompensated (43.4%) stages of hepatic failure.

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Review Article

Journal of Forensic Chemistry and Toxicology
Volume 6 Number 1, January–June 2020
DOI: http://dx.doi.org/10.21088/jfct.2454.9363.5219.6

DNA testing Falsehood: Conditions and Facts

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Abstract

DNA as an evidence plays a very important role in establishment of human identity through genetic profiling both in criminal and civil disputes. DNA has emerged as a powerful investigative tool as no two persons can have same DNA profile except for identical twin. The proper collection of any biological samples is must for effective utilization of DNA as medicolegal evidence. Single biological sample such as blood, semen, saliva, and hairs etc without any contamination can easily identify a single person. But, if there is mixing/contamination of the biological samples or there is insufficient sample collection from the object or surface for genetic profiling then the results can be ambiguous. In such situations, reports are inconclusive, also it could be difficult to exclude genetic sample of perpetrator from such mix profile results. Furthermore, the new age treatment therapies such as In-Vitro Fertilization (IVF), Blood transfusion, bone marrow transplantation and organ transplants can also hinder to present the actual identity or genetic profile of a person due to genetic mixing. Therefore, biological samples collected from crime scene can either match with suspect or acquit him/her from suspicion. Modern DNA forensic methods are powerful and sensitive, carelessness or ignorance in proper history and handling procedures for biological evidence can result in an unfit sample for analysis.

Keywords: DNA evidence; Genetic profiling; Reliability; Transplant therapies; Fallacies.

How to cite this article:
Nidhi Sharma, Neha Sharma, Arijit Dey, et al. DNA testing Falsehood: Conditions and Facts J Forensic Chemistry Toxicol. 2020;6(1):53–57

Introduction

DNA is a powerful investigative tool because no two individuals have same DNA profile except identical twin. DNA evidence plays a very important role in identification of human identity through genetic profiling in civil dispute and criminal cases. The most reliable tests study as a part of the DNA testing is Short Tandem Repeats (STRs).[1] The method is called multiplex polymerase chain reaction (PCR). PCR is a method of amplifying and sequencing DNA. It is the method used to diagnose hereditary and infectious diseases for clinical aspects. DNA testing is considered to be the only completely accurate method of determining parentage in the medical community.Over the years, genetic parental testing technology has advanced substantially. During 1920's, paternity was determined through blood type. Blood types are not exclusive for genetic relationships; while they can be used to exclude certain people but cannot conclusively determine the parentage.[2,3] After more perceptive knowledge regarding DNA, an easier and correct techniques has been developed. Current methodology utilizes study of a particular section (short repeats) of DNA on chromosomes which determines genealogical heritage i.e. genetic characteristics passed from generation to generation. In forensic cases DNA testing, establishment of paternity or maternity of child were involved and revers parentage for identity of deceased.[4] The analysis of DNA testing worldwide is usually based on comparison of profiling of biological evidence with reference samples by using multiplex PCR technology. In this paper we are highlighting the possibilities of fallacies during DNA profiling.

Strengths of DNA testing in Forensic community

Although 99.9% of human DNA sequences are the same in every person in the world, there is still enough difference in order to distinguish one person from another. Using a method called DNA testing, also known as DNA profiling where experts or scientists analyses a long chain of DNA to identify specific "loci." These loci are very similar when they are comparing the loci of two closely related people, but the differences are much greater among unrelated people. Thus, in criminal prosecutions, DNA evidence is often offered to link the presence of accused with being at the scene of crime, as well as be often used by the defendant to prove his/her actual innocence.[5]

In contrast to this all DNA evidence is not equal. Sometimes its clear single biological sample such as blood or semen that can identifies a single person. If it's contains more than one person's biological samples or it is just a few skin cells left on an object as a genetic material, then it can be more ambiguous for experts. In such situations, report were not conclusive, or the defendant could not be excluded from the mix profile result.There is variation in statistical approaches used to evaluate the strength of the evidence where involvement of a particular person is decided than the approaches used must be supportable.[6,7]

A. Social and legal source of evidence

DNA analysis has brought a strong change in the world in medicine, especially forensic domain. So majority of forensic cases analysed by DNA testing involve disputes and crime via paternity or maternity of child or to identify unknown deceased. In this regards new methods have been developed, validated, and put into use to help in criminal investigations. New approaches for interpreting evidence via probabilistic modelling are being introduced. The validity and accuracy of older and current methods are even challenging. [7] The DNA analysis is based on comparison of result of biological evidence with reference samples such as blood /buccal swab. Sometimes stored or preserved biological and other intimated items of individuals like tooth brush, cloths, shaver, other object collected at crime scene are being send for DNA testing to establish the identity of deceased, victim, accuse; under such circumstances authentication of test is usually problematic.[8]

B. The technical reliability of DNA evidence depends on various aspects

1. Quantity and quality of the sample analysed
2. Laboratory equipment / technique in analysing the sample
3. Polymerase chain reaction (PCR) based testing is relatively insensitive to degradation
4. Analysis of poor quality DNA samples may lead to uncertain results requiring substantial interpretation by the forensic scientist
5. When a DNA sample contains a mixture of several persons' DNA, and the forensic scientist does not account for this, the resulting DNA profile may be incorrect.

C. Laboratory Accreditation

1. Accuracy of DNA analysis depends on the quality control and quality assurance procedures in the forensic laboratory.
2. Quality control refers to measures to help ensure that each DNA analysis result (and its interpretation) meets a required standard of quality.
3. Quality assurance refers to monitoring, verifying and documenting laboratory performance[9]

D. Human errors during sample handling

As it is very sensitive technique the DNA samples may be cross contaminate with other human DNA depending on nature of crime where crime scene samples may contain a mixture of fluids (biological and non-biological) or tissues from different persons. Moreover the crime scene sample may be contaminated during sample handling and transferring from the crime scene to the laboratory; or carry-over contamination can happen during PCR-based testing if the amplification products of one test are carried over into the mix for a subsequent PCR test. Sample mishandling, mislabelling or contamination is more likely to compromise a DNA analysis than an error in the analysis. Samples can be contaminated at any stage of collection, transportation and analysis of DNA samples. Laboratory staff unintentionally could make errors in conducting DNA analysis, in interpreting or reporting the results of the analysis, or in entering the DNA profile result after

analysing the allelic data. This might result from failure to comply with an established procedure, misjudgement by the expert, or some other mistake. While protocols and precautions can be introduced to minimise the opportunity for error during analysis or interpretation, the potential for human error cannot be fully eliminated. [10]

E. Tempering or indirect transfer with the samples at crime site

A suspect's DNA profile might match the profile found at a crime scene as a result of tampering with the crime scene, or subsequent substitution of DNA samples. This might occur where the actual offender, a police investigator, or another person deliberately leaves a suspect's genetic sample at the crime scene. [11] Alternatively, it is possible that a suspect's sample might later be substituted for the actual crime scene sample to falsely implicate the suspect in the offence. There may be possibilities of such instances during practice and procedure for the collection of crime scene samples and handling of these samples for transfer to the laboratory, and at the laboratory itself, the steps to minimise the opportunity for tampering must be assured. In contrast, complexity and different possibilities of DNA transfer make the identification of contamination incidents quite difficult as biological material can not only be transferred by means of direct but also by means of indirect transfer during sample collection and transportation.

A match between the crime scene profile and a defendant's profile does not prove that the defendant committed the particular offence. There may be several alternative explanations for a match, because there is possibility that sample was deliberately left at the crime scene during or immediately after the offence and could be 'planted' at the crime scene. False positive result may be possible due to error during DNA handling.[12]

F. Samples of close relatives

Close genetic relatives have more common genes than unrelated persons. Therefore, it is possible that an innocent person's DNA profile could match with the profile obtained from a crime scene, where the offender was in fact that person's sibling or other close relative. However, the chance of such a coincidence will decrease inversely as the number of loci examined along the DNA molecule increases [13]. In this condition sample originated from a close relative of the suspect; or it originated from an unrelated person who by coincidence has the same DNA profile as the suspect.[14]

G. Cases of Mix DNA profiling

The evaluation and interpretation of forensic DNA mixture evidence has greater interpretational challenges due to increasing complex mixture evidence. Such type of challenges are occurs when low quantity or degraded DNA evidence shows dropouts of locus and allele, allele staking and stutter artefacts instead of true alleles. There are common concerns that methods utilized for interpretation of complex forensic DNA mixtures may not be implemented properly in some casework. Similar questions are being raised which lead to some confusion about mixture interpretation for current and previous case works.[15]

H. Disputed Parentage test

Parentage testing mainly relies on autosomal STRs DNA profiling, the cases of monozygotic twin, motherless paternity or fatherless maternity (where child and one parent are available), in endogamous and consanguineous generally showed inconclusive DNA profile because of common alleles where as in cases of mutation or degradation showed incomplete DNA profile. The conventional DNA test methodology by 15 STR loci can lead to false inclusion in very closely related parent and child or siblings as they share more obligatory alleles than the unrelated.As parentage testing determines an alleged individual is biological parent of disputed child are based on likelihood ratio, if alleged parent are sharing common alleles it is difficult to eliminate.[16,17] Similarly for monozygotic twins involved in sexual assault cases DNA testing is challenging and even the sample of product of conception (POC) can be contaminated or produced mixed DNA profile.In cases of child swapping in hospital or other places and adopted child may give mismatched DNA results.

I. Cases of rape survivor

Rape is the great social problem of society and it is heinous crime in many develop and underdeveloped countries. It has been reported that rape cases are often committed by persons who are known to the victim and 5%-15% of assaults are perpetrated by a stranger or unknown to victim. The biological evidences play important role in sexual assault cases. In such crime for DNA profiling, biological samples of victim and accused required which includes epithelia cell and spermatozoa

(semen). In rape cases improper or delayed collection (after 24 to 72 hours) of samples and low quantity of DNA can give inconclusive DNA profiling results. On other side if involvement of more than one person (gang rape), generates a mix DNA profile.The technique used for differentiation of male and female DNA is not always successful. [18]

J. Methods of treatment: Bone marrow/Organ transplant and IVF

Advancements in treatment procedures and new methods of treatment have evolves to save lives by transplant therapies. An organism having two or more genetically distinct cells is called chimera. In cases of bone marrow or organ transplantation therapy, chimeras testing can be performed for acceptance or rejection of patients who have received a hematopoietic stem cell transplant. The test involves identifying the genetic profiles of the recipient and of the donor and then evaluating the extent of mixture in the recipient's blood or bone marrow. There are cases when man has transplant bone marrow, his DNA changed to that donor who has live far away from the recipient.[19–21] In in-vitro fertilization (IVF) biological father can be donor for the children or any other complication during such procedure can be misinterpreted DNA profile. Other hematological disease condition where blood transfusion is the only treatment procedure like thalassemia, anemia, and also some form of chemotherapy may also cause some misinterpretation of results.[22–24] It can be inferred that in criminal offence cases, some specific medical treatments can hinder the actual identity of person due to mix profile.

Conclusion

In the DNA profiling procedures to choose a suspect, investigators try to match the sample for loci and the suspect's loci as closely as possible. But often, crime scene samples are imperfect and the DNA breaks down, so the loci are weak and unable to generate good profile. That makes finding of 100 percent match very challenging. Modern DNA forensic methods are powerful and sensitive, but great care must be taken to prevent unfairness of justice. The methodology for DNA test opted by the expert is also a crucial, good practice of laboratory protocol is also important to make the uniformity in DNA profile. Carelessness or ignorance in handling procedures as well as case false history for biological evidence can result in an unfit sample for analysis.

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Review Article

Journal of Forensic Chemistry and Toxicology
Volume 6 Number 1, January–June 2020
DOI: http://dx.doi.org/10.21088/jfct.2454.9363.6120.7

Exploring Barriers and Challenges in Drug Facilitated Sexual Assault (DFSA) Cases: A Short Review

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Abstract

Drug Facilitated Crimes is an emerging discipline in the field of forensic toxicology. The most widespread DFCs are Drug Facilitated Sexual Assaults (DFSA); sexual crimes which occurred with involvement and administration of drugs. Drugs such as flunitrazepam, lorazepam, GHB, nitrazepam, midazolam, temazepam, ketamine are added to the food/drink of the victim during any party, or at the bus stands or railway stations without the knowledge of the victim and inhibits person's ability to resist and prevent her from remembering the assault. These drugs being depressants in nature cause symptoms of drowsiness, impaired perception, nausea, vomiting, unconsciousness, anterograde amnesia and mislead the police to form an assumption that the person has only drunk and not drugged. The metabolism of these drugs is too rapid and the drugs get excreted from the body before the incident is reported to the Police Officer. These fast-acting drugs therefore leads to various problems in the detection and analysis of drug facilitated crimes. The paper focuses on such problems and challenges associated with these drug facilitated sexual assault cases.

Keywords: Drug facilitated sexual assaults; Metabolism; Detection; Challenges; Crime.

How to cite this article:
Neha Jain, A. C. Rajvanshi. Exploring Barriers and Challenges in Drug Facilitated Sexual Assault (DFSA) Cases: A Short Review. J Forensic Chemistry Toxicol. 2020;6(1):59–63

Introduction

Recent advancements in the field of forensic toxicology in present crime scenario has lead to the emergence of new discipline of forensic toxicology called as Drug Facilitated Crimes (DFC). The most widespread DFCs are Drug Facilitated Sexual Assaults (DFSA) a term used to define sexual crimes occurred with involvement and administration of drugs. In these cases victim is made incapable of fighting or consenting legally against the acts of sexual crimes due to the pharmacological effects of drugs. It is a complex and a highly prevalent problem presented to Law Enforcement Agency, Police Department in the country nowadays. Drug-facilitated sexual assault occurs when alcohol or drugs are used to compromise an individual's ability to consent to sexual activity. These substances make it easier for a perpetrator to commit sexual assault because they not only inhibit a person's ability to resist but prevent her from remembering the assault.[1,15]

Any person stranger, friend or colleague can be a victim of these crimes as these DFSA crimes can happen with anyone by any friend, stranger, colleague, neighbour, relative, or any other person for that matter. The cases suspected of DFSA are reported at any place, be it a workplace or any hotel room or at the time of a date, or such acts can take place at assailant's house or victim's own house or room or at any club or pub or during a party or gathering depending onto the perpetrator.[1,6,12]

Drugs such as flunitrazepam, lorazepam, GHB, nitrazepam, midazolam, temazepam, ketamine are added to the food/drink of the victim during any party, or at the bus stands, railway stations without the knowledge of the victim. The drug is secretly administered to a person usually through a food or drink. The main objective of the perpetrator is to make the victim helpless to respond against such acts. These drugs start their action within 30 minutes of ingestion and make the person prone to assault. These drugs being depressants in nature cause symptoms namely drowsiness, impaired perception, nausea, vomiting, unconsciousness, complete loss of motor functions, and anterograde amnesia etc. similar to other depressants specifically alcohol. These symptoms mislead the police to form an assumption that the person has only drunk and not drugged. These drug assisted crimes can also occur by the voluntarily administration of drug by the victim, of which the perpetrator take advantage and commits such assaults. [1,6,4]

Current Scenario in India v/s Global Scenario of the Problem

In India drug facilitated sexual assaults are committed using a large number of benzodiazepines namely flunitrazepam (rohypnol), nitrazepam, alprazolam, temazepam, diclazepam. There are reported a few incidents of such assaults revealing the use of these sedatives for spiking the drinks of the girls in cases of revenge and love affairs. The prevalence of involuntary administration of these drugs in such cases increased from 25% to 33% over the past 2-years. These drugs get readily absorbed in the gastro-intestinal tract and their effects can be synergistic if taken in combination with alcohol in smaller doses. Oxazepam, diazepam, lorazepam, and clonazepam, temazepam are some of the drugs detected in majority of cases. These drugs are always found in combination with other substances.[6,11,19,20]

Flunitrazepam known as Rohypnol by trade name is used most frequently in cases of drug facilitated sexual assaults and classified as Schedule IV drug of benzodiazepine. It is a central nervous system depressant drug produces sedation to severe coma. The drug is reported to be ten times more potent than the commonly occurring benzodiazepine diazepam. The drug becomes active within 15-20 min of administration and lasts for 4-6 hours. The drug is abused prevalently in India among teenagers and the students as it gets easily dissolved and mixed in drinks/food because of its tasteless and odorless properties. These properties make its detection difficult in routine drug screening procedures. A main problem in such cases is the challenge for detection of flunitrazepam in different body fluids even if present in fairly large concentration.[1,6,7,8]

Other than benzodiazepines there are also drugs reported to be abused for this purpose for example non- benzodiazepines, sedatives (zolpidem, zopiclone), ketamine, GHB, barbiturates, opioids, antihistamines, chloral hydrate, antipsychotics, anticholinergics, and various centrally acting muscle relaxants agents in facilitating the sexual assault. Various substances will get added up in this list depending on the discovery and availability of the new drugs.[2-5]

Ketamine, a Schedule III controlled substance in United States and a short acting anaesthetic for use in humans and animals, is another predatory drug used by perpetrators for committing sexual assault. A large number of cases showing abuse of ketamine suspected of DFSA that have been reported in various countries (Canada, United states, United Kingdom etc.) The drug is usually given to the victim by mixing in the drink or in combination with alcohol prior to the commission of acts involving sexual assault, robberies etc. Ketamine being fast acting in nature i.e. the onset starts within about 0.5-2h intramuscularly and 4-6 hrs orally, only lasts up to about 30-45 minutes. Ketamine administration in the person leads to visual disturbance, hallucinations and the victim undergoes a dream like state showing confusion, anterograde amnesia and delirium effects. Some memories of the assault may sometimes be experienced by the victim after ingestion of the drug few days or weeks after. Due to the short time span of ketamine, it is not detected in routine toxicology screening and may give a false positive result for Phencyclidine.[1,3,6]

A study conducted in Paris reported that out of 119 cases analyzed, 8% suspected of drug facilitated sexual assault showed positive results for GHB. Another study conducted in Netherland and Ireland reported about 73% and 56% cases of drug facilitated sexual assault which shows the abuse of alcohol and other drugs respectively. Their study also reported that benzodiazepines mainly diclazepam and its metabolite lorazepam is detected in the biological samples in a high frequency due to its higher window of detection. Another study conducted in France from June 2003 to May 2004 reported 128 cases of drug facilitated crimes and about 18% of those cases showed the abuse of zolpidem, clonazepam followed by bromazepam, nordazepam and midazolam.[12]

These drugs can be used either directly in combination with alcohol or can be introduced secretly into alcoholic or non-alcoholic beverages without noticeably changing the taste or colour because of their readily dissolving nature. The metabolism of these drugs is too rapid and the drugs get excreted from the body before the incident is reported to the Police Officer. Thus the fast acting drug leads to various problems in the detection and analysis of the drug facilitated crimes.[7]

Reasons for delay in collection of sample

One of the major impediment is the delay in examination of DFSA victims present for medical attention and the victim's unwillingness about reporting of the incident due to a sense of discomfort, feeling of humiliation and embarrassment. This unnecessary delay in reporting limits the chance of detecting the drugs which has been ingested by the victim either due to the intentional consumption or by covert action of the perpetrator. The delay also arises due to the onset of effects (amnesia) of the drugs under the influence of which the victim remains uncertain about the facts that accounts for rape and sexual assault. This delay in such cases is a significant contributing factor.[4,6,10,11]

Detection window for the drugs used in sexual assault cases

Easy availability of wide variety of drugs to the perpetrators and the information available on internet has increased the rate of occurrence of these crimes. Any inadequacy in sample collection makes the detection of these drugs difficult. In these cases, by the time biological samples are collected more than half of the drug is passed out from the victim's body and only a small residue is left. For example delay in the collection of blood sample for more than one or two hours or urine sample beyond four or more days after drug administration may give negative results. Storage conditions of such biological samples suspected of containing drugs of interest also affects their stability due to the potential interaction of the drug in the matrix. The detection of the drug in traces is quite difficult and sometimes, impossible with the usual standard analysis toxicology protocol. The matrix chosen for the detection also affects the timeline of analysis of these drugs. Therefore, oral fluid can be chosen over other conventional matrixto avoid impeachment of privacy, specific storage conditions and invasiveness. Using oral fluid as a sample matrix eliminates the requirement of large sample volume and tedious extraction procedures. The drug taken even in small amount can easily be detected using oral fluid as a sample not only in DFSA cases but also in cases of drug driving.[4, 6, 9, 11]

The drugs used for this purpose possess the tendency to get metabolized rapidly in the body and the rate of metabolism of these drugs namely lorazepam, triazolam and alprazolam, zopiclone and zolpidem in the individuals is so quick that making an assessment of the dose and the exact time of their exposure is difficult. This rapid metabolism leads to the formation of respective metabolites and the detection of these drugs and their metabolites is usually hindered due to an extensive delay in collection of sample.[4,9,10]Similarly the detection window for GHB is about 6-8 hours in blood and 10-18 hours in urine, the drug can be detected in its parent form within this time span only. Another problem involves in the analysis of biological samples of these cases is of ketamine due to the pharmacology of the drug and lack of rapid and sensitive screening methods. Similarly flunitrazepam get absorbed orally and reached to a peak concentration within 30- 90 minutes and gets metabolized to 7-aminoflunitrazepam. Other sedative hypnotics drugs namely zolpidem also gets rapidly metabolized in the body into its inactive metabolite and can be detected into bloodstream up to 7-8 hours.[12,3] All these problems leads to an extensive delay in reporting of these cases therefore an appropriate strategy in terms of prevalence of these drugs used needs special focus for investigating such crimes.

Legal Provisions and Penalties in India and Other countries

The DFSA crimes committed using administration of such drugs are increasing at an alarming rate in different countries of the world including India therefore legal penalties need to be strictly imposed in these cases under consideration as a heinous crime.

In European countries the details of the drugs commonly abused, specifically for performing sexual acts is not mentioned in the criminal code but the legal system has the provision for punishments and penalties to control the crimes associated with administration of any poison or drug or any substance added to the drink covertly for commission of robbery or any homicide.[12]

Drug Facilitated Sexual Assault (DFSA) cases is punishable in United States under Drug-Induced Rape Prevention and Punishment Act 1996 (Public

Law 104-305). The Act modified 21 U.S.C., stated that penalty of up to 20 years imprisonment and fine will be given to those who intend to commit a crime of violence (including rape) by distributing a controlled substance to another individual without that individual's knowledge.[13]

Indian law does not recognise date rape as a specific crime and therefore in Indian Law, Section 375 of IPC, stated "A man is said to commit "rape" who, except in the case hereinafter excepted, has sexual intercourse with a woman with her consent, when, at the time of giving such consent, by reason of unsoundness of mind or intoxication or the administration by him personally or through another of any stupe¬fying or unwholesome substance, she is unable to understand the nature and consequences of that to which she gives consent" is the only section that covers the nature of DFSA crimes. [18,16]

In view of the said provision of the Code, consent given by an intoxicated/drugged women is of no avail whether she has drugged/intoxicated herself or by the accused or anybody else. Once it is proved that the women is drunken/intoxicated/drug facilitated, the consent even if given is no consent. There is no recent verdict on the said provision due to various reasons as an Indian women informs to her Kiths and Kins/ the police officer after lapse of time when, in fact, the effect of drug/intoxicant is undetectable.

To prove that the victim was under influence of drug/intoxicant, if the victim approaches the police promptly, it can be detected through forensic aid, the police officer while forwarding the victim should make specific request to the doctor for making such arrangement/taking such body fluid(s) by testing of which a forensic opinion may be furnished by the expert to prove the effect of drug/intoxication at the relevant time when the women was subjected to rape. If the forensic opinion corroborates the version of the prosecutrix, the prosecution case may be properly proved coupled with the victims statement.

Some special and separate provisions needs to be implemented in such cases as the national database of sexual offenders created by National Crime Records Bureau in October 2018 also does not provide any evidence of DFSA cases. Although the widespread increase of drugs facilitated sexual assault cases dealt with ketamine abuse, the Drug and Cosmetics Act, include it in the stringent schedule X in December 2011 to curb its easy availability.[17]

Future Challenges and Predictive Problems

The current scenario of drug facilitated sexual assault cases poses a great challenge to the women safety. These cases remain unsolved by forensic science laboratories during examination of the victim's biological samplesfor the presence of drug because of lack of information about evidence of drug administration. This inadequacy attributes to decrease in reporting of the cases dealing with drug facilitated crimes.[14]

The upcoming challenges associated with these drugs facilitated sexual crimes involve the introduction of new drugs in the market each year capable to impair the memory of the victim.[4] Sometimes administration of a single dose and its rapid metabolism causes an extensive delay in collection of suitable sample and reporting because the drug gets dissociated and changed into some other metabolic form the detection limit of which is beyond the purview of toxicological analysis.[16]

A thorough investigation of the circumstances of each case has to be carried out in such scenarios for a better probability of success.

Acknowldegement: Authors are extremely grateful to Sh. Y.M. Upadhyay, Director of the Institute for his constant encouragement during the preparation of this review article.

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Review Article

Journal of Forensic Chemistry and Toxicology
Volume 6 Number 1, January–June 2020
DOI: http://dx.doi.org/10.21088/jfct.2454.9363.6120.8

Utilizing Simulation Technology to Train Doctors in Indian Society of Toxicology Life Support Course at Poison Control Centre

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Abstract

Simulation has been widely adopted as a training and assessment tool in medical education. Conventional teaching methods may be inadequate to properly train healthcare providers for rare but potentially lethal events in Toxicology such as managing associated polytrauma in alcoholic drink and drive cases, Acute Brain stroke due to cocaine toxicity, acute coronary syndrome in Cannabis and cardio-respiratory arrest in Opioid abuse. We have observed that Simulation based training, by enhancing provider skills, can subsequently decrease medical errors and increase patient safety.

Keywords: Toxicology; Poisoning; Overdose; Simulation; Training.

How to cite this article:
Vivekanshu Verma, Remya, Prateek Rastogi, et al., Utilizing simulation Technology to Train Doctors in Indian Society of Toxicology Life Support Course at Poison Control Centre. J Forensic Chemistry Toxicol 2020;6(1):64–72

Introduction

Indian Society of Toxicology have created ISTOLS-Indian Society of Toxicology Life support course with international training skills for the purpose of educating consultants and resident doctors, as well as Nurses and paramedics in life saving skills course for managing toxicology and various trauma management certified courses for fixed duration on regular basis, to update their skills and knowledge. Medical simulation offers the challenge of integrating medical knowledge, attitudes, and skills in case scenarios, procedure-based learning activities, and role playing.[(1)]

Mannequins come in various shapes and sizes and can serve different purposes, including replication of the clinical deterioration and imitation of an adult in distress secondary to acute medical event.[(2)] Fidelity describes the extent to which the appearance and behavior of the mannequin imitate the appearance and behavior of an actual patient.[(3)] Simulation based training, by enhancing provider skills, can subsequently decrease medical errors and increase patient safety.[(4)] Simulation is especially effective in developing skills in procedures that require eye–hand coordination and ambidextrous maneuvers, such as securing airway in compromised airway due to vomiting and aspiration in unconscious state, vascular access in cardiovascular collapse, shock and cardiac arrest and the use of laryngoscopes for endotracheal intubation in respiratory compromise.[(5)]

ISTOLS is one of the unique CME Bedside simulation training organized by Pioneers in the field of Toxicological emergencies, addressing the common poisonings found in Indian subcontinent. Participants learned to identify poisoning, recognise drug overdoses, recall

relevant antidotes with routes and doses, utilize the resources available bedside in rescuing critically ill intoxicated patients. Participants underwent simulation training bedside for understanding the pathophysiology of envenomation and got practical tips in administering antidotes, anticipating adverse events of therapy and preparing mind maps for the future practical situations during real life management of intoxications.

We have recently conducted ISTOLS on 21st December in Poison Control Centre, Amrita Institute of Medical Sciences by training 24 resident doctors and consultants in critical care, emergency, forensic medicine and toxicology.

Photo. 1: Our ISTOLS simulation team of instructors at Poison Control Centre AIMS Kochi – Dr Ramakrishnan, Dr Vivekanshu, Dr Athira Aji, Dr Remya S, Dr Nirmal (From left to Right) and Dr Vijay Vasudev Pillay (sitting in middle) on 21-12-19 during Annual IST conference alongwith IST Toxic Medley of simulated plant parts, venomous animals, fishes, insects, smells as station for participants.

Fig. 1: Common Poisonous mushrooms, simulated by colouring the edible mushrooms with sketch pen to look alike Amanita, Jack O' Lantern, Psilocybe, thus learning Toxicology in non-toxic manner.

Fig. 2: Dhatura seeds simulation by chilli seeds.

Fig. 3: Poisonous animals (Dart Frog) Venomous insects (Black widow spider, Scorpion, Red scorpion, Honey Bee, Wasp, Spanish Fly, Centipede, Tarantula), non-venomous (Common Spider, Cockroach, Red Beetle) of rubber used for simulation training including beetle, spider, scorpion and dart frog. Thus learning Toxicology in non-toxic manner.

On 21st November 2019, Similarly, we conducted ISTOLS in Grande Internationale Hospital, Kathmandu, Nepal successfully by training 34 resident doctors and consultants in critical care, emergency, forensic medicine and toxicology

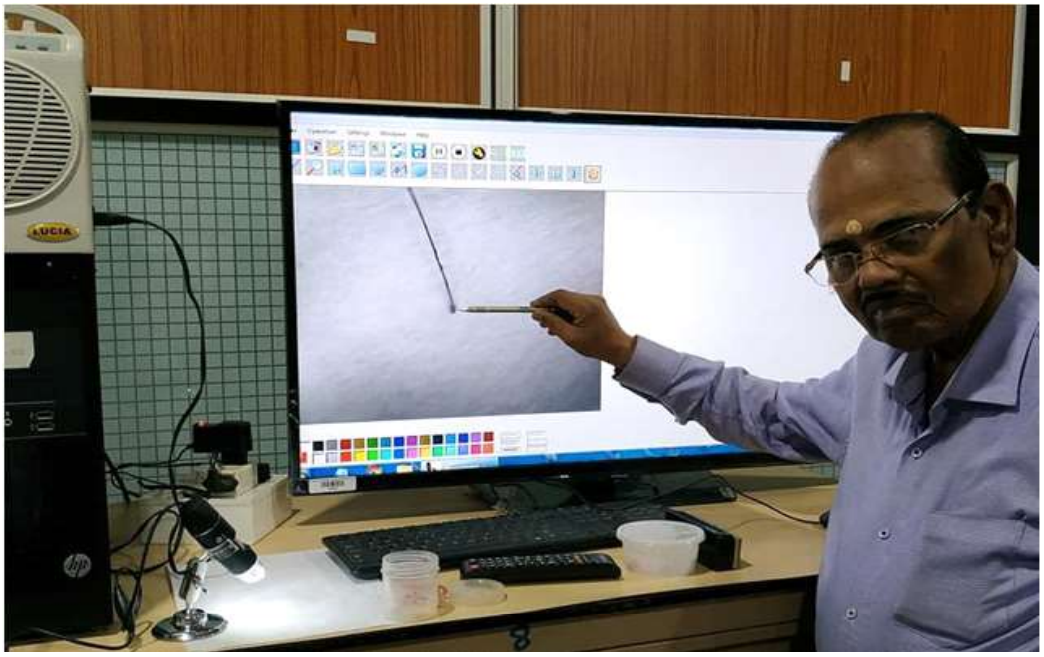

Fig. 4: Demonstrating bedside point of care, using Digital microscope magnified 1000 times on-spot, identifying human hair of thallium poisoning victim in ISTOLS by Dr Gopalan, Simulation training Lab in charge, AIMS Kochi.

On 29th July 2019, in Sarvodaya Hospital, Faridabad, we have conducted ISTOLS workshop successfully by training 55 resident doctors, NRHM Rural Medical Officers of Haryana and consultants in critical care, emergency, forensic medicine and toxicology.

Fig. 5: Marine envenomation teaching by venomous fishes, Fugu, Sting Ray, Star fish with sting, Jelly fish with tentacles, Blue-ring octopus, shellfish and non-venomous Octopus, scenarios simulated by similar look alike toys, thus learning Toxicology in non-toxic manner

Fig. 6: Different Venomous Snakes for identifying 4 Toxidromes, by big 4, Cobra and Viper, and non-venomous Green Snake, Brown Snake; utilising look alike toys, thus learning Toxicology in non-toxic manner

Fig. 7: Simulating rattling sound of rattle snake by colliding magnetic pellets and differentiating with death rattle in victim, simulated by rattle toy sound.

On 23rd March 2019, in Amrita Institute of Medical Sciences (AIMS), Poison control centre, Kochi, we have Bottom figure 7. Caption, add space to the bottom para below on page 71medicine and toxicology. conducted ISTOLS workshop successfully by training 25 resident doctors, NRHM Rural Medical Officers of Kerala and consultants in critical care, emergency, forensic medicine and toxicology.

Fig. 8: Toxic smell simulation by common household substances, like shoe-polish (aromatic Nitrobenzene), Napthalene balls, Perfume scents (poison lily, jasmine), turpentine oil (Organophosphates), rotten egg (Hydrogen Sulphide), almond oil (Cyanide), Burnt Rope (Cannabis), Poppy seeds (Opium), Ketotic odour (fruit juice), Tobacco (snuff), Camphor, Alcohol (Handrub), Arsenic/ Phosphorus (actual Garlic bulb from kitchen garden). Last year, we have conducted ISTOLS course on 24th-25th June 2018 in Medanta-the Medicity successfully by training 21 resident doctors, NRHM Rural Medical Officers (RMO) of Haryana, Rajasthan and Delhi NCR consultants in critical care, emergency, forensic medicine and toxicology.
On 13th January 2019, in Victoria Hospital, Bengaluru, we have conducted ISTOLS workshop successfully by training 55 resident doctors, NRHM Rural Medical Officers of Karnataka and consultants in critical care, emergency, forensic medicine and toxicology.

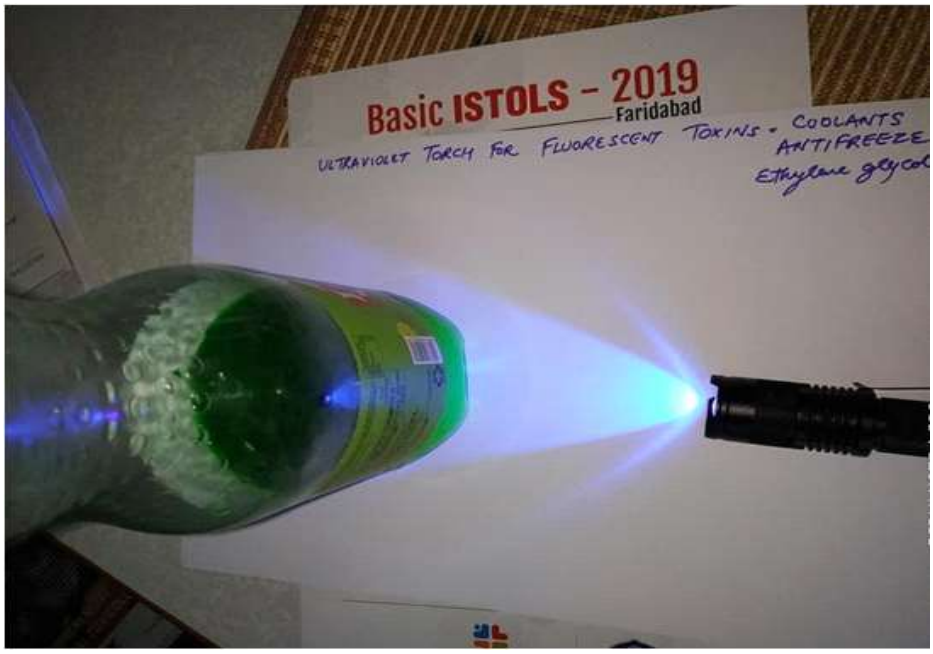

Fig. 9: Ultraviolet fluorescence of Car AC coolant- ethylene glycol- toxic alcohol (glows in UV torch) for on-spot diagnosis Last year, we have conducted ISTOLS on 24th-25th June 2018 in Medanta-the Medicity successfully by training 21 resident doctors, NRHM Rural Medical Officers of Haryana and consultants in critical care, emergency, forensic medicine and toxicology.
Last year, we have conducted ISTOLS course on 24th-25th June 2018 in Medanta-the Medicity successfully by training 21 resident doctors, NRHM Rural Medical Officers (RMO) of Haryana, Rajasthan and Delhi NCR consultants in critical care, emergency, forensic medicine and toxicology.

Fig. 10: Grey coloured cement powder simulating celphos powder of Aluminium phosphide

We have similarly conducted another ISTOLS on 7th April 2018 in SMIMSSikkim Manipal Institute of Medical sciences, Gangtok, Sikkim successfully by training 37 resident doctors in critical care, emergency, forensic medicine and toxicology, pharmacology, internal medicine and NRHM Rural Medical Officers posted in peripheries of Sikkim State.

Fig. 11: Raw mango (from Garden) simulating look alike, Cerbera odollam suicide tree fruit.

We took pre-test and Post-test for all the RMOs and observed during above mentioned ISTOLS trainings in States of Sikkim, Karnataka, Kerala, Haryana, Rajasthan, Uttar Pradesh and RMOs internationally, posted in our neighbour country Nepal that, Rural Medical Officers who received high fidelity training on a human patient simulator performed significantly better on the advanced life support, in written examination, in medicolegal documentation of right antidotes indicated to right patient, in right dose, frequency and by right route in right manner; as well as improved selfconfidence of RMOs in handling mass casualties by toxic disasters during a mock resuscitation. Pre-test and posttest analysis reported improvement in overall performance by doctors after implementation of simulator training sessions. Figure No. 12. **Bar Diagram** showing impact of simulation teaching on participant's scores of Pre-test and Post-test by simulation training of RMOs

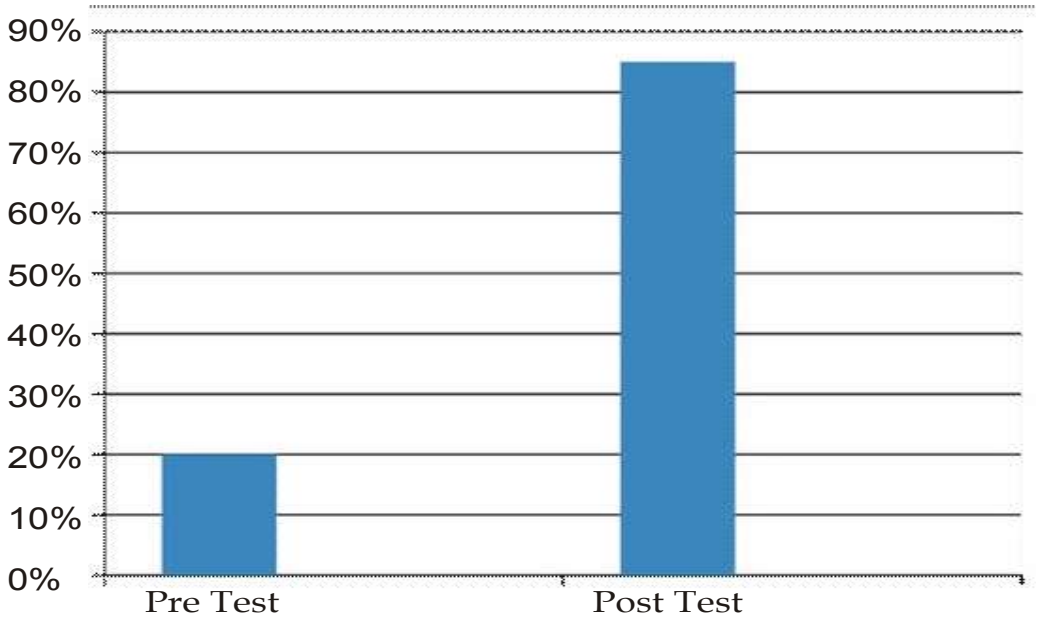

Fig. 12: Bar Diagram showing impact of Toxicology simulation teaching on overall scores of Pre-test and Post-test of Rural Medical Officers posted in Kerala, Karnataka, Haryana, Rajasthan, DelhiNCR, Uttar Pradesh, Sikkim and Nepal.

We observed that 94% of RMOs benefitted from increased confidence and perceived technical and non-technical skills during toxicological emergencies following simulation based learning. These novice learners in clinical toxicology demonstrated improved triage and intervention scores after each virtual reality toxidrome drill. RMOs who received human patient simulator training in addition to advanced life support showed significantly higher adherence to international standards as compared with those who received basic life support alone. It was observed that the prior simulation training positively impacted their clinical skills during the resuscitation, including rapid problem recognition, correct choice and dosage of specific therapy, and coordination of team efforts. The differences of percentage in RMO's responses towards life support skills with and without the implementation of simulation practice were calculated and recorded. After the implementation of simulation training, RMOs showed more anticipation in the implementation of simulation training by hands-on practice on manikins and there was an increase from 63.89% to 89.44%. Among RMOs, 65.35% of them liked life support skills as a subject even before the simulation training were implemented. After the application of the simulation in training, 95.49% of RMOs were interested to learn life support skills in managing poisoning patients. There was an increase of 30.14% of RMOs, who had developed their liking towards life support skills in management of toxicological emergencies.

We compared 'low-fidelity' versus 'high-fidelity' simulation on RMO's ability to successfully perform nasogastric lavage and endotracheal tube placement. The low-fidelity mannequin consisted of a relevant body part model that allowed for tube insertion, in contrast to the high-fidelity simulator (anatomically correct mannequin that reacts to tube insertion with physical responses such as change in vital signs, gagging/coughing sounds, etc). RMOs who received high fidelity training scored significantly higher than residents trained by the low-fidelity simulator.

RMOs were subsequently evaluated on their peripheral line placement technique on manikins. The 'trained' RMO group outperformed the 'untrained' RMO group on the majority of clinical aspects of venous catheterization, including fewer attempts to find the vein, identification of anatomical landmarks, and total overall performance score. The 'trained' RMO group also scored higher on a post-test, supporting a correlation between knowledge gain and improved clinical performance. Before the training, the RMOs used to undermine themselves: 'we know nothing about clinical toxicology.' Moreover, the RMOs felt that they were not capable of managing without a critical care expert. After the training, it was quite evident that RMOs have a very clear role to perform, while managing poisoning cases in emergency. Every RMO learned to contribute their important role to the team work in resuscitation of a dying patient due to fatal poisoning.

The participating RMOs' positive feedback for the overall training program indicated that 95% of RMOs were satisfied with the training program and believed that skills learned could be applied well in their field of work. The RMOs' positive response directly correlated to their post-training evaluation undertaken by trainers, which positively signified effective learning of skills during the training intervention. Learning features, such as training in teams, skills training, and realistic repeated scenarios with consecutive debriefing for reflective learning, including a systems approach to human error, were crucial for enhanced teamwork during bedside toxidrome drills on high fidelity manikins. Developing clear communication and teamwork were found to be the key learning principles guiding their practice. The most important findings from the RMOs' group discussions were the importance of team training as learning feature, and the perception of improved ability to use a teamwork approach to toxidrome diagnosis and management.

Conclusion

There is ample evidence that simulationbased educational interventions in training in Toxicology to Doctors working in Emergency and Intensive Care Units, increases their retention of knowledge for cardiopulmonary resuscitation, Antidote management, trauma and toxicology care, airway management, procedural skills, team-training, and toxic disaster management.

Conflict of interest: Nil

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Case Report

Journal of Forensic Chemistry and Toxicology
Volume 6 Number 1, January–June 2020
DOI: http://dx.doi.org/10.21088/jfct.2454.9363.6120.9

Death due to Accidental Electrocution: Social Aspects: A Case Report

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Abstract

Background: The passage of electric current through human body can produce multiple effects, commonly known as Electrocution. The passage of electric current through the body produces wide range of effects, varying from insignificant localized spasm, little or no contact burns, fatality with little or no burns or extreme severe burning. *Case Report:* A detailed history of the incident revealed that the bike rider skid and fell on the water logged road in rainy season and came in contact with the high tension wire and was electrocuted. The second person tried to attend to the bike driver and he also came in contact with the same wire and died instantly. Autopsy revealed external electrocution injuries on both deceased. Both these deaths were accidental in nature and were preventable. Histopathological examination of the electric contact point showed elongation and streaming of nuclei with focal inter-epidermal and epidermal-dermal separation. *Conclusion*: This present article focuses on need for proper development of water drainage system especially during rainy season and also to generate public awareness regarding regular supervision of roadside Electric pole and wires which could prevent similar accidents. A detailed history regarding the incidence, scene visit, circumstantial evidence, statement of witness and proper postmortem examination with the histopathological examination are recommended prior to concluding the cause of death in case of alleged death due to electrocution. The data can be used for further planning and implementation of adequate measures to prevent accidents and thereby benefit the society.

Keywords: Electrocution; Rainy season; High tension wire; Water logging; Electric contact point.

How to cite this article:
Hemant Kumar, Vanika, Ruchi K, et al. Death due to Accidental Electrocution: Social Aspects: A Case Report. J Forensic Chemistry Toxicol 2020;6(1):73–78

Introduction

The passage of electric current through human body can produce multiple effects, commonly known as Electrocution, varying from a localized muscle spasm to internal organ damage and even sudden death of the person. Electric fatalities are usually accidental in nature.[1] In India, with high population density, most people tend to flout the building rules with regards to electricity, high tension wires pass by roadsides in the electric poles, which may lead to accidental electrocution.[2] Severity of electrocution injuries mainly depends on the path traversed by the current along with type and duration of its contact with the body. High voltage electrical injuries are relatively uncommon but contribute to occupational fatalities due to hazardous exposure during their tasks at workplaces.[3] In case of death due to alleged electrocution, proper history of incidence with examination of scene of occurrence and a detailed postmortem examination by an autopsy surgeon may help to conclude the cause of death and its manner, for the purpose of compensation and help to implement future preventive policies to reduce such incidences.The authors describe one such case of accidental death due to electrocution in a water logged road in rainy season, and discuss the preventive aspects.

Case Details

Case 1: A 28-year old male deceased was brought

to mortuary of JPNATC, AIIMS, New Delhi, after being recovered from road. The victim was driving his bike on road in rainy season (September), when his bike skidded and he fell down on a water-logged road, where water had stagnated after a heavy rainfall. A high tension wire had broken from a nearby high tension pole (11000 KW), and came in contact with the abovementioned victim, who instantly was electrocuted (Fig. 1).

The autopsy revealed that the deceased was 165cm in length, averagebuilt and well-nourished male, with allhis clothing mud stained and soaked with water at several areas (Fig. 2). Rigor mortis was fully developed all over the body and the postmortem lividity was present over back except pressure areas and was fixed (Fig. 3).On external examination, electric contact points were present obliquely over posterior aspect of right forearm over an area of 27 cm by 6 cm, back of right chest over an area of 10 cm by 2 cm and posterior aspect of left leg over an area of 8 cm by 2 cm(Fig. 4—6).Margins of all these injuries had shallow crater with ridges at the circumference. Base and floor of injuries were blackened, charred and pale at places. Periphery of the injuries was erythematous and peeled off at places. On internal examination, all internal organs were congested and the heart was cyanotic and rigid. The brain was mildly oedematouswith congested vessels. Histopathological Examination of electric contact point showed elongation and streaming of nuclei with focal inter-epidermal and epidermal-dermal separation with coagulation changes (homogenization)and necrosis seen in dermis (Fig. 7). Brain showed focal areas and edema and few axonal bulbs predominantly in sub-meningeal areas. Lung showed autolytic changes, hemorrhagic areas and destruction of alveolar spaces (Fig. 8). Heart showed focal areas of deep eosinophilic changes with coalesces of myocardial fibres.The cause of death was opined as Shock due to Electrocution.

Case 2

A 38-year old male deceased was brought to the mortuary of JPNATC, AIIMS, New Delhi. The victim was a pedestrian, walking on the roadside nearby the bike rider and when he saw him fall down,then the latter went to help the fallen bike rider and accidentally he also came in contact with the same high tension wire which was broken from a pole and was in contact with water (Fig. 9).

The autopsy revealed that the deceased was 168 cm in length, averagebuilt and well-nourished male. All clothing wasmud stained and soaked with water at several areas. Rigor mortis was fully developed and the postmortem lividity was present over back except pressure areas and was fixed(Fig. 10). On external examination, electric contact points were present obliquely over the body over left shoulder tip over an area of 14 cm by 8 cm, anterior aspect of neck over an area of 12 cm by 10 cm and antero-lateral aspect of right arm over an area of 18 cm by 4 cm (Fig. 11—13). Margins of the wound were shallow crater with ridges at its circumference. Base and floor of the injury were blackened, charred and pale at places. On internal examination, all the visceral organs were congested and stomach contained about 100 ml of semi-digested food particles. Histopathological Examination of Brain showed focal areas of gliosis, sub-meningeal edema; Liver showed focal fatty changes and mild focal chronic inflammatory infiltrates in portal triad; Lung showed autolytic changes, hemorrhagic areas and irregular alveolar spaces filled with fluid/ hemorrhage (Fig. 14); Heart showed focal overlapping/ coalescence of myocardial fibres and eosinophilic cytoplasm (Fig. 15). Electric contact point showed inter-epidermal and epidermal-dermal separation, coagulation, necrosis in epidermis, nuclear elongation in epidermis homogenization of dermis along with nuclear elongation in epithelium of hair follicle (Fig. 16). The cause of death was opined as Shock due to Electrocution.

Fig. 1: Scene of Incidence

Fig. 2: Deceased Bike rider

Fig. 3: External appearance of deceased.

Fig. 4: Electrocution Injury—right forearm.

Fig. 5: Electrocution Injury—back of right chest.

Fig. 6: Electrocution Injury—back of left leg.

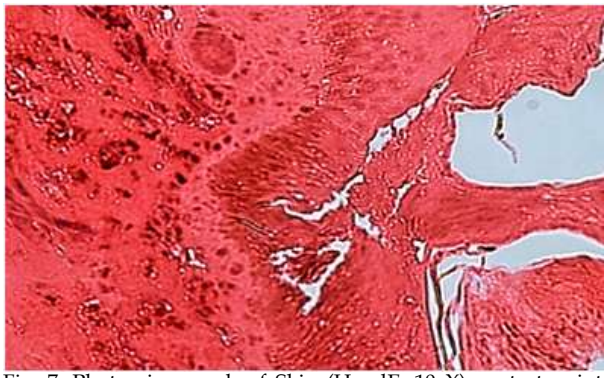

Fig. 7: Photomicrograph of Skin (HandE, 10 X) contact point showing inter-epidermal and epidermal-dermal separation, coagulation and necrosis in epidermis.

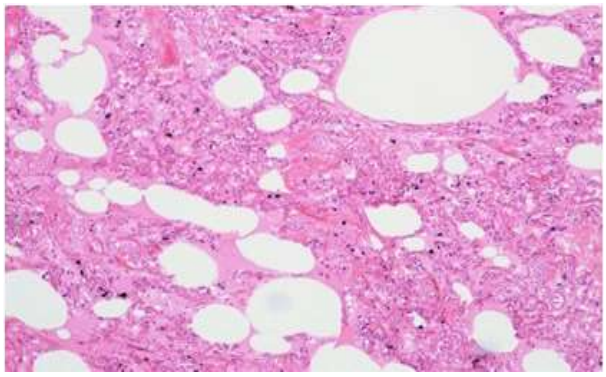

Fig. 8: Photomicrograph of Lung (HandE, 40X) showing the destruction of alveolar space.

Fig. 9: Deceased pedestrian.

Fig. 10: External appearance of deceased.

Fig. 11: Electrocution Injury—right arm.

Fig. 12: Electrocution Injury—front of neck.

Fig. 13: Electrocution Injury—left shoulder.

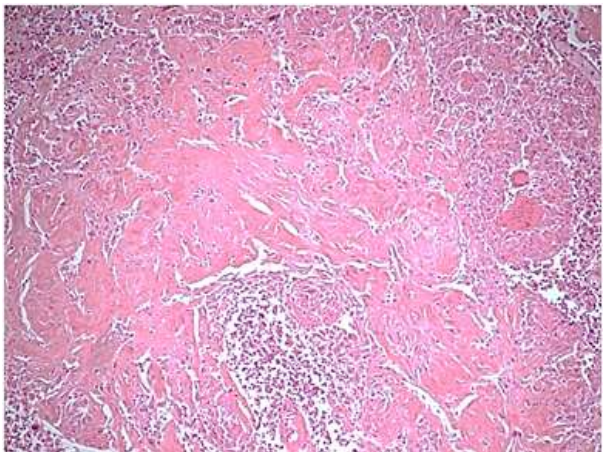

Fig. 14: Photomicrograph of Lung (HandE, 10X) showing autolytic changes, hemorrhagic areas and irregular alveolar spaces filled with fluid/hemorrhage.

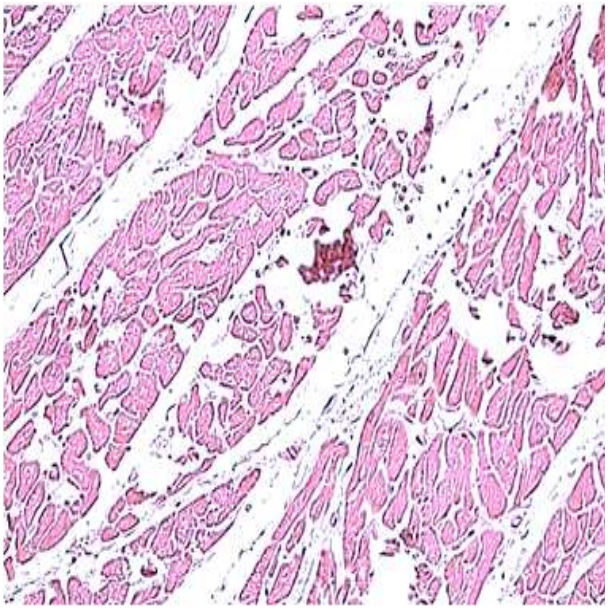

Fig. 15: Photomicrograph of Heart (H and E, 40X) showing focal areas of hyper-eosinophilic changes, overlapping myocardial fibers.

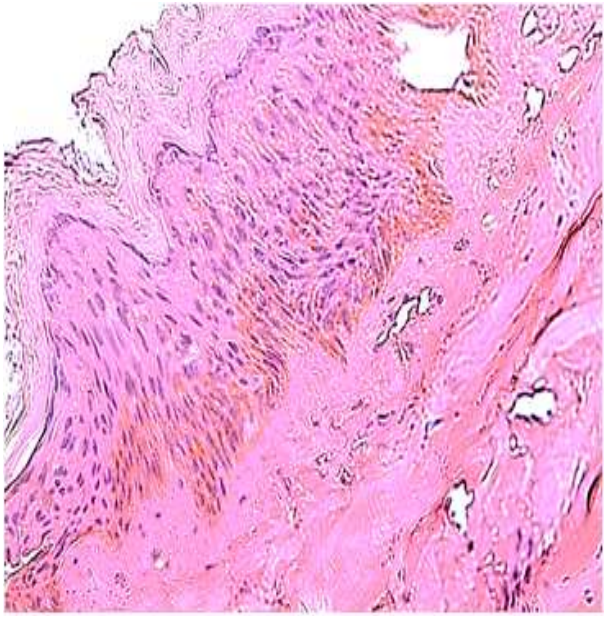

Fig. 16: Photomicrograph of Skin (HandE, 40X) Contact point showing nuclear elongation in epidermis, homogenization of dermis with nuclear elongation in epithelium of hair follicle.

Discussion

A detailed history of incident revealed that the bike rider skid and fell on water logged road in rainy season and came in contact with the high tension wire and was electrocuted. The second person tried to attend the bike driver and he came in contact with the same wire and died instantly. A study by Hussain et al.[4] showed that high voltage electrocution accounts to 18—26% of deaths due to electric current and 40% of victims were aged between 21—40 years, to which both victims of present case also belonged. In their study, Pathak et al.[5] observed that accidental incidences were typically higher in monsoon season (47.5%) as compared to other seasons, which shows that there is a characteristic seasonal variation in electrocution. Accidental electrocution may be encountered when an individual disregards warning signs or ignores the presence of high voltage cables when he is engaged in some activity near the cable.[6] Diagnosis of high voltage electrocution is sometimes difficult in absence of history and circumstantial evidence, as pathognomonic features like electric marks and joule burns are often seen with low or medium voltage current involvement and also entry and exit marks are seen in 20% cases.[7]

Electrical burns occur due to the conversion of electric current to heat within the body, and this can be either entry or exit marks. According to the circumstantial evidence, both the deceased came in contact with the current wire that carries about 11000 KW from a pole and was in contact with water. Multiple electrical marks were identified during the autopsy of both the victim. Those marks showed characteristic appearance of dry, firm to hard craters with ragged edges along with 1stand 2nddegree burns also with flash effect over the back of the body. Histopathological examination of skin around the burnt area done with normal Haematoxylin and Eosin (H and E) staining showed characteristic sub-epidermal separation, nuclear streaming and partial separation of the dermis due to micro blisters. The skin over crocodile flash burns showed thinning with focal separation of epidermis with nuclear streaming, which was due to the cooking effect of tissues with the strong heat and the large vacuoles were also identified within epidermal layer. Elongated and tightly packed nucleus in the basal layer of epidermis causes streaming of the nuclei which give rise to nuclear palisading effect and by far it is the most characteristic histological feature seen in electrical burns and are seen in majority of cases.[8] In both the cases, all the above mentioned histological changes were present in the skin.

Electrical injuries leading to myocardial damage are either due to direct cellular damage or by inducing cardiac arrhythmias. Ventricular fibrillation is the most common mechanism of death identified in electrocution.9 Necrotic and fragmented myocardial cells with round or square shaped nuclei were seen in the microscopic section of heart, and this finding along with the absence of cellular reaction suggests an instantaneous death. This feature of myofibril breakdown was seen in 90%of cases in a study done by Fineschi et al.[10] Cause of death in both the cases were opined as death due to electrocution and manner of death was accidental. There is lack of general public awareness regarding safety measures near electric poles and wires and also about the initial response to an electrocuted person. Hence, public should be made aware of basic preventive steps such as not to touch electrocuted person and not to attempt to give first aid and call for help in such circumstances. Roads should be better maintained, along with regular supervision of drainage system to avoid water logging on roads.Better maintenance of electric poles and wires and strict adherence to safety measures can reduce the fatality due to electrocution, which is mostly accidental and easily preventable.

Conclusion

The cases described in the article occurred as a result of electrocution due to broken high tension wire, along with poor drainage system of water which caused water logging on the road in rainy season. This present article focuses on need for proper development of water drainage system especially during rainy season and also to generate public awareness regarding regular supervision of roadside Electric poles and wires, which could prevent similar accidents. Insulating/isolating high tension electric wires would also reduce these instances. A detailed history regarding the incidence, scene visit, circumstantial evidence, statement of witness and proper postmortem examination with the histopathological examination are recommended prior to concluding the cause of deathin case of alleged death due to electrocution. This article can be used for further planning and implementation of adequate measures to prevent accidents and thereby benefit the society.

Funding: None

Conflict of Interest: None declared

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Case Report

Journal of Forensic Chemistry and Toxicology
Volume 6 Number 1, January–June 2020
DOI: http://dx.doi.org/10.21088/jfct.2454.9363.6120.10

Creatine Creating Muscles or Creating Malady in Indian Youth: Medicolegal Issues

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Abstract

Most of Indian Urban Youth are keen of bulky bicep muscles, are getting misguided by online advertisements of "Creatine Creating Muscles". Supplement abuse is rampant in the fitness world. Enthusiasts often take them without proper clearance from medical professionals and often feel the consequences later, in the end of the month. Relevant scientific studies warns the Harmful Effects on kidneys of even healthy youth, associated with consuming creatine supplement for creating muscular body building alongwith physical workouts by weight lifting in Gymnasiums. The doctors get baffled to see otherwise healthy, young males hospitalised for an avoidable emergency, by regulating creatine use only after medical expert's supervision and monitoring.

Keywords: Creatine; Kidney injury; Body building; Creatinine; Dialysis.

How to cite this article:
Vivekanshu Verma, JidhinJanardhan, Rana Abhyuday Singh. Creatine Creating Muscles or Creating Malady in Indian Youth: Medicolegal Issues. J Forensic Chemistry Toxicol 2020;6(1):79–82.

Introduction

Increasing the body pool of creatine increases creatine concentration in serum and also urinary excretion rate.[1] The main metabolic waste product of creatine is Creatinine, which is metabolized further to form methylguanidme. Both creatinine and methylguanidine have been implied as kidney toxins.[2,3]

Dehydration due to excessive sweating during gym workouts, lack of proper rehydration, along with consuming pain killers for muscle ache after workouts add further add to damage to kidneys.[1,4,5]

Case Series

Two such cases of kidney injury associated with creatine abuse in healthy urban males doing Gym workouts have been reported in Medanta Hospital in Gurugram in past 6 months.

The first index case involved a young healthy male from Najafgarh in February 2019, unaware of his single functional kidney, experienced a sudden shut down of kidney and a decrease in glomerular filtration rate, after abusing creatine powder for one month, for body building. An Urban Male, 26 yrs age, weighing 60 kg, well built, bulging biceps and back muscles, was brought by his father in midnight at our Hospital Emergency from Najafgarh Hospital with Complains of shortness of breath and altered behavior since 2 days. Admitted in Najafgarh hospital and treated as Anxiety disorder. No history of any knows Allergy.

ECG showed=Hyper-acute tall T waves suggesting Hyperkalemia

ABG: pH=6.88	Hb=7.3gm/dl (anemia)
PCO2=18.0	Wbc=16.48 x 10^3
PO2= 49.2	RBC=2.39 million/cumm
HCO3= 3.3	Haemetocrit=24.1%
Hb = 7.4	Urea= 289 mg/dl(Critically High)
Na+ = 124	creatinine=20.60mg/dl(Critically
K+ = 6.15	High)
Ca+2 = 0.79	Potassium : 6.1mmol/dl(Critically
Chloride = 116	High)
Lactate= 1.02	Calcium=5.3mg/dl
	CPK=1005 (Critically High)
	CKMB mass=16.80 ng/ml (normal)

On detailed History by his father and friends: Reduced urine output since 2-3 days, No significant Past history, No love affair, No History Drug abuse, No Alcohol, Educated, Graduate, doing Business, no history of financial loss.

H/o Gymnasium: Body building exercise in his home town gymnasium recently

H/o intake of self-medication of Over the Counter Creatine powder approx. 120gm/day as nutrition supplement for Body building since last one month. No history of suspicious Events like trauma, assault.

- USG Abdomen- Solitary Shrunken kidney
- NCCT brain- WNL.

Management: Airway Secured by endotracheal intubation, Breathing supported by artificial ventilation, Inj. SODA BICARBONATE for correction of metabolic acidosis, antihyperkalemic measures in form Glucose, Insulin and Potassium infusion, Salbutamol nebulization, Calcium administration. Nephrologist advised urgent dialysis for supporting kidney functions. Patient was kept admitted for 7 days in ICU for repeated dialysis to filter out all the excess toxins and discharged in stable condition on biweekly dialysis dependent state.

Case No. 1, unaware about his solitary functional kidney consumed excessive Creatine supplements by self, and did lots of vigorous exercise without proper hydration, developed kidney injury, remained only a step away from dying, but timely intervention saved his life but necessitated life-long dialysis. His Kidneys were damaged irreversibly and they will never return to their normal functioning in spite of best of medical care.

The second case reported in June 2018, involved acute kidney injury in a healthy young male wrestler after creatine abuse. A professional state level wrestler was hospitalized in Medanta for dark coloured urine associated with high dose of Creatine after long term regular use for 2 months for muscle gain, requiring urgent dialysis. Further examination revealed that the reason behind the severe condition of a seemingly healthy person was the health supplements he had been taking for the last four months on the advice of his gym trainer. Patient recovered luckily and was able to participate in his tournament, as his kidney function resolved upon discontinuance of the creatine use.

In February 2019, another case of limb vessel blockage associated with creatine abuse in a healthy urban male from Mathura, was hospitalized in Medanta with pain and discoloration of right lower limb associated with high dose of Creatine after long term regular use along with Gym exercise since one year. On investigation, his limb vessels were choked, causing gangrene of lower limbs, and in order to save his life, vascular surgeon had to amputate of both lower limbs. Patient was discharged after 26 days of hospitalisation in stable condition with limb prosthesis to let him walk with support.

Discussion

Similarly, recent cases on the change in renal function associated with creatine use have been reported in international medical journals.

Herlitz LC, et al. (2016) reported a case series involving 10 healthy bodybuilders abusing steroids and creatine> 10gm/day, who developed focal segmental glomerulosclerosis resulting an elevation in serum creatinine and a decrease in glomerular filtration rate.[6]

The second case reported by Koshy, K.M., et al. (1999) involved the development of acute interstitial nephritis in a healthy young man. A professional baseball player was hospitalized for renal dysfunction.[7]

Another case reported by Robinson S.J., et al. (2000) of acute quadriceps compartment syndrome and rhabdomyolysis in a weight lifter using high-dose creatine supplementation.[8]

Almukhtar, S.E., et al. (2015) reported a case series of Four bodybuilders who injected anabolic steroids and ingested commercial protein creatine (15 g/day) products presented with high serum creatinine levels. Renal biopsies revealed acute tubular necrosis. Four weeks after discontinuing injections and supplements, serum creatinine was in the normal range.[9]

The findings highlight a risk for acute and potentially chronic kidney injury among young men abusing anabolic steroids and using excessive amounts of Creatine supplements.

So, All Commercial containers with creatine supplements should bear a warning label advising against use of these products by any person diagnosed with, or at risk for renal dysfunction.

Some relevant case studies on animals fed on creatine, were found significant: A mouse study has reported on oral creatine after a single high dose of 50 mg/kg, is metabolized to methylamine, which is further metabolized to formaldehyde. Formaldehyde is responsible for crosslinking proteins (e.g.Lysine residues) in vivo and chronic methylene administration has been reported to cause oxidative damage in rats.[10]

In similar animal studies, for autosomal dominant polycystic renal disease has shown that oral supplementation of creatine salts resulted in greater cyst growth and renal impairment.[11,12]

But, to promote and market the commercial products of creatine supplements, the manufacturers have supported the Creatine drug trials to get favourable position statements and their employees have published some studiesin favour of nil side effects of Creatine on kidney (Kreidir et al. 2017), which can't be relied blindly as co-authors of these articles had Conflict of interest, and authors have received externally-funded grants from creatine industry to conduct research on creatine, and last but not the least is that, some co-authors were already hired in advisory board by companies that sells creatine.[13]

Recommended Dose of Creatine: Taking dosages greater than 2 g/day is potentially harmful to the kidneys. Creatine is sold in a powder or liquid form in dosages by Gym Trainers, sometimes greater than 10 g/day, without any written prescription.[14]

So Safety of Creatine use and risk of kidney injury in youth is pertinent, and more requires double blind clinical trials, to save the lives of youth seeking body building supplements, especially for professional games and tournaments. Not surprisingly, there is a high prevalence of doping (77.8%) among competitive bodybuilders.[15]

Some of the psychological research studies bodybuilding without focusing on the "obsession with muscle." Schneider et al. (2016) explained the drive for muscularity in their study.[16]

Recent Canadian study by Boychuk, et al (2016) reported that Creatine supplementation does not alter neuromuscular recovery after eccentric exercise.[17]

Conclusion

The consensus of Medical Experts conclude that creatine in high dose, if consumed for long term, can adversely affects renal function in healthy individuals doing lots of aerobic exercises, causing excessive water loss due to sweating resulting in dehydration.

Creatine use in individuals with the history of pre-existing renal disease or an increased risk should be restricted from the use of creatine, and every person seeking bodybuilding should undergo preventive health check-up including kidney function tests, urine tests and ultrasound of kidneys. And individuals consuming Creatine supplement should be monitored regularly with Creatine phosphokinase assay, Blood gas and electrolytes tested under medical supervision, to avoid acute kidney injury.

Conflict of Interest: Nil

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Standard journal article

[1] Flink H, Tegelberg Å, Thörn M, Lagerlöf F. Effect of oral iron supplementation on unstimulated salivary flow rate: A randomized, double-blind, placebo-controlled trial. J Oral Pathol Med 2006; 35: 540-7.

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Article in supplement or special issue

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Corporate (collective) author

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Reference from electronic media

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