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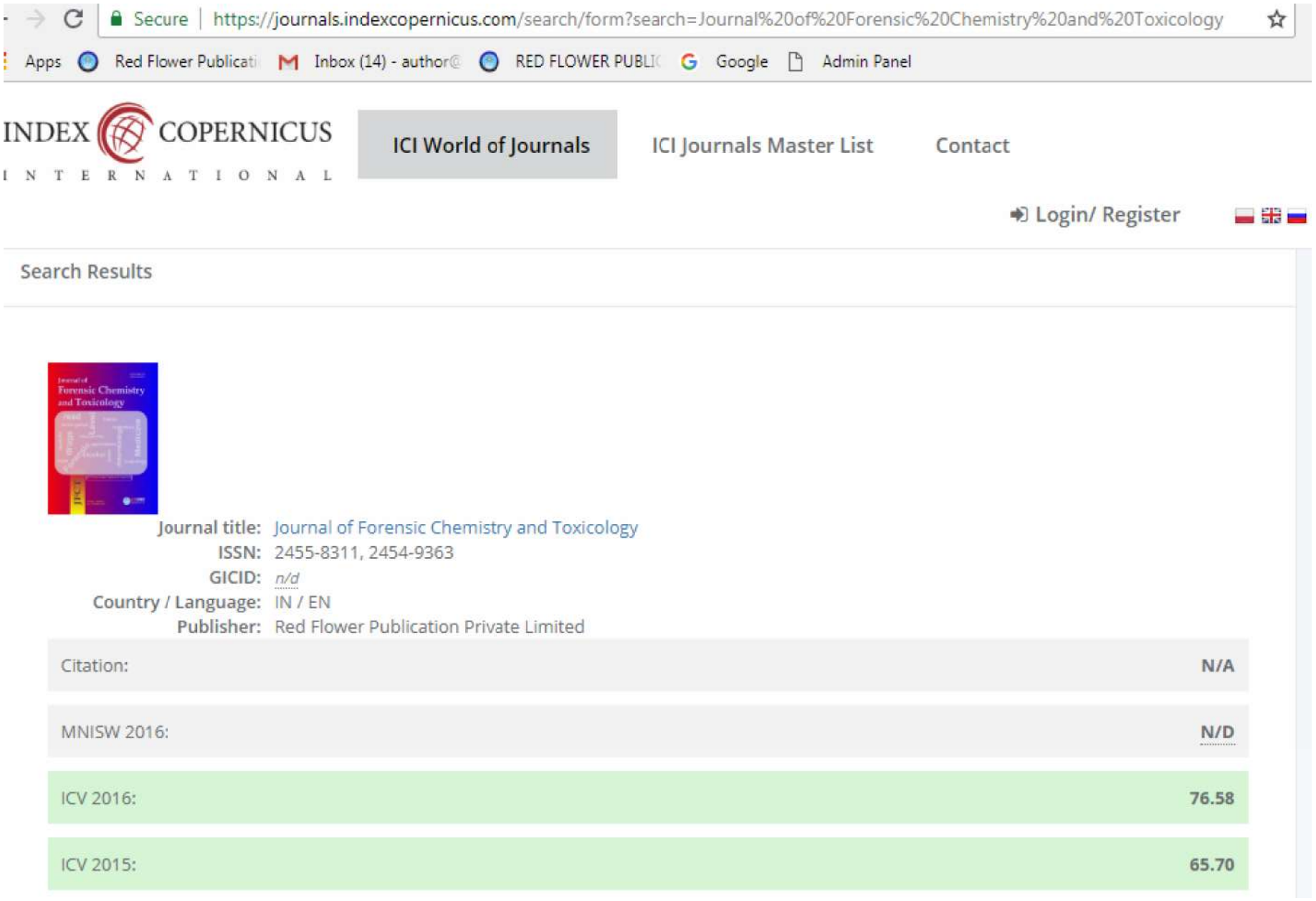

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Toxological Viscera Analysis in India: Current Scenario, Problems & Suggestions- One Year Study of Autopsy Cases Where Viscera Had Been Preserved

Abhishek Yadav[1], Arijit Dey[2], Kulbhushan[3], Neha Sharma[4], S.K. Gupta[5]

Authors Affiliation: [1]Assistant Professor [2]Senior Resident [3]Assistant Professor [4]Senior Resident [5]Prof. and Head of Department, Department of Forensic Medicine and Toxicology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi-110029.

Reprints Requests: Abhishek Yadav, Assistant Professor, Dept. of Forensic Medicine, AIIMS, New Delhi-110029.

E-mail: drayad_in@yahoo.com

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Abstract

Chemical and Toxoclogical analysis of viscera is conducted in a Medicolegal Death investigation to rule out poisoning/intoxication in the death of the deceased. The analysis is done only in Forensic Science Laboratories established by Indian Government. The Viscera reports are usually received after a period of considerable delay and opinion about the case is kept pending till then. Keeping the same thing into consideration, this study was undertaken to analyze the system of Viscera analysis after autopsy and identifying the factors and problems hampering the timely Chemical Analysis. The study was conducted in Department of Forensic Medicine, All India Institute of Medical sciences, New Delhi. All the Medicolegal Autopsy Cases in the period of one year from 1st January 2013 to 31st December 2013 where viscera was preserved were studied. Data was collected and analyzed from the PM reports and the subsequent viscera reports from FSLs received in the Department till 30th April 2017. The viscera analysis report was received in only 45.5% cases till 30th April 2017 and only 6.2% cases analysis reports were received within six months of conduction of postmortem. Poisons and Drugs were detected in 134 (41.5%) case out of which Ethyl and Methyl Alcohol constitutes 78.6% cases and only in those cases quantification was performed. The reasons of the delay in analysis of viscera and its effects on Medicolegal Death Investigation are discussed. It was concluded that there is a need of establishing Toxological Laboratories associated with the district hospitals and Medical Colleges where postmortem are being conducted.

Keywords: Toxicology; Poisoning; Viscera; Medicolegal Autopsy; Chemical Analysis.

Introduction

In Medicolegal Autopsy practice viscera is preserved for Toxological and chemical analysis in poisoning cases, sudden deaths, unclear history or to rule out concomitant poisoning/intoxication[1-4]. The preserved viscera are handed over to the Investigating Officer of the case. The Indian Government has established many Forensic Laboratories controlled by either State or Central Government where the viscera are tested. The report of the Viscera analysis is again sent to the autopsy surgeon by the IO for opinion about the cause of death so as to complete the legal course of the case. A negative viscera report creates a dilemma for the autopsy surgeon when there is no other pathology or injuries found during the postmortem and there is suspicion of foul play in the case with specific allegations of poisoning. Similarly a false positive report can also raise an unwarranted suspicion in a case. The authors of the study have themselves encountered these problems many times in their course of duties. The Viscera reports are usually received after a period of considerable delay and the case is kept pending till then. Keeping the same thing into consideration, this study was undertaken to analyze the system of Viscera analysis after autopsy and identifying the factors and problems hampering the timely Chemical Analysis.

Material and Methods

The study was conducted in retrospective and prospective manner in the Department of Forensic

Medicine, All India Institute of Medical sciences, New Delhi. All the Medicolegal Autopsy Cases in the period of one year from 1st January 2013 to 31st December 2013 where viscera was preserved were taken for study. Data was collected and analyzed from the PM reports. We further analyzed the subsequent viscera reports from FSLs received in the Department till 30th April 2017 regarding factors like nature of poisons detected, time lag between autopsy & receipt of analysis of reports, quantification of poisons (if detected), number of positives & negative cases etc.

Results and Observations

A total number of 1713 autopsy were conducted in the year 2013 between 1st January 2013 to 31st Dec 2013 and viscera was preserved in 710 (41.4%) autopsy cases **(Figure-1)**.

Out of these 710 cases, the viscera analysis report was received in only 323 (45.5%) cases till 30th April 2017 **(Figure-2)**.

The time duration of submitting the viscera report by the IO in the Department was calculated from the date of the autopsy and their percentage was calculated out of the total 710 cases of Viscera preservation. It was found that the analysis reports were received in only 2.7% cases within three months, in 3.5% cases from three to six months, in 28.6 % cases from 6 months to 1 year, in 9.3% cases between 1-2 year and in 1.4% cases after 2 years **(Table-1)**.

The viscera report was still awaited in 54.5% cases. Out of 323 cases in which the report was received, Poisons and Drugs were detected in 134 (41.5%) cases. **(Table-2)**.

Out of 134 positive reports, Ethyl Alcohol was reported in most of the positive cases (67.1%) followed by the combination of Ethyl and Methyl Alcohol (11.2%) and Aluminium Phosphide (6.7%) **(Table-3).**

One important observation was that the quantification was done in 107 cases out of 134, but all of them consists of ethyl and Methyl Alcohol.

Table 1: Time duration of receiving viscera analysis report from autopsy date

Time duration	Number	Percentage out of total 710 cases of viscera preservation
Within 3 months	19	2.7
3—6 months	25	3.5
6—12 months	203	28.6
1—2 years	66	9.3
More than 2 years	10	1.4
Total	323	45.5

Table 2: Viscera reports positive for poison/drugs

Report positive for poison/drugs	Number	Percentage
Yes	134	41.5
No	189	58.5
Total	323	100.0

Table 3: Types of poisons detected

Poison detected	Number	Percentage	Percentage out of total 710 cases of viscera preservation
Ethyl alcohol	90	67.1	12.6
Ethyl alcohol and methyl alcohol	15	11.2	2.1
Methyl alcohol	2	1.5	0.3
Aluminium phosphide	9	6.7	1.3
Dichlovos	4	3.0	0.6
Organo phosphorus compounds	4	3.0	0.6
Carbon monoxide	1	0.7	0.1
Corrosive acid	2	1.5	0.3
Others	2	1.5	0.3
More than one poison	5	3.8	0.7
Total	134	100.0	18.9

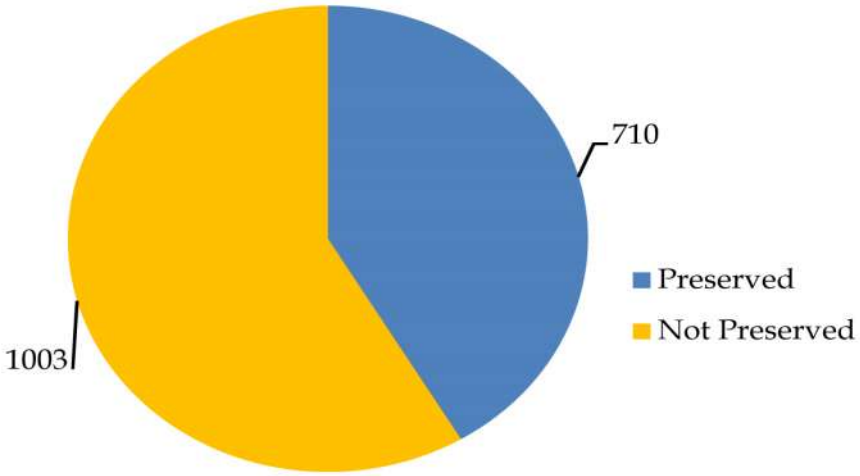

Fig. 1: Percentage of Cases in which Viscera was preserved

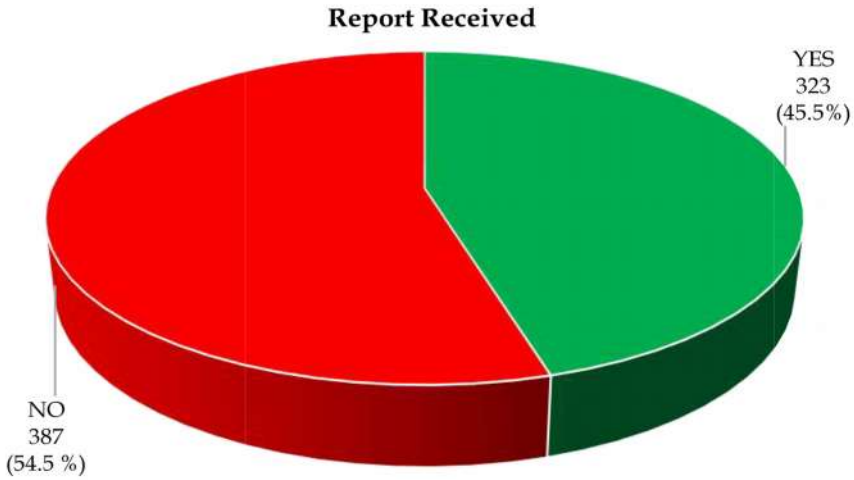

Fig. 2: Percentage of Viscera analysis reports received till 30th April 2017

Discussion

The Department of Forensic Medicine, AIIMS has the jurisdiction for conducting the autopsy of the Medicolegal cases of two districts of Delhi, namely South and Southeast besides hospital admission deaths. Out of the total 1713 autopsy cases viscera was preserved in 710 cases, which is an important finding which needs deliberations as it implies that the police investigation could not be concluded in 41.4% **(Figure 1)** of the Medicolegal cases for the want of viscera report. To understand the reason for such high number of viscera preservation, we will illustrate few examples of the type of cases in which the viscera is generally preserved[1-4]:

1. Suspected poisoning.
2. Sudden Natural Deaths with no hospital admission.
3. Accidental deaths with the suspicion of deceased/driver being intoxicated or under influence of alcohol/drugs.
4. Homicides to know about the toxicological/ intoxication status of the victims to correlate the chain of events.
5. Equivocal cases of hanging to differentiate between suicidal and homicidal manner.
6. Suicides after intoxication
7. Suspected Deaths of females due to Dowry harassment.

So, we can deduce that determining the toxological status of a deceased is essentially required in variety of cases even other than the poisoning cases. This is further supported by our finding that out of the total 323 viscera analysis report 41.5 % reported the presence of a for a poison or drugs **(Table-2)**. Ethyl alcohol alone and in combination with Methyl

Alcohol was detected in 78.3% of the total positive reports in different concentrations **(Table-3)**. This again indicates that the significance of keeping Viscera as the presence of alcohol can hugely impact a Criminal Trial in a court of law in an accident case to fix the culpability. Presence of alcohol can also be linked as a confounding factor in suicidal cases, a deciding factor in Insurance cases and an important circumstantial evidence in Homicide cases.

The cutoff date for analyzing the viscera reports was fixed as 30th April 2017 which is about more than three years if calculated from the end of the study year 2013. The viscera report was not received in 54.5 % cases till cutoff date. It implies that in about 387 cases of Medicolegal death conducted in a year **(Figure-2)**, the investigation was still pending even after three years have passed since autopsy was done. It is a matter of grave concern as the data collected is only for the two districts of the county in a single year. There were 640 districts in India as per the data of 2011 census[5]. If there are 387 cases pending in two districts of the National capital even after three years, one can very well imagine the number of incomplete investigations across all the districts of the country which may run into lakhs per year. The first and the foremost reason for the delay is insufficient number of Forensic Science Laboratories (FSLs) in the country. There are only 7 FSLs under Central Government and about 31 FSLs in different states of the country[6,7] while Medicolegal postmortem are conducted at all major District Hospitals and Government Medical College. So a gross mismatch is clearly visible between the scientific demand and available infrastructure for analysis of viscera.

The viscera preserved routinely during autopsy for Toxological analysis consist of Stomach with contents sealed in one jar, parts of liver with gall bladder, kidneys, and spleen sealed in another jar, about 20-50 ml of blood in one container and a solution of preservative in another container. The preservative used in most of the cases is saturated solution of common salt[1-4]. The human tissue starts to degrade after death and the preservation of the viscera can only slow the process but does not completely stall it[1-4]. An Honorable High Court of Calcutta had queries regarding the procedure of preserving the Viscera and its analysis for which an amicus curiae was appointed[8]. The amicus curiae consulted the experts who informed that viscera can be preserved only if properly refrigerated and will decompose in six months. Currently the viscera are collected by the police officers and stored in the police station at room temperature. They are submitted in the FSLs as per the waiting list according to the priority of the case. Delay in processing of Viscera leading to decomposition of the tissues is a well established reason for a negative analysis[9-11]. In our study viscera analysis reports of only 6.2% of the total 710 cases were received within six months **(Table-1)**, the ideal time in which the analysis should have been conducted. Further analysis of the viscera in the pending 387 cases after more than three years have passed seems nothing but a mere formality as the tissues would have been already decomposed and now will not be of any aid in Medicolegal investigation. Few previous studies[12-14] done specifically in poisoning cases have reported about the non detection of poison. Malik[15] also pointed about the pendency in the viscera reports. But no study specifically tried to understand the reasons and the solutions to address this issue. One more important finding which needs to be mentioned is that the quantification was done only in the cases where Ethyl alcohol and methyl alcohol were detected. Many poisons like Lead, Organo-phosphorus, Pesticides, Arsenic etc have been reported to be present in general population[16-20]. So in absence of the quantification attributing cause of death due to a specific poison is a questionable issue and importance of the viscera report is reduced to a mere corroborating evidence.

The above mentioned findings and discussion mandates the need of overhauling the current mechanism of viscera analysis and infrastructure. Indian is a developing Nation and is in a continuous process of improvisation. By this study we intend to highlight the drawbacks in the system not to criticize but only to improve the process of delivery of Natural justice.

Recommendations

1. There is a clear and urgent requirement of establishing more Laboratories for Toxological analysis in India at both State and National Level to cope up with the increasing work load so as to ensure accurate analysis of viscera and timely conclusion of Medioclegal Death Investigation.
2. A toxicology unit which can handle the analysis of the commonly found poisons and drugs should be established associated with Government Medical Colleges and District Hospitals where the postmortem are being conducted.
3. The laboratories should take measures to quantify the poison/drug detected in the Viscera so as to increase the positive evidentiary value of the analysis.

Compliance with Ethical Standards

Funding

There was no funding involved with the study.

Conflict of Interest

There is no conflict of interests of any of the author.

Ethical approval

The ethical approval was taken from Institutional ethics Committee.

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Derivative Ultraviolet Spectrophotometric Studies on Ignitable Liquids

Gurvinder Singh Bumbrah[1], Rajinder Kumar Sarin[2], Rakesh Mohan Sharma[3]

Authors Affiliation: [1] Department of Forensic Science, Punjabi University, Patiala-147002, India. [2]Former Director, Central Forensic Science Laboratory, Ramanthapur, Amberpet, Hyderabad, Andhra Pradesh-500013, India.

Reprints Requests: R.M. Sharma, Department of Forensic Science, Punjabi University, Patiala-147002, India.

E-mail: rmsforensics@pbi.ac.in

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Abstract

The present article describes the potential utility of derivative ultraviolet (UV) spectrophotometric technique in the discrimination of different kinds of ignitable liquids in their neat states. Derivative UV spectrophotometry is capable of differentiating these ignitable liquids as derivative spectra have more number of points for comparison than their corresponding normal zero order spectrum. Derivative spectra are relatively simple. The technique is rapid, simple, cost-effective and can be used for the screening purpose at the initial stage of investigation. Taking into account the results obtained in the present work, it is possible to suggest the use of this technique to distinguish these ignitable liquids, if recovered from arson scene or clandestine laboratories.

Keywords: Derivative Ultraviolet Spectrophotometry; Clandestine Laboratory; Arson; Ignitable Liquids; Petroleum Products; Solvent Extraction.

Introduction

An ignitable liquid (IL) is a volatile, inflammable liquid with a flash point of less than 200^0F [1]. It includes charcoal lighter fluids, paint thinners, cleaning solvents, engine fuels, lamp oils, polishes and lubricants [1, 2]. IL's can be broadly classified into 2 classes: petroleum and non-petroleum based IL's. Petroleum based IL's are refined from crude oil and includes petrol, kerosene and diesel. These IL's are made up of hydrocarbons. Non-petroleum based IL's are those derived from other sources and includes oxygenated solvents and naturally derived products such as turpentine. Non-petroleum based IL's can further be divided into 2 subclasses: oxygenated solvents and natural products derived from plants. Oxygenated solvents are rich in oxygenated compounds. These solvents can be single compound product (e.g. methanol, isopropanol) or complex mixtures (e.g. lacquer thinner, enamel reducer). Natural products extracted from plants categorized under the subclass of non-petroleum based IL's and includes turpentine and citrus oil extract [1].

IL's are frequently used in different daily-life situations varying from domestic use to commercial or industrial use. However, these substances can be used in criminal activities such as in committing arson, in illegal preparation of prohibited drugs and improvised explosive devices (IED's). In cases of arson, IL's are frequently used to initiate the fire due to their easy availability, simple handling, storage, transportation and cost effectiveness. These substances are frequently used as a fire accelerant in arson and in bride burning cases [1, 3]. The unexplained presence of IL's strongly indicates a fire of suspicious origin. Detection and identification of these IL's are therefore helpful in determining origin and cause of fire [4].

Different analytical techniques such as Infrared (IR) spectrophotometry [5], nuclear magnetic resonance (NMR) spectroscopy [6], thin layer chromatography (TLC) [7], gas chromatography (GC) and gas chromatography – mass spectrometry (GC-MS) [8-10] can be used to detect and identify traces of IL's in samples of forensic importance. However, these techniques have many shortcomings. The

drawbacks of IR spectrophotometry include the requirement of a clean, pure and moist free sample for analysis. Lack of reference database and limited access are the major limitations of NMR spectroscopy. TLC can be used to analyse only dye components of sample. However, GC and GC-MS are frequently used for the analysis of IL's due to its high sensitivity, resolution and specificity. Despite the reasonable success of GC, it suffers from certain problems: technique is destructive in nature, peaks generated from substrate and burning and pyrolysis products of it causes interference in identification, evaporation of IL's during fire causes loss of its low boiling components which further raises problems in interpretation of chromatograms [11]. Since some IL's contain number of components so long run time is required to resolve these components and hence method is time consuming. The complex nature of chromatograms makes the comparison process tedious and raises the question against the reliability of interpretation and identification. Peaks from the background substrates such as cloth, wood etc. also enhance the complexity of chromatogram and further complicate the interpretation of chromatogram [12]. Most of these problems associated with GC can be minimized or eliminated through the use of UV spectrophotometry in derivative mode.

Derivative ultraviolet spectrophotometry is an analytical technique in which normal zero order spectrum of sample is mathematically differentiated into a derivative (first or higher derivatives) and thereby enhances the "fingerprint" of a sample and provides cleaner spectrum. It isolates qualitative and quantitative information from overlapping bands of the analytes and interferences and useful for analysis of mixture of multi-components. This technique improves resolution bands, eliminates the influence of background or matrix and provides more defined fingerprints than traditional ordinary or direct absorbance spectra. It can separate superimposed curves for quantitative measurements and is able to suppress matrix effects [3,13,14]. Second derivative UV spectrophotometry improves the detectability of spectral features [15].

Lawrence [15] used second derivative UV spectrometric method to differentiate amphetamine and phenethylamine. Transformation of spectrum into derivative form enhances the resolution of peaks and thereby improves the specificity of method. Davidson and Elsheikh [16] determined ephedrine and pseudoephedrine in different kinds of pharmaceutical preparations using second and fourth derivative UV spectrophotometry. Lawrence and MacNeil [17] used second derivative UV spectrometry to differentiate amphetamine, ephedrine, meperidine, phentermine and phenethylamine. Gill et al. [18] used derivative UV spectroscopy to detect amphetamine in liver extract. It is observed that second derivative UV spectra can be used to eliminate the broad background absorption. Verweji and Bonte [19] detect the carboxyhaemoglobin in blood samples by using second derivative spectrophotometry. Cruz et al. [20] also determined carboxyhaemoglobin and total haemoglobin in carbon monoxide intoxicated patients by using third derivative spectrophotometry. Randez-Gil et al. [21, 22] simultaneously determined nitrazepam and clonazepam in urine and blood plasma samples by high order (fourth and fifth) derivative spectrophotometry. Lawrence and Kovar [23] used amplitude difference D_{308}-D_{300} and D_{286}-D_{282} to determine the concentrations of cannabinol and Ä^{9}-tetrahydrocannabinol in mixtures containing up to 99% of Ä^{9}- tetrahydrocannabinol and 50% of cannabinol. A group of scientists [24, 25] identified and determined cocaine in binary mixtures of cocaine and local anaesthetics using derivative UV spectrophotometry. It was observed that technique was useful to distinguish cocaine-lidocaine, cocaine-procaine, cocaine-tetracaine and cocaine-benzocaine mixtures. Kuo et al. [26] simultaneously determined diquat and paraquat in blood, urine and tissue samples using second order UV derivative spectrophotometry. Sharma et al. [27] analyzed some commonly abused over the counter drugs by derivative ultraviolet spectrophotometric method. Kaur et al. [28] analyzed some undetonated explosives by derivative ultraviolet spectrophotometry. Saini et al. [29] compared some lipstick smears by ultraviolet-visible spectrophotometry operated in derivative mode and pointed out that derivative spectrophotometry provides more points for comparison than conventional ultraviolet spectrophotometry. Meal [30] analysed the fire debris samples by using second derivative ultraviolet spectroscopy and observed a unique and easily recognizable second derivative UV spectrum of petrol, kerosene and diesel. Absence of minima at 251nm and maxima at 261nm in second derivative spectrum of kerosene differentiate it from diesel. Zerlia et al. [31] analyzed different petroleum products using ultraviolet spectrometry and suggested that present method can be used as a tool for rapid screening of petroleum products in petroleum field without performing chromatographic separation prior to analysis by ultraviolet spectrometry. Bumbrah et al. [32] detected residues of petroleum products residues in simulated fire debris samples by derivative UV spectrophotometry. Dixit et al. [33] used second derivative ultraviolet spectrophotometry to determine

naphthalene and its derivatives in petroleum fractions.

It is observed that very little work had been done on the analysis of IL's using derivative ultraviolet spectrophotometry. Therefore, in the present study, UV spectrophotometry in normal and derivative mode is used to analyse IL's. In this paper, we describe the potential utility of normal and derivative UV spectrophotometry in the analysis of IL's**.**

Materials and Method

Reagents and Samples

The commercially available IL's including thinners and petroleum products (petrol, kerosene and diesel) analysed in the present study were purchased from local market, petrol pump and oil depot of Patiala city, Punjab and details of samples are given in Table 1. Cyclohexane of analytical grade and Whatman filter paper were purchased from Loba Chemie, Ambala. All samples were stored in glass vials (20ml) with screw caps (Labbox, Mataro, Barcelona, Spain) and kept at 4^0C until their analysis.

Data Acquisition

Double beam UV-VIS spectrophotometer with model 1700 PharmaSpec (Shimadzu Corporation, Kyoto, Japan) was used to record the absorbance of samples. UVProbe Version 2.32 (Shimadzu Corporation, Kyoto, Japan) software was used to record and process the normal and higher order derivative UV spectra of all samples. Quartz cells of 1cm path length were used. Instrument was operated in spectrum mode to record the zero, first and second order derivative spectra of samples. All samples were scanned from 320 to 245nm region of ultraviolet band. Sample concentrations were adjusted to provide a sample absorption maximum of within unity. Cyclohexane was used as extracting solvent as well as reference. The following instrumental parameters were kept constant throughout the present study:

- Measurement mode – Absorbance
- Scanning range – 320-245nm
- Absorbance recording range – 0.00A ~ 1.00A
- Scan speed – Fast
- Scan mode – Auto
- Number of scans – 1
- Display mode – Overlay

Sample Preparation

Before preparing the samples, all IL's were filtered using Whatman filter paper in order to remove any contaminants or sediments present in it. Neat samples of IL's were prepared by dissolving 20µl filtered IL in 10ml of cyclohexane and were subjected to UV spectrophotometry and spectrum was recorded in the range of 320 to 245nm. Three different samples of each IL in their neat state was analysed three times. In this way, total of 90 spectra were recorded.

Results and Discussion

In the present study, ultraviolet spectrophotometry in normal and derivative mode is used to analyze different kinds of IL's including petroleum products. The potential utility of this technique in screening of different IL's is observed. Table 1 reflect the characteristic peak wavelengths of different IL's in their neat states along with type of spectrum recorded.

Table 1: Description of ignitable liquids including sample code, name, class, colour, brand, manufacturer and location

S. No.	Sample Code	Name	Class	Colour	Brand Name	Manufacturer	Location
1.	S1	Crude	Thinner	Colorless	Local	NR	Patiala
2.	S2	Shine	Thinner	Colorless	SL	Kayson Thinners & Chemicals	Jalandhar
3.	S3	Lusture	Thinner	Dark Purple	Ramble	Ramble Cosmetics	Delhi
4.	S4	Welcome	Thinner	Colorless	New Wembley	New Wembley Products	Delhi
5.	S5	Axalta	Thinner	Colorless	Axalta	Axalta Coating India Pvt. Ltd.	Gujarat
6.	S6	Coats	Thinner	Dark Yellow	NR	NR	Patiala
7.	S7	Nails	Nail Polish Remover	Colorless	Camieo	NR	Delhi
8.	S8	Petrol	Petroleum Product	Orange	Hindustan Petroleum	Hindustan Petroleum	Patiala
9.	S9	Kerosene	Petroleum Product	Blue	Public Distribution Services	NR	Patiala
10.	S10	Diesel	Petroleum Product	Yellow	Hindustan Petroleum	Hindustan Petroleum	Patiala

The zero order spectrum of sample S1 shows a broad absorption region at 261.5nm (Figure 1). However, in case of its first and second order derivative, the spectra are bipolar with more points for comparison. In its first order derivative spectrum, maximum absorbance occurs at 252.0nm (Figure 2) while in second order derivative spectrum of sample S1, this characteristic peak shifts to 272.0nm (Figure 3). In case of sample S1, as such no specific pattern is observed in shifting of wavelength region of characteristic absorption peak with change in derivative order. It is observed that number of maxima and minima points increases with derivative order (i.e., from zero to second order derivative). The second order derivative spectrum has more number of maxima and minima points than their corresponding zero and first order derivative spectra (Table 2). The zero order spectrum of sample S2 shows a broad absorption region at 262.0nm (Figure 1). In its first order derivative spectrum, peak of maximum absorbance shifts from 262.0nm to 251.5nm (Figure 2) while in its second order derivative spectrum, this characteristic peak further shifts to 273.0nm (Figure 3). Similar trend, as in case of sample S1, is observed in number of maxima and minima points in respective spectra with their derivative orders (Table 2).

In zero order spectrum of sample S3, peak of maximum absorption is present at 278.0nm (Figure 1) which is shifted to 256.0nm in its first order derivative spectrum (Figure 2). In second order derivative spectrum of sample S3, this characteristic peak further shifts to 298.5nm (Figure 3). Neither increase nor decrease is observed in number of maxima points with derivative order. All spectra of sample S3 have only one maxima point irrespective of derivative order. However, second order derivative spectrum of sample S3 has more number of minima points than their corresponding zero and first order derivative spectra. Sample S4 show no characteristic peak between 320 and 245nm in its zero order spectrum (Figure 1). However, only one peak at 266.5nm is observed in its first order derivative spectrum. Some hidden peaks are resolved and observed at 272.5, 265.5, 257.5 and 252.0nm in its second order derivative spectrum (Table 2). It is observed that second order derivative spectrum of sample S4 have more points, between 320 and 245nm, for comparison than their corresponding zero and first order derivative spectra. The zero order spectrum of sample S5 shows a broad absorption region at 265.5nm (Figure 1). However, in its first order derivative spectrum, peak of maximum absorbance shifts from

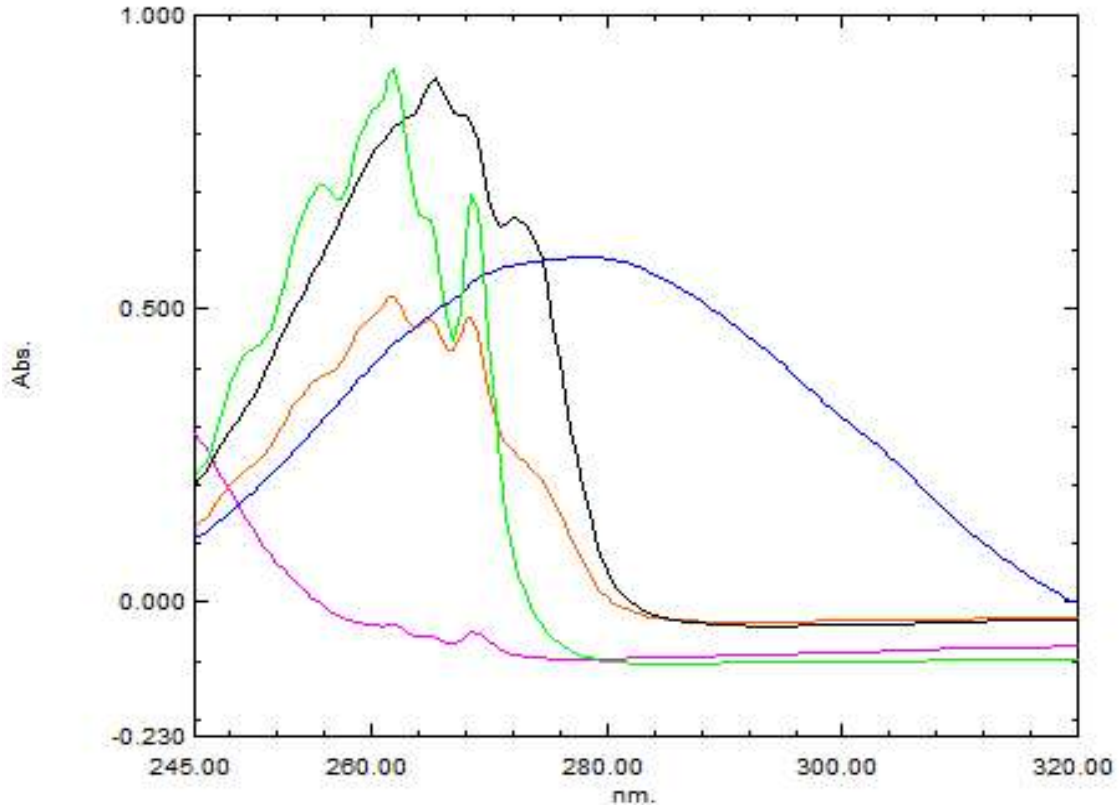

Fig. 1: Normal UV spectra of S1 (Orange), S2 (Green), S3 (Blue), S4 (Pink) and S5 (Black)

Table 2: Characteristic peaks of ignitable liquids in their normal and higher order derivative spectra (1st and 2nd)

S.No.	Sample Code	Spectrum Order					
		Zero		First		Second	
		Maxima (nm)	Minima (nm)	Maxima (nm)	Minima (nm)	Maxima (nm)	Minima (nm)
1.	S1	261.5[a]	292.0	252.0[a]	276.5, 274.5, 271.5	280.0, 272.0[a], 265.0, 257.5, 251.0, 245.0	275.0, 268.0, 260.5, 254.0
2.	S2	262.0[a]	285.0	251.5[a]	272.0, 266.5, 263.5	273.0[a], 265.5, 258.0, 251.5, 245.5	269.0, 261.5, 254.5, 248.5
3.	S3	278.0[a]	-	256.0[a]	307.5, 301.5, 296.5	298.5[a]	304.0, 280.0, 275.5, 270.5, 263.0
4.	S4	-	277.0	266.5[a]	272.0	272.5, 265.5, 257.5, 252.0	269.0, 262.5
5.	S5	265.5[a]	293.0	254.5[a]	277.0	279.0[a], 270.5, 256.0, 249.5	274.5, 266.5, 253.5, 247.0
6.	S6	272.5, 270.5, 268.0[a]	319.5, 317.0	258.0[a]	294.5, 288.0, 278.0	298.0, 282.5[a]	293.5, 288.0, 273.5, 266.5
7.	S7	275.0[a], 258.5	-	270.0[a]	304.5, 286.5	289.5[a]	283.5, 275.0
8.	S8	261.5[a]	310.0	250.0[a], 246.0	277.0	279.0[a], 271.5, 265.0, 251.0, 245.0	274.5, 267.5, 260.5, 254.5, 248.0
9.	S9	272.5[a], 270.5, 268.5	317.5	257.5[a]	295.5, 289.0, 281.0	298.5, 283.0[a], 255.5, 252.5	293.0, 278.5, 276.0, 273.0, 269.5, 266.5, 262.0, 260.0
10.	S10	258.5[a]	315.0	245.0[a]	277.0, 271.5, 268.5	280.0[a], 270.5	274.5, 266.5, 259.0, 254.0, 247.0

"a" – Indicates wavelength of maximum absorbance
"-" – Not detected

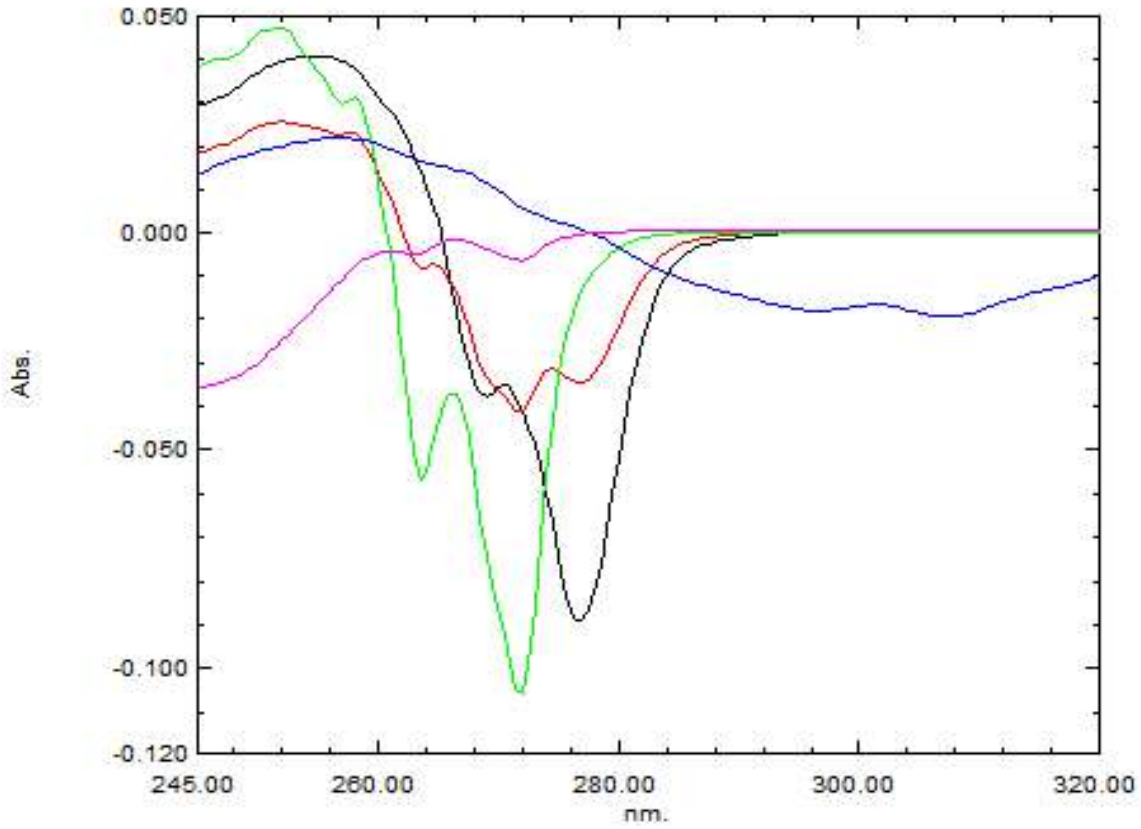

Fig. 2: First order derivative UV spectra of S1 (Red), S2 (Green), S3 (Blue), S4 (Pink) and S5 (Black)

265.5nm to 254.5nm (Figure 2) which is further shifted to 279.0nm in its second order derivative spectrum (Figure 3). The second order derivative spectrum of sample S5 also shows three maxima points at 270.5, 256.0 and 249.5nm respectively. Same numbers of maxima and minima points are present in all spectra of sample S5. The second order derivative spectrum of sample S5 contains more number of maxima and minima points than their corresponding zero and first order derivative spectra.

The zero order spectrum of sample S6 shows a characteristic absorption peak at 268.0nm (Figure 4). It also shows two maxima points at 272.5 and 270.0nm. However, in case of its first order derivative spectrum, maximum absorbance occurs at 258.0nm (Figure 5) while in second order derivative spectrum of sample S6, this characteristic peak shifts to 282.5nm (Figure 6). It is observed that number of maxima points decreases from zero to first order derivative spectrum. However, the number of minima points increases with increase in derivative order. The second order derivative spectrum has more number of minima points than their corresponding zero and first order derivative spectra (Table 2). In zero order spectrum of sample S7, peak of maximum absorption is present at 275.0nm (Figure 4) which is shifted to 270.0nm in its first order derivative spectrum (Figure 5). In its second order derivative

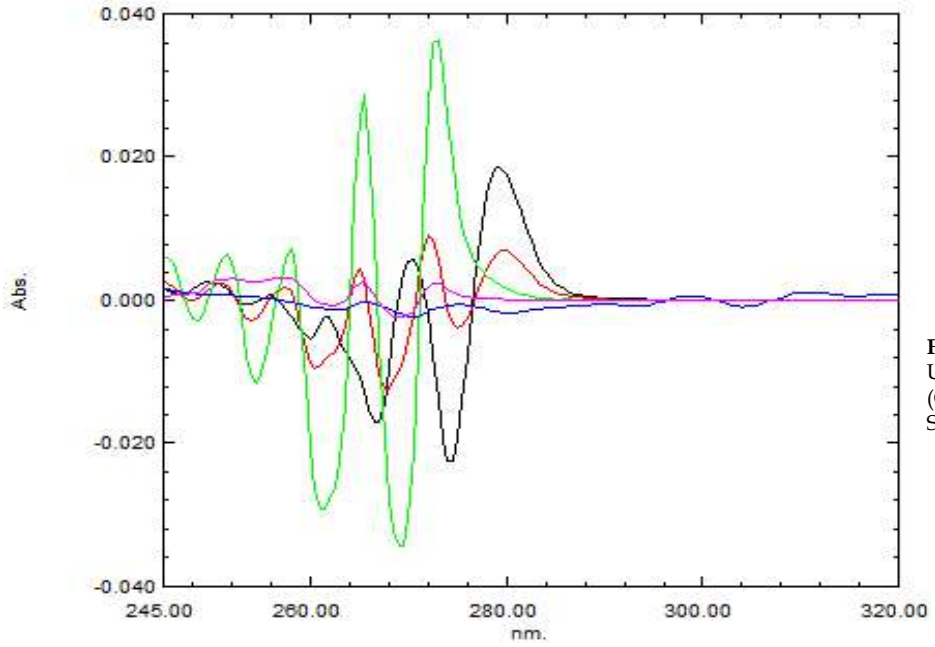

Fig. 3: Second order derivative UV spectra of S1 (Red), S2 (Green), S3 (Blue), S4 (Pink) and S5 (Black)

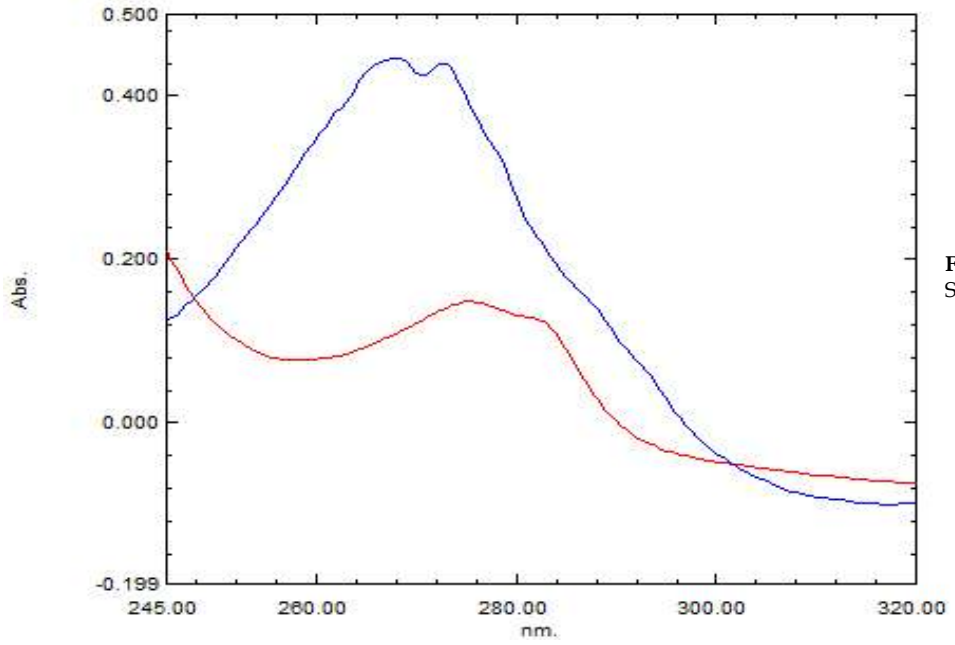

Fig. 4: Normal UV spectra of S6 (Blue) and S7 (Red)

spectrum, this characteristic peak shifts from 270.0 to 289.5nm (Figure 6). The first and second order derivative spectra of sample S7 have same number of maxima and minima points but at different wavelengths.

The zero order spectrum of sample S8 shows a broad absorption region at 261.5nm (Figure 7). This characteristic absorption peak shifts from 261.5nm to 250.0nm in its first order derivative spectrum (Figure 8) which is further shifted to 279.0nm in its second order derivative spectrum (Figure 9). The second order derivative spectrum of sample S8 also shows maxima points at 271.5, 265.0, 251.0 and 245.0nm respectively. It is observed that number of maxima points increases with increase in derivative order of spectrum. The second order derivative spectrum has more number of minima points than their corresponding zero and first order derivative

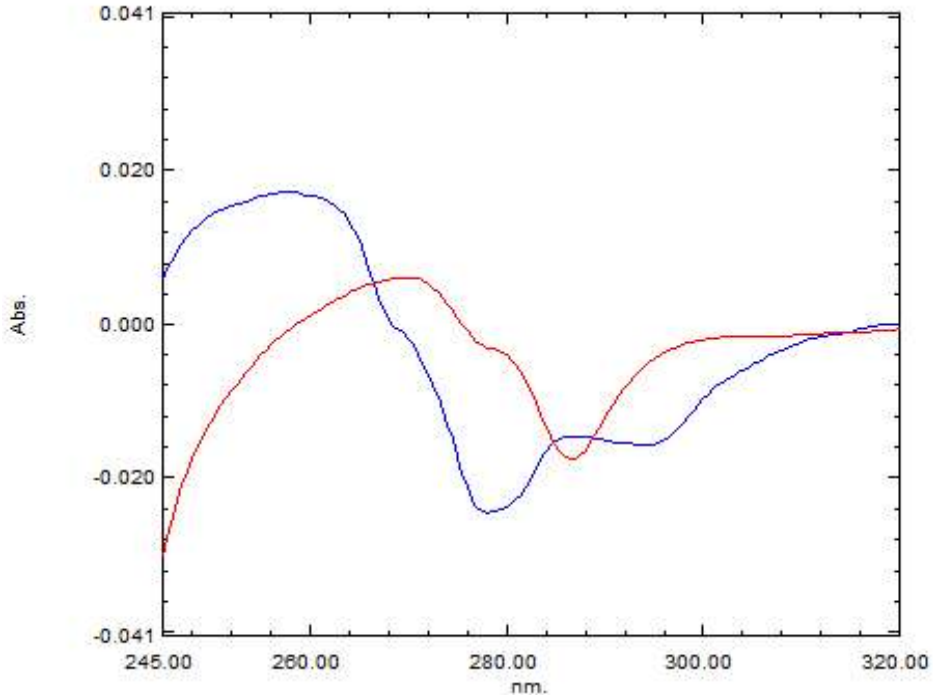

Fig. 6: Second order derivative UV spectra of S6 (Blue) and S7 (Red)

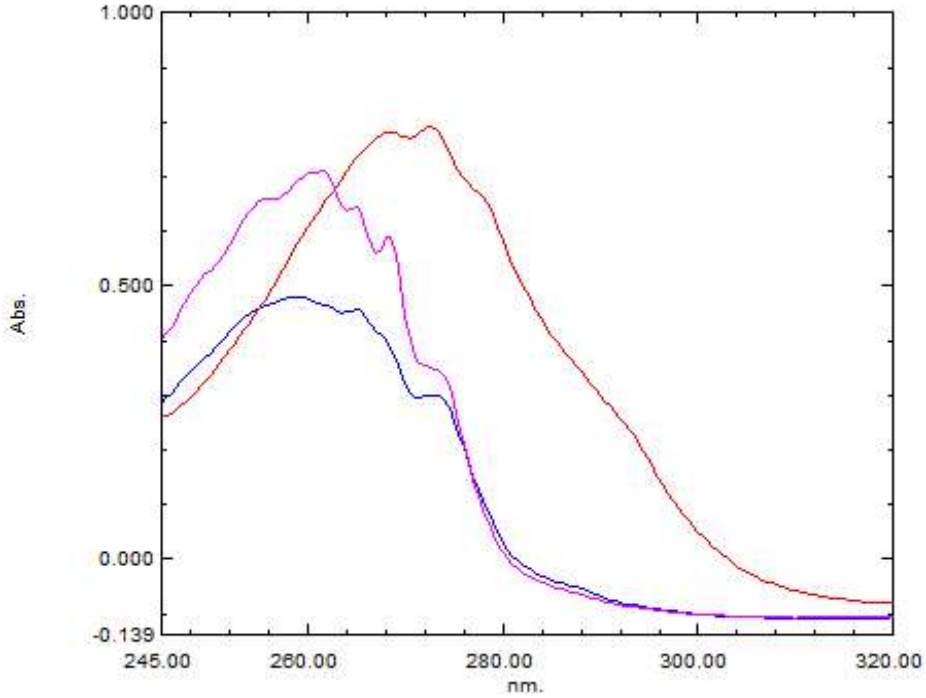

Fig. 7: Normal UV spectra of S8 (Pink), S9 (Red) and S10 (Blue)

spectra (Table 2). Meal [30] observed characteristic strong minima at 274nm in second order derivative spectrum of petrol. In a similar study, Bumbrah et al. [32] observed characteristic strong maxima at 251.3nm and minima's at 274.1 and 248.0 in second order derivative spectrum of petrol. The zero order spectrum of sample S9 shows a characteristic absorption peak at 272.5nm (Figure 7). It also shows two maxima's at 270.5 and 268.5nm. However, in case of its first order derivative spectrum, maximum absorbance occurs at 257.5nm (Figure 8) while in its second order derivative spectrum, this characteristic peak shifts from 257.5nm to 283.0nm (Figure 9). Second order derivative spectrum of sample S9 also

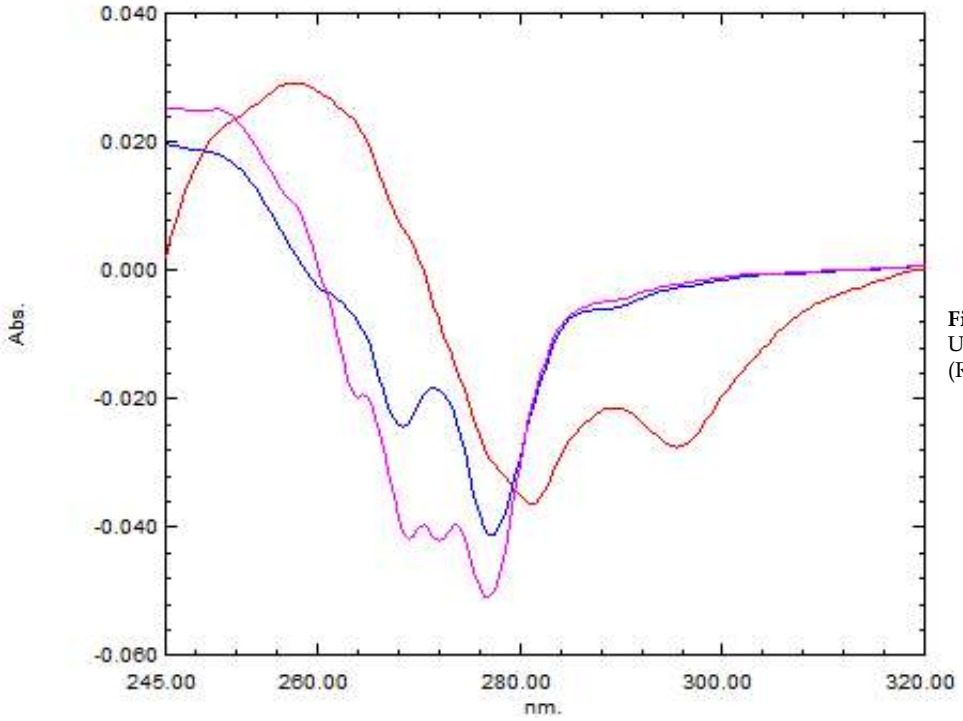

Fig. 8: First order derivative UV spectra of S8 (Pink), S9 (Red) and S10 (Blue)

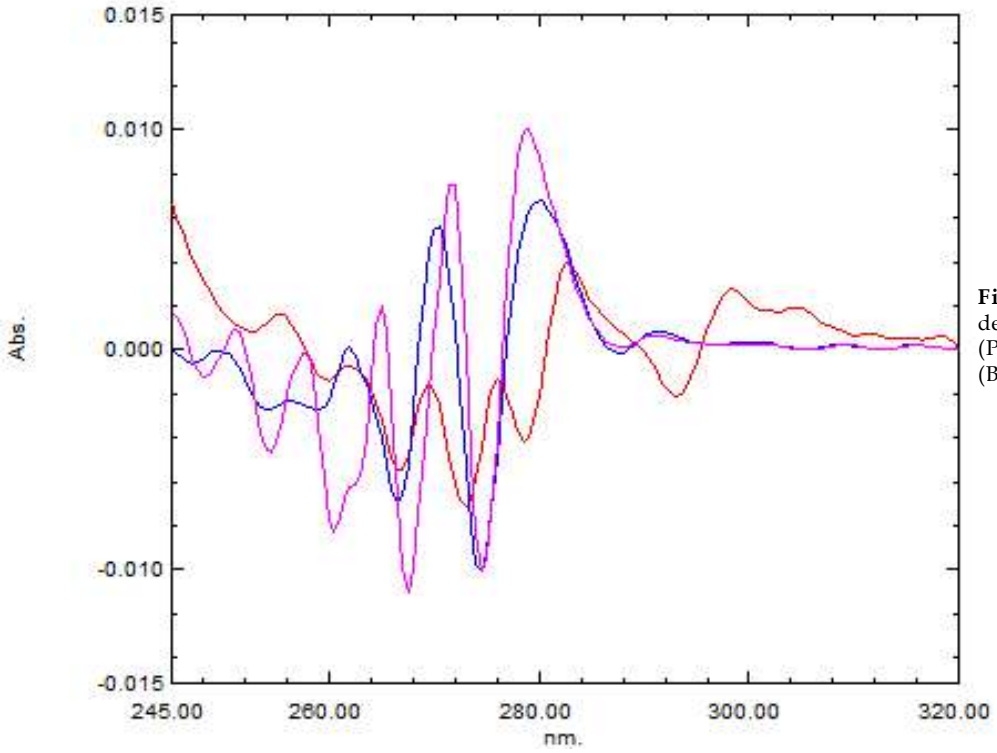

Fig. 9: Second order derivative UV spectra of S8 (Pink), S9 (Red) and S10 (Blue)

shows maxima's at 298.5, 255.5 and 252.5nm respectively. It is observed that number of minima points increases with increase in derivative order of spectrum. The second order derivative spectrum has more number of minima points than their corresponding zero and first order derivative spectra. However, similar correlation is not observed between number of maxima points and derivative order (Table 2). Meal [30] observed characteristic strong minima at 276nm in second order derivative spectrum of kerosene which is in accordance with the present study. Bumbrah et al. [32] also observed strong maxima points at 272.7nm, 268.1nm and 257.8nm in zero and first order derivative spectra of kerosene. Strong minima points at 317.5nm and 289.1nm are also observed in zero and first order derivative spectra of kerosene. In zero order spectrum of sample S10, peak of maximum absorption is present at 258.5nm (Figure 7).

This characteristic absorption peak shifts from 258.5nm to 245.0nm in its first order derivative spectrum (Figure 8) which is further shifted to 280.0nm in its second order derivative spectrum (Figure 9). It is observed that number of minima points increases with increase in derivative order of spectrum. The second order derivative spectrum has more number of minima points than their corresponding zero and first order derivative spectra (Table 2). Bumbrah et al. [32] also observed strong maxima and minima points at 270.2nm and 247.2nm in second order derivative spectrum of diesel.

It is observed that appearance of strong maxima and minima at 272.0nm and 268.0nm in second order derivative spectrum of sample S1 are specific and can be used to differentiate it from other samples. Similarly, maxima at 273.0nm and minima's at 261.5nm and 248.5nm, in second order derivative spectrum of sample S2, are specific. In second order derivative spectrum of sample S3, strong minima's at 304.0nm and 280.0nm can be used to differentiate it from other samples. Although the zero order spectrum of sample S4 is unique in itself and did not match with rest of samples yet the presence of strong maxima and minima at 252.0nm and 262.5nm in its second order derivative spectrum are useful to differentiate it.

In second order derivative spectrum of sample S5, presence of maxima and minima at 249.5nm and 253.5nm can be used to differentiate it from other samples. In second order derivative spectrum of sample S6, maxima and minima at 282.5nm and 288.0nm are specific. Similarly, in second order derivative spectrum of sample S7, maxima and minima at 289.5nm and 283.5nm can be used to discriminate it from other samples. Sample S8 can be easily distinguished from other samples by observing strong maxima and minima's at 271.5nm, 267.5nm and 248.0nm in its second order derivative spectrum. Similarly, sample S9 can be easily discriminate from other samples by observing strong maxima's and minima at 283.0nm, 255.5nm and 278.5nm in its second order derivative spectrum. In second order derivative spectrum of sample S10, presence of strong minima at 259.0nm is specific and can be used to discriminate it from other samples.

It is important to note that peak shifts from zero to first order derivative spectrum are close while a significant change is observed in peak shifts from first to second order spectrum of all samples. Thus, peak shifting is a useful parameter to distinguish different IL's on the basis of their second order derivative spectra. Therefore, peak shifting along with the visual inspection of normal and higher order derivative spectra are useful to discriminate IL's. The presence or absence of maxima or minima point in derivative spectra (first and second) can be used to establish the differentiation between samples and can be helpful in exclusion of suspected IL. Although the technique is incapable of identifying the IL yet it is very useful and effective in screening. Derivative spectra not only provide more points for comparison than their corresponding normal zero order spectrum but it also enhances the certainty in exclusion of samples. Transformation of normal UV spectrum into derivative (first and second) spectra also enhances the discriminating potential of this technique.

Derivative UV spectrophotometric technique can be very useful and helpful for the screening of IL's. In addition to this, the technique can be used for initial screening purpose and final identity can be established by analyzing the same sample with more sophisticated instrumental techniques such as GC-MS and GC-MS-MS.

Conclusion

Derivate Ultraviolet spectrophotometry is a simple, easy, cost-effective, non-destructive, well established analytical technique for the analysis of IL's as it provides much better fingerprints of IL's than the conventional UV spectrophotometry by resolving the overlapped and hidden peaks in spectra of IL's. It is a rapid and reliable screening technique and can be used to exclude the sample by visual comparison of derivative spectra of sample with their corresponding standard at the initial stage of analysis process.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Review Article

Neurotoxic Effect of Insecticides on Human Nervous System

Vikash[1], Anil Kumar Verma[2], A.K. Jaiswal[3], Sudeep Mishra[4]

Authors Affiliation: [1]Department of Forensic Science, Galgotias University, Greater Noida, India. [2,4]Institute of Pesticide Formulation Technology, Sec-20, Udyog Vihar, Gurugram,Haryana, India. [3]Department of Forensic Medicine and Toxicology , AIIMS, New Delhi, India.

Reprints Requests: Vikash, Department of Forensic Science, Galgotias University, Greater Noida - 201310 , Uttar Pradesh, India.

E-mail: vikashratawjia.pmt@gmail.com

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Abstract

Prologue- Pesticides is a generic term for a variety of agents that may be classified more specifically on the basis of pattern of use and organism killed such as insects, weeds, fungi, and rodents. A number of pesticides can cause neurotoxicity. It is not surprising that these agents also have neurotoxic effects on large mammals including humans. Despites man's persistent efforts to develop mechanism of actions in selectivity and specificity of these agents towards certain species while reducing toxicity to other forms of life, all pesticides possess an inherent degree of toxicity to human being.

This family of chemicals such as the organophosphates, the carbamates, the pyrethroids, the organochlorines, and other compounds directly target nervous system to show their mechanism of toxicity. Insecticides interfere with chemical neurotransmitter or ion channels in nerve cell, and usually cause reversible neurotoxic effects, that could nevertheless be lethal. The effects of pesticides on the nervous system is as neurotoxins, or may contribute to chronic neurodegenerative disorder, one of the most common notably is Parkinson's and Alzhemier Disease. This brief review summarizes some of the main neurotoxic insecticides, their effects and mode of action.

Keywords: Neurotoxicity; Neurotransmitter; Organophosphates; Carbamates; Organochlorines; Pyrethroids.

Introduction

The term "agricultural chemicals" has largely been replaced by the term "pesticides," defined as economic poisons, regulated by federal and state laws that are used to control, kill, or repel pests [1]. It can be defined as any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating pests. Pests can be insects, rodents, weeds, and a host of other unwanted organisms [2]. Thus, pesticides occupy a rather unique position among the many chemicals that we encounter daily, in that they are deliberately added to the environment for the purpose of killing or injuring some form of life. Ideally, their injurious action would be highly specific for undesirable targets; in fact, however, most pesticides are not highly selective, but are generally toxic to many non target species, including humans.

Thus, the use of pesticides must minimize the possibility of exposure of non target organisms to injurious quantities of these chemicals [3]. As there are dozens of drugs with different therapeutically indications and different mechanisms of action, several different classes of pesticides exist, with different uses, mechanisms and hence, toxic effects in non target organisms. The most common classification of pesticides relies on the target species they act on. The four major classes (and their target pests) are those of insecticides (insects), herbicides (weeds), fungicides (fungi, molds), and rodenticides (rodents).

In addition, for regulatory purposes, plant growth regulators, repellents, and attractants (pheromones) often also fall in this broad classification of chemicals. Furthermore, within each class, several subclasses exist, with substantially different chemical and toxicological characteristics. For example, among

insecticides, one can find organophosphorus compounds, carbamates, organochlorines, pyrethroids, and many other chemicals.

Depending on what a compound is designed to do, pesticides have been sub classified (as shown in Table: 1) into a number of categories [4].

- Generally, a new pesticide takes some five to seven years to bring it to market once its pesticidal properties have been verified. Many tests must be conducted to determine such things as the compound's synthesis, its chemical and physical properties, and its efficacy and there are numerous toxicity tests are undertaken including those for acute toxicity and chronic effects such as reproductive anomalies, carcinogenesis, and neurological effects and those for environmental effects.
- In the United States, the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) was passed in 1962 (amended in 1974, 1978, and 1988.This act divides all pesticides in four broad classes depending on their toxicity.

The label of each pesticide has to contain a signal word depending on its toxicity. The criteria established by the FIFRA are given in (Table-2) [5].

Table 1: Classification of Pesticides, with Examples

Class	Principal Chemical Type	Example, Common Name
Fungicide	Dicarboximide	Captan
	Chlorinated aromatic	Pentachlorophenol
	Dithiocarbamate	Maneb
	Mercury	Phenyl mercuric acetate
Herbicide	Amides, acetamides	Propanil
	Bipyridyl	Paraquat
	Carbamates, thiocarbamates	Barban
	Phenoxy	2,4-D
	Dinitrophenol	DNOC
	Dinitroaniline	Trifluralin
	Substitute urea	Monuron
	Triazine	Atrazine
Insecticide	**Chlorinated hydrocarbons**	
	DDT analogous	DDT, DDD
	Chlorinated alicyclic	BHC
	Cyclodiene	Aldrin
	Chlorinated terpenes	Toxaphene
	Organophosphorus	Chlorpyrifos
	Carbamate	Carbaryl
	Thiocyanate	Lethane
	Botanicals	
	Nicotinoids	Nicotine
	Rotenoids	Rotenone
	Pyrethroids	Pyrethrin
	Synthetic pyrethroids	Fenvalerate
	Synthetic nicotinoids	Imidacloprid
	Fiproles	Fipronil
	Juvenile hormone analogs	Methroprene
	Growth regulators	Dimilin
	Inorganic	
	Arsenicals	Lead arsenate
	Fluorides	Sodium fluoride
	Microbial	Thuricide, avermectin
Rodenticides	Anticoagulants	Warfarin
	Botanicals	
	Alkaloids	Strychine sulphate
	Glycosides	Scillaren A and B
	Fluorides	Fluoroacetate
	Inorganics	Thallium sulphate

What's the LD_{50}???

It's the Lethal Dose of a chemical compound, measured as milligrams of chemical per kilogram of body weight (mg/kg), which would theoretically kill 50% of an exposed population.

Table 2: Criteria of Pesticide Toxicity, Established by the Federal Insecticide, Fungicide and Rodenticide Act of 1962 [5]

S. No.	Category	Toxicity	Acute Oral LD50	Inhalation LD50
1	Danger& poison	High	0-50 mg/kg	Up to 0.2mg/L
2	Warning	Moderate	50-500mg/kg	0.2-2 mg/L
3	Caution	Low	500-5000mg/kg	2-20 mg/L
4	Caution	Relative safe	more than 5000 Mg/kg	more than 20 mg/L

- In India, a predominantly agricultural country, handling of insecticides is governed by *The Insecticides Act 1968 and The Insecticides Rules, 1971 (amended in 1993). Section 19 of The Insecticide Rules, 1971* classifies insecticides on a similar basis. Section 19 also insists on affixing a label to the insecticide container in such a manner that it cannot be ordinarily removed. Among other things, it must contain a square, occupying not less than one sixteenth of the total area of the face of the label, set at an angle of 45° ***(diamond shape).*** This square is to be divided into two equal triangles, the upper portion of which shall contain the "signal word" and the lower portion the specified colour. The classification of insecticides, signal words to be used, and the colour of the "identification band" on the label according to The Insecticide Rules, 1971 of India are given below :(see fig:1).

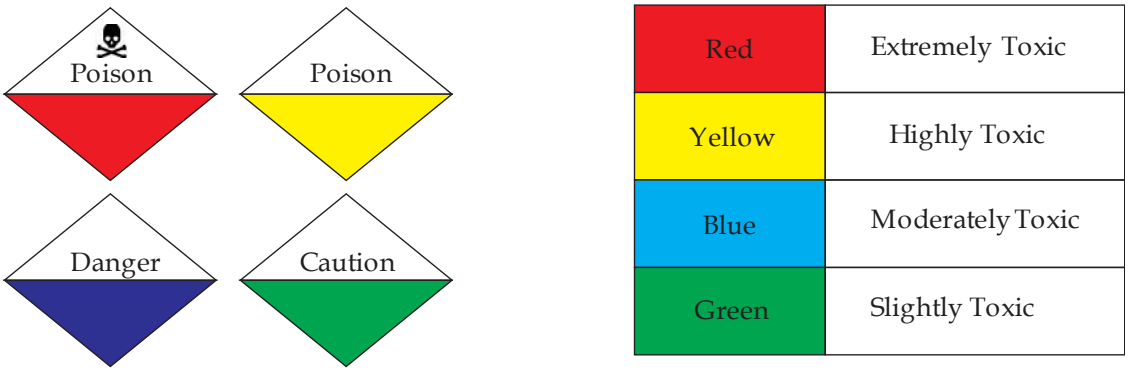

Fig. 1: Toxicity classification of pesticides using signal word and identification band color

Human Poisoning

Fig. 2: Exclusive news of mass poisoning through pesticides in India

Agrochemical poisoning remains one of the major causes of morbidity and mortality around the world today [6]. Peoples are exposed to low levels of pesticides every day. You can be exposed to pesticides in a variety of places including your home, at school, or at work. Pesticides can get inside your body from eating, drinking, breathing, them in, and by skin contact. Different pesticides affect human health in different ways. For example, some pesticides may affect the nervous system, while other may show their affect on skin and eyes. Yet, from a global perspective, the major problem with pesticides remains that of acute human poisoning.

- The ***World Health Organization (WHO)*** estimated that there are around three million hospital admissions for pesticide poisoning each year, that result in around 220,000 deaths (WHO, 1990). The 2002 annual report of the *American Association of Poison Control Centres (AAPCC)* Toxic Exposure Surveillance System listed a total of 2,380,028 human exposures to poisons occurring in the United States during the year 2002 alone. Most occur in developing countries, particularly in Southeast Asia, and a large percentage is due to intentional ingestion for suicide purposes [7].

Insecticide

An **insecticide** is a substance used to kill insects. They include ovicides and larvicides used against insect eggs and larvae. Insecticides are used in agriculture, medicine, industry and by consumers. Insecticides are claimed to be a major factor behind the increase in agricultural 20th century's productivity. Nearly all insecticides have the potential to significantly alter ecosystems; many are toxic to humans; some concentrate along the food chain [8]. All of the chemical insecticides in use today are neurotoxicants, and act by poisoning the nervous systems with specific molecular target of insecticide in organisms with their different mode of action (Table-3, 4). The central nervous system of insects is highly developed and not unlike that of mammals, and the peripheral nervous system, though less complex, also presents striking similarities. Thus, insecticides are mostly not species-selective with regard to targets of toxicity, and mammals, including humans, are highly sensitive to their toxicity [9].

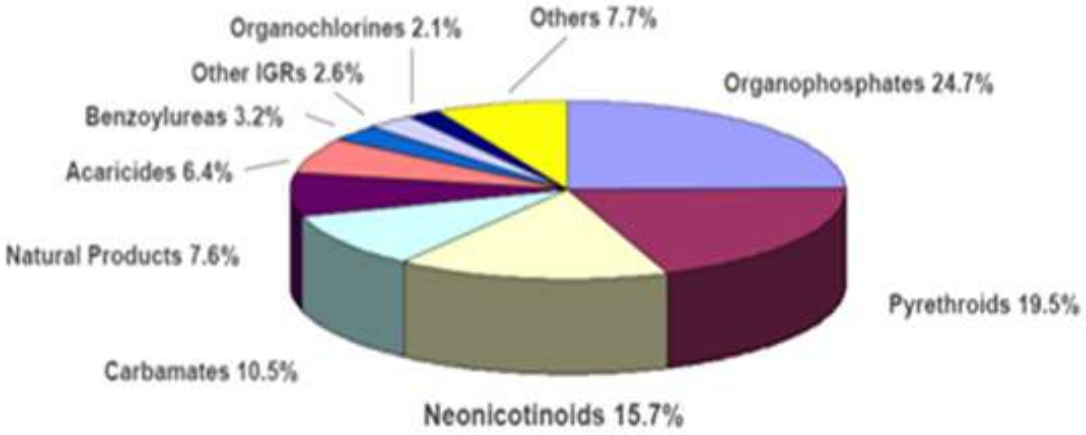

Fig. 4: Global Insecticide Sales in 2003

Table 3: Molecular Targets of the Major Classes of Insecticides

Toxicity Target	Insecticide	Effect
Acetyl cholinesterase	Organophosphates	Inhibition
	Carbamates	Inhibition
Sodium channels	Pyrethroids (Type I and II)	Activation
	DDT (Organochlorine)	Activation
Nicotinic acetylcholine	Nicotine	Activation
Receptors	Neonicotenoids	Activation
GABA receptors-gated	Organochlorine (Cyclodiene type)	Inhibition
Chloride channels	Pyrethroid (Type II)	Inhibition

Toxicological Effects of Insecticides on Nervous System

Human Nervous System???

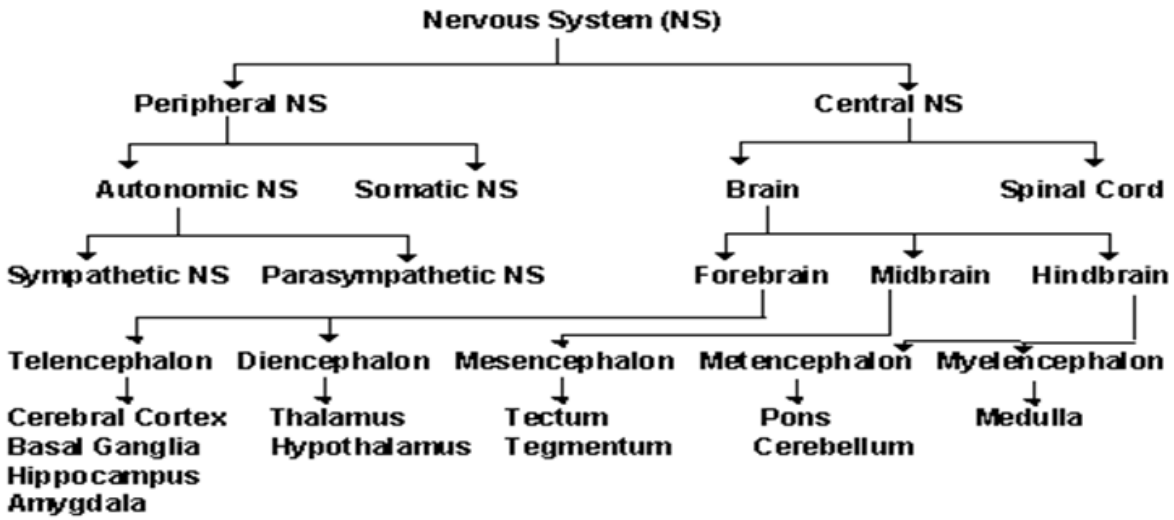

Fig. 5: Typical view of arrangement of Physiological System of Nervous System

The nervous system functions as a fast-acting means of transmitting important information throughout the body. The nervous system has two components :(shown in fig-5)

1. The *peripheral nervous system* receives and transmits incoming signals (taste, smell, sight, sound, and touch) to the central nervous system, and transmits outgoing signals to the muscles and other organs, effectively telling them how to respond.
2. The *central nervous system (CNS)* interprets the signals and coordinates the body's responses and movements. The CNS is composed of the brain and spinal cord in humans and a series of ganglia, or nerve bundles, in insects.
3. A *neuron* is a single nerve cell. It connects with other neurons and with muscle fibers (the basic units of muscles). These connecting neurons (or connecting neuron and muscle fiber) do not touch, however, and instead have a slight gap between them called a ***synapse***.

Incoming signals (the pain from a sharp object, the sight of a predator, or the odor of food, etc.) are transformed by the neuron into an *electrical charge* that travels down the length of the neuron. The charged particles (called ions) that deliver the charge move through *channels* in the membrane of the neurons.

There are four main types of channels to allow different ions to move along the neuron: *sodium channels, potassium channels, calcium channels*, and *chloride channels*. Many of the channels have gates that open or close in response to a certain stimulus, which is an important mechanism through which some pesticides work. This process repeats over and over until the signal has reached the CNS to be interpreted. Impulses from the CNS to the peripheral nervous system continue in the same way until the signal reaches the appropriate muscles or organs. (***Please see the fig: 6 to understand the mechanism of signal transfer in neuron)***

Humans have many different neurotransmitters that work at different sites throughout the nervous system. Some neurotransmitters are:

The first neurotransmitter is acetylcholine discovered by Otto Loewi got Nobel Prize. Many functions of body are associated with this. It is also present in sensory neurons of nervous sys

Inhibitory: they result in the signal being blocked from travelling to a connecting neuron. In this way, the body ensures that the signal has the desired effect in each muscle or organ, since many different reactions are involved in even a simple movement. e.g.: GABA , Dopamine, Serotonin, Endorphin).

Excitatory: they result in the signal being sent on through the synapse to a connecting neuron. It is not necessary that they are always exciting but they also stimulate the brain. (Epinephrine or Adrenaline, Norepinephrin, Acetylcholine, Glutamate,) [10].

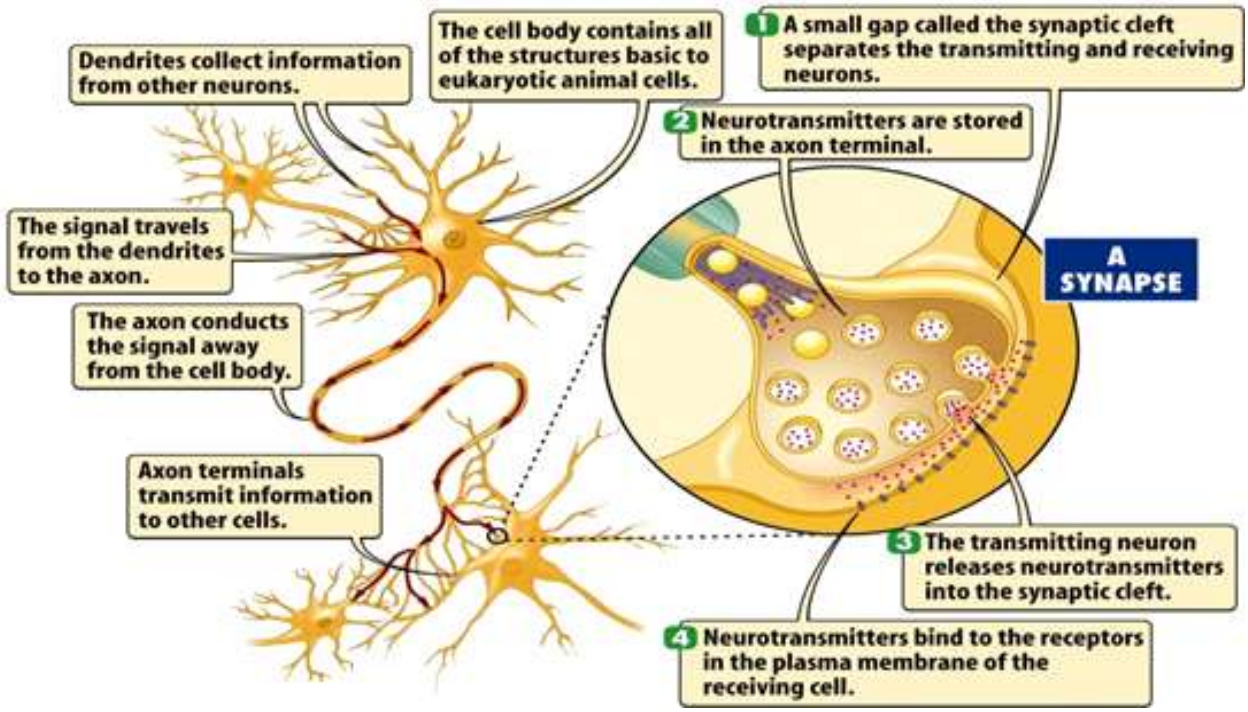

Fig. 6: Mechanism of signal transfer in human nerve cells (Neuron)

Definition of Neurotoxicity

Neurotoxicity is the damage to the brain or the peripheral nervous system by exposure to natural or man-made toxic substances or chemicals**.** This can eventually disrupt or even kill neurons, key cells that transmit and process signals in the brain and other parts of the nervous system.

Mechanism Action of Neurotoxic Effect of Insecticides

The mechanism of action of individual neurotoxin compounds have begun with the identification of the cellular target. In the nervous system, this has most often been one of four targets: the neuron, the axon, the myelinating cell, or the neurotransmitter system. As a result, neurotoxic compounds may be identified which cause neuronopathies, axonopathies, myelinopathies, or neurotransmitter-associated toxicity [11]. Many of the neurotransmitters that humans have, *acetylcholine (ACh)* and *gamma-amino butyric acid (GABA*) are important targets of some insecticides. ACh can either excite or inhibit its target neurons. Depending on the particular neuron and the specific receptors at the site, ACh can cause particular neurons to "fire," continuing the nerve impulse transmission, or it can cause the nerve impulse to stop at that particular site. In contrast, GABA is an inhibitory neurotransmitter. When GABA is the neurotransmitter activated at a synapse, the nerve impulse stops.

Some insecticides interfere with the normal action of these neurotransmitters. Other insecticides attacking the nervous system work by other means.

This means the insecticide does not release the bound cholinesterase. Fortunately, the body continually produces cholinesterase, although it may take several weeks to again reach the desirable circulating level. Applicators using cholinesterase-inhibiting pesticides regularly should consider having their cholinesterase level monitored [12].

Organophosphorus Insecticides

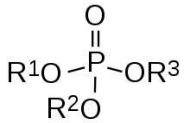

Fig. 7: General Chemical Structure of Organophosphorus Compound

General Review

Organophosphorus pesticides (OPs) are phosphoric acid esters or thiophosphoric acid esters and are among the most widely used pesticides for insect control. During the 1930s and 1940s Gerhard Schrader and co-workers began investigating OP compounds. They realized that the insecticidal

Table 4: Insecticides Modes of Action

Common name (examples of trade names)	Chemical Family (GROUP)	Targeted system/process	Mode of action
Chlorpyrifos (Lorsban) Acephate (Orthene) Chlorpyrifos methyl (Reldan) Diazinon Dimethoate (Rebelate) Disulfoton (Di-Syston) Ethyl parathion (Parathion) Fenitrothion (Sumithion) Fenthion (Baycid, Baytex) Isofenphos (Oftanol, Pryfon) Malathion Methamidophos (Monitor) Methidathion (Supracide) Mevinphos (Phosdrin) Monocrotophos (Azodrin) Naled (Dibrom) Phorate (Thimet) Phosmet (Imidan)	Organophosphates	Nervous system	Cholinesterase inhibitor
Aldicarb(Temik) Aldoxycarb(Standaz) Bendiocarb (Garvox) Carbaryl (Sevin) Carbofuran (Furadan) Carbosulfan(Advantage) Methiocarb (Mesurol) Methomyl (Lannate) Promecarb (Carbamult) Propathrin (Danitol) Propoxur (Baygon)	Carbamates	Nervous system	Cholinesterase inhibitor
Bifenthrin (Brigade, Capture, Empower, Talstar) Cyfluthrin (Baythroid, Countdown, Cylense, Laser, Tempo) Cypermethrin (Ammo, Barricade, Cymbush, Cynoff, Ripcord) Deltamethrin (Decis, DeltaDust, DeltaGard, Flythrin, Suspend) Esfenvalerate (Asana, Hallmark) Fenpropathrin (Danitol) Fenpropathrin (Danitol) Fluvalinate (Mavrik) Gamma-cyhalothrin (Proaxis) Lambda-cyhalothrin (Demand, Karate, Matador, Scimitar, Warrior) Permethrin (Ambush, Astro, Coopex, Outflank, Pounce, Pramex, Talcord) Tau-fluvalinate (Mavrik) Tefluthrin (Evict, Fireban, Force, Raze) Tralomethrin (Scout X-TRA, Tralex)	Pyrethroid	Nervous system	Sodium-channel modulator
Acetamiprid (Assail, Chipco, Pristine) Clothiamidin (Poncho) Imidacloprid (Admire, Advantage, Confidor, Gaucho, Marathon, Merit, Premier, Provado)	Neonicotinoid	Nervous system	Nicotinic Acetylcholine receptor stimulant Agonists/Antagonist
Endosulfan (Thiodan, Thoinex) Methoxychlor Eldrin Dieldrin Endrin Isobenzan Chlordane Chlorinated terpenes (Toxaphene) DDT and its analogs	Organochlorine insecticides of Cyclodiene types(Alicyclics)	Nervous system	Affected the chloride channel by inhibiting the GABA-receptor
Lindane (BHC)	Organochlorine Insecticide	Nervous system	Sodium-channel modulator
	Gamma-HCH (Hexachlorocyclohexane)	Nervous system	Affected the chloride channel by inhibiting the GABA-receptor

properties of these compounds and by the end of the World War II had made many of the insecticidal OPs in use today, such as ethyl parathion [*O,O*-diethyl *O*-(4 nitro phenyl) phosphorothioate]. Chlorpyrifos [*O*, *O*-diethyl *O*-(3, 5, 6-trichloro-2-pyridinyl) phosphorothioate] (as shown in fig: 8) became one of the largest selling insecticides in the world and had both agricultural and urban uses [13]. Other examples in this category are Acephate, Malathion, Methamidophos etc.

Chemically, organophosphates are derived from phosphoric acid (H3PO4) and two chemical groups are available [14]:

i. *Alkyl phosphates* -e.g. tetraethyl pyrophosphate (TEPP), hexaethyl tetraphosphate (HETP), octamethyl pyrophosphoramide (OMPA), dimefox, isopestox, sulfotepp, demeton, malathion (Kill bug; Bugsoline), etc. and

ii. *Aryl phosphates*- e.g. parathion (Follidol; Kill phos; Ekato), paraoxon, methyl parathion (Metacide), chlorthion, diazinon (Diazion; Tik 20).

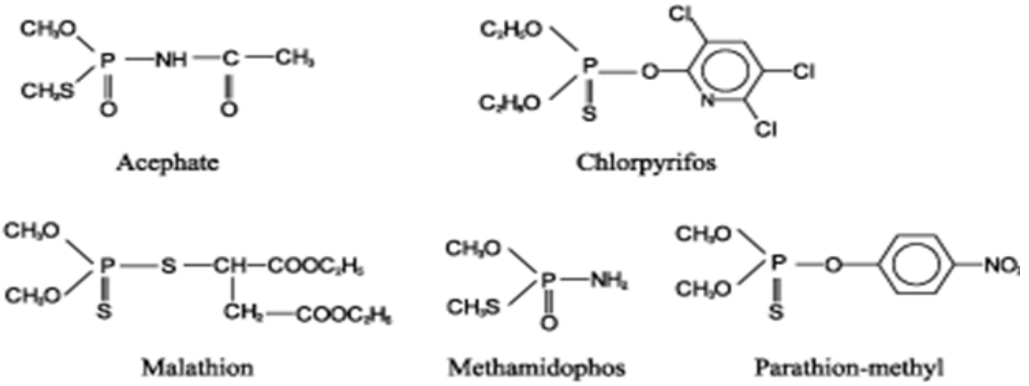

Fig. 8: Chemical Structure of Organophosphorus Insecticides

Neurotoxic Mode of Action of Ops Insecticides

OPs are toxic because of their inhibition of the enzyme acetyl cholinesterase. Acetylcholine is a neurotransmitter that affects the preganglionic and postganglionic parasympathetic synapses (**muscarinic actions**), sympathetic preganglionic synapses including the adrenal medulla (**nicotinic actions**) and the neuromuscular junctions (**nicotinic actions**). It is also a transmitter in the central nervous system. *At the synapses, it is hydrolysed by the enzyme, acetyl cholinesterase, thus producing acetate and choline*. The toxic effects of organophosphates are due to the inhibition of acetyl cholinesterase (**that is why they are called as cholinesterase inhibitors)** [see Fig: 10*]* ***resulting in the excessive accumulation of acetylcholine at the synapse***. [15].

- Normally the cholinesterase rapidly hydrolyze the neurotransmitter acetylcholine (fig: 9) into inactive fragments of choline and acetic acid after the completion of neurochemical transmission. The neurotransmitter acetylcholine is present in the terminal endings of all postganglionic parasympathetic nerves, at myoneural junctions, and at both parasympathetic and sympathetic ganglia. The major toxicity of organophosphate compounds is the covalent binding of phosphate radicals to the active sites of the cholinesterase, transforming them into enzymatically inert protein.

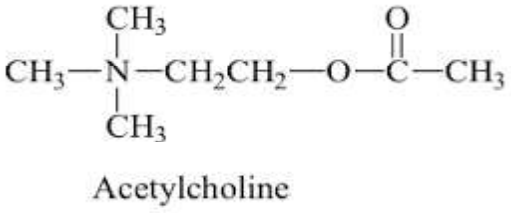

Fig. 9: Chemical structure of Acetylcholine

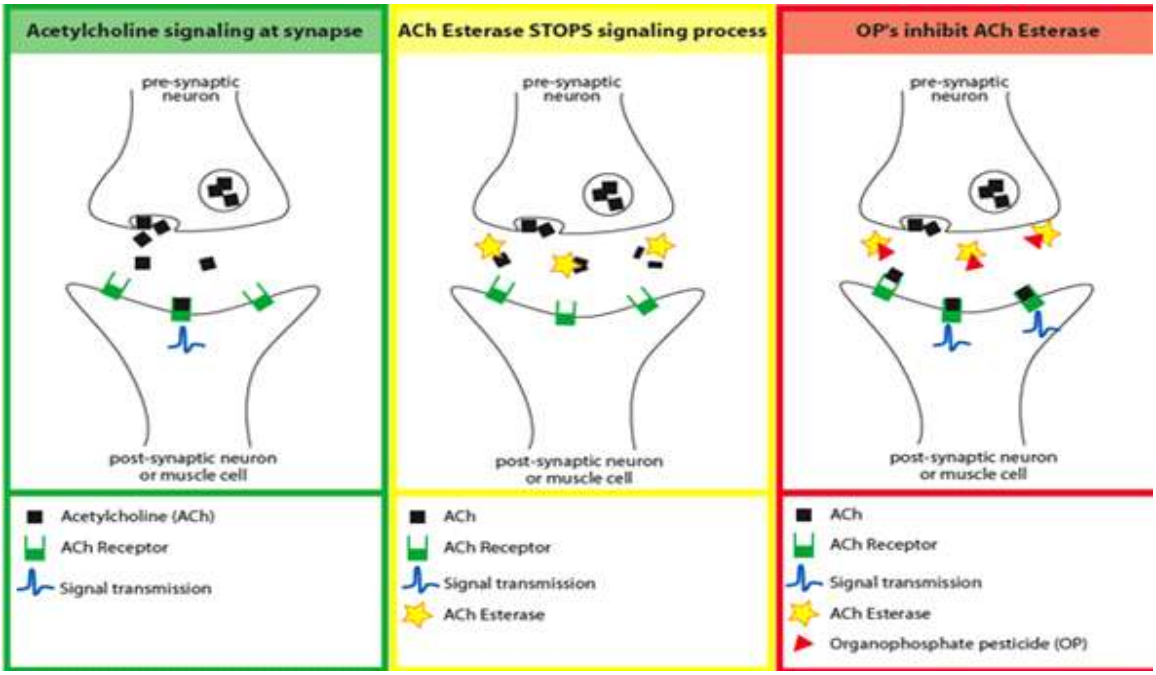

Fig. 10: Schematic Diagram of mode of action of OPs Poisoning

Carbamates Insecticides

Fig. 11: General Chemical Structure of Carbamates Compound

General Review

The carbamate insecticides are esters of *N*-methyl (or occasionally *N*,*N*-dimethyl) carbamic acid (H2NCOOH). The first recognized anti-ChE was in fact a carbamate, physostigmine (also called eserine), obtained in pure form in 1864 by Jobst and Hesse from the Calabar bean. ***Like organophosphates, carbamates are inhibitors of AChE, but instead of phosphorylating, they carbamoylate the serine moiety at the active site*** (Fig: 12). This is a reversible type of binding, and therefore, their toxicity is less severe and of lesser duration. Because they do not penetrate the CNS to any great extent, the CNS toxicity of carbamates is relatively low [16]. One of the most widely used carbamate insecticides is carbaryl (1-napthyl methylcarbamate), a broad spectrum insecticide. Carbaryl is not considered to be a persistent compound, because it is readily hydrolyzed. It is used widely in agriculture, including home gardens where it generally is applied as a dust or solution, such as aldicarb (Temik), aminocarb (Matacil), approcarb (Baygon), carbaryl (Sevin), carbofuran (Furaxdan) as shown in(see Fig:13).Absorption occurs through all routes [17].

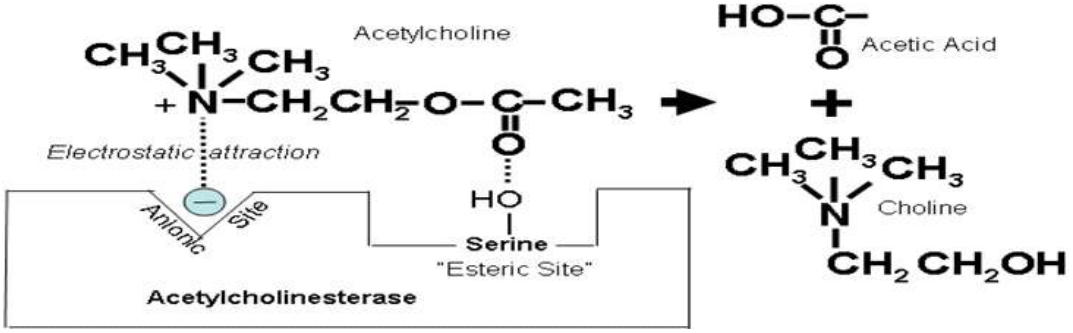

Fig. 12: Breakage of Acetylcholine into Acetic Acid and Choline (by cholinesterase enzyme)

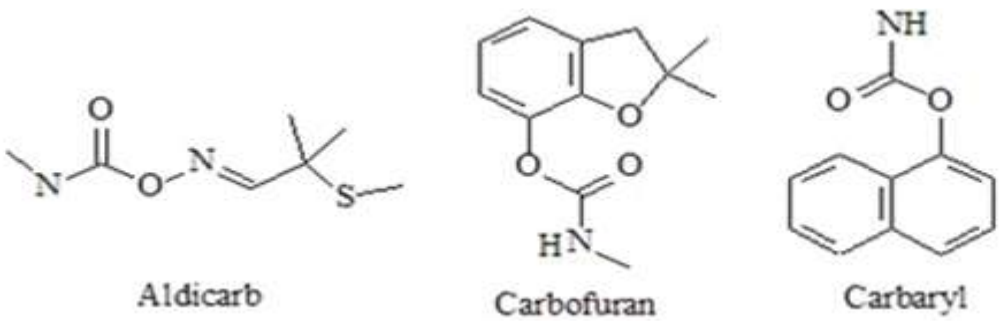

Fig. 13: Chemical structure of Carbamates Insecticides

Neurotoxic Mode of Action of Carbamate Insecticides

Like the OP insecticides, the mode of action of the carbamates is acetyl cholinesterase inhibition, but carbamylate the serine moiety at the active site instead of phosphorylation with the important difference that this is reversible type of binding (Fig 12). The inhibition is more rapidly reversed than with OP compounds. Because there is rapid reactivation of the carbamylated enzyme in the presence of water and hence symptoms are less severe and of shorter duration. Also, as carbamates do not penetrate the CNS effectively toxic features related to CNS are not much prominent in the event of poisoning. [18]

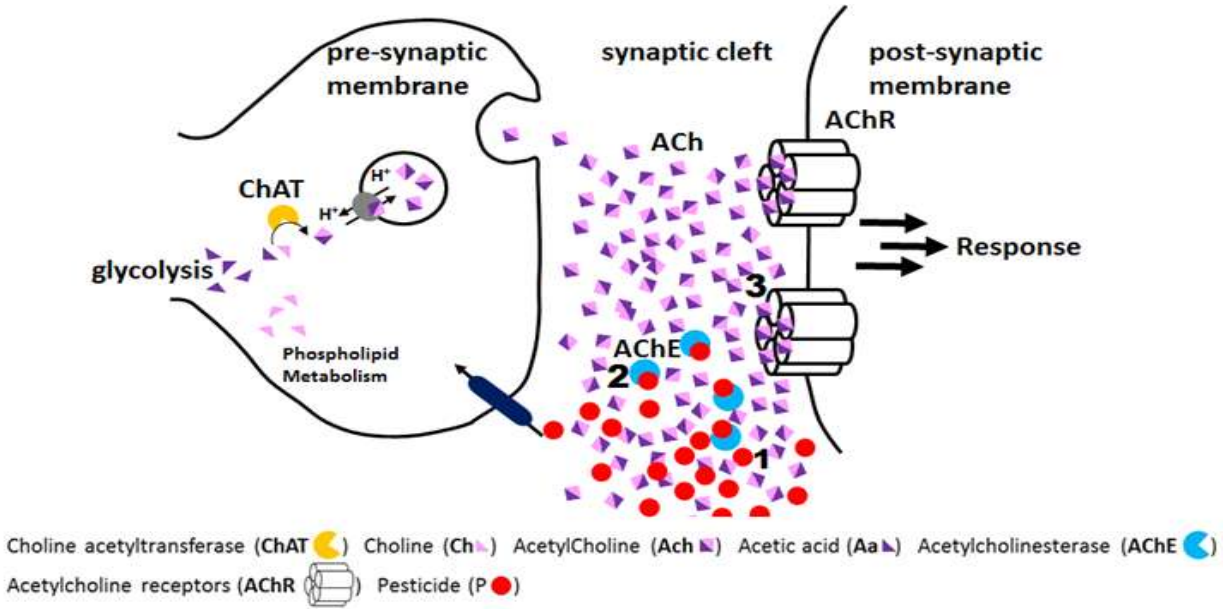

Fig. 14: Schematic Diagram of mode of action of Carbamates Poisoning

Pyrethroid Insecticides

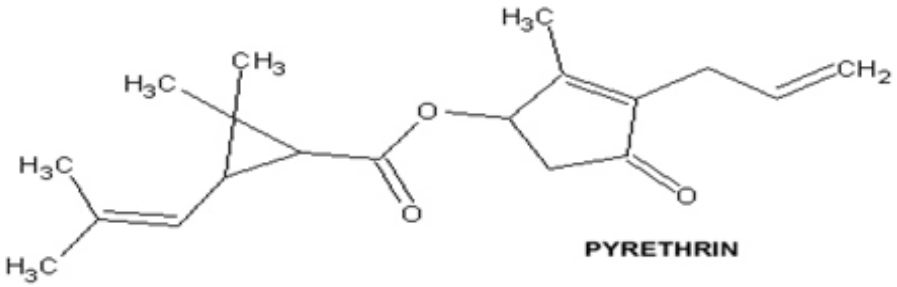

Fig. 15: Chemical structure of Pyrethrin

General Review

Pyrethrins were first developed as insecticides from extracts of the flower heads of *Chrysanthemum cinerariaefolium*, whose insecticidal potential was appreciated in ancient China and Persia. However, because pyrethrins were decomposed rapidly by light, synthetic analogs, the pyrethroids were developed. Pyrethroids are used widely as insecticides both in the house and in agriculture. Pyrethroids are known to alter the normal function of insect nerves by modifying the kinetics of voltage-sensitive sodium channels, which mediate the transient increase in the sodium permeability of the nerve membrane that underlies the nerve action potential [28]. There are two broad classes of pyrethroids depending on whether the structure contains a cyclopropane ring [e.g., cypermethrin {(±)-á-cyano-3-phenoxybenzyl (±)-*cis*,*trans*-3-(2,2-dichlorovinyl 2,2-dimethyl cyclopropanecarboxylate)}] or whether this ring is absent in the molecule [e.g., fenvalerate{(*RS*)-á-cyano-3-phenoxybenzyl(*RS*)-2-(4-chlorophenyl)-3-methylbutyrate}] [19].

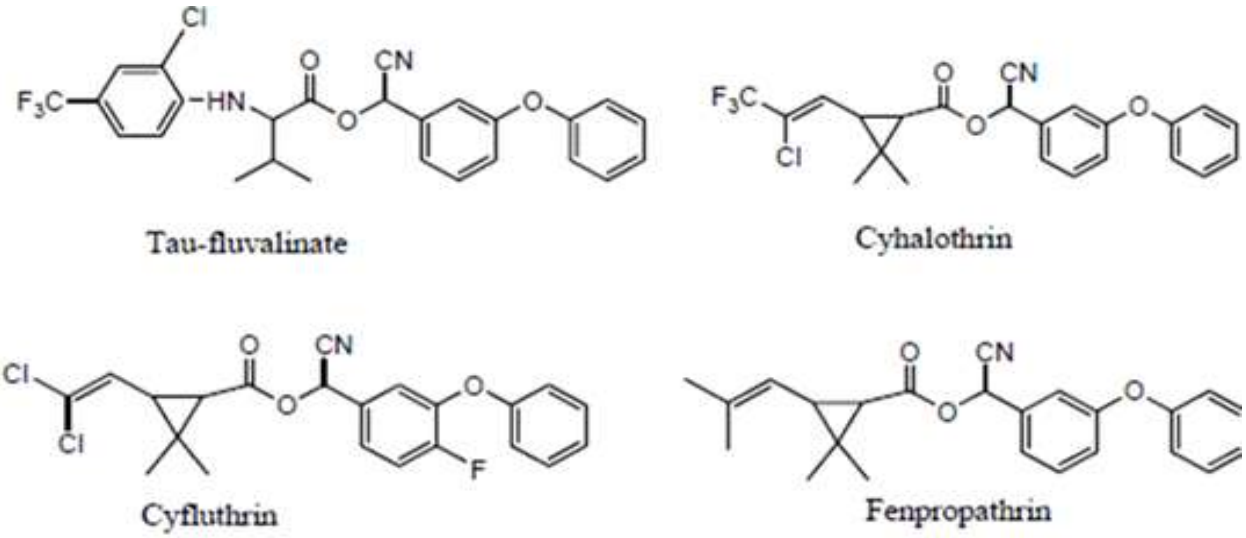

Fig. 16: Chemical Structure of Pyrethroid Insecticides

Neurotoxic Mode of Action of Pyrethroid Insecticides

Based on its sign and mechanism action Pyrethroid has been divided into two types:

- Type I (T) and Type II (CS) Syndrome (Figure:17)

A key structural difference between type I and type II pyrethroids is the presence only in the latter of a cyano group at the carbon of the alcohol moiety of the compound [20].

The mode of action of pyrethroids in mammals is the disruption of the voltage-gated sodium channels that maintains the electrical charge of nerve cell membrane [21]. Pyrethroids bind to the á subunit of the sodium channel and slow the activation (opening), as well as the rate of inactivation (closing) of the sodium channel, leading to a stable hyperexcitable state. Sodium channels then open at more hyperpolarized potentials, and are held open longer, allowing more sodium ions to cross and depolarize the neuronal membrane [22][Fig:18].

- Type I compounds prolong channel opening only long enough to cause repetitive firing of action potential (repetitive discharge).
- Type II compounds hold the channels open for such long periods that the membrane potential ultimately becomes depolarized to the point at which generation of action potential is not possible (depolarization-dependent block). Type II pyrethroids also bind to, and inhibit GABA-gated chloride channels.

These differences in the time of opening of sodium channels are believed to be at the basis of the differences observed between the T and CS syndromes [23].

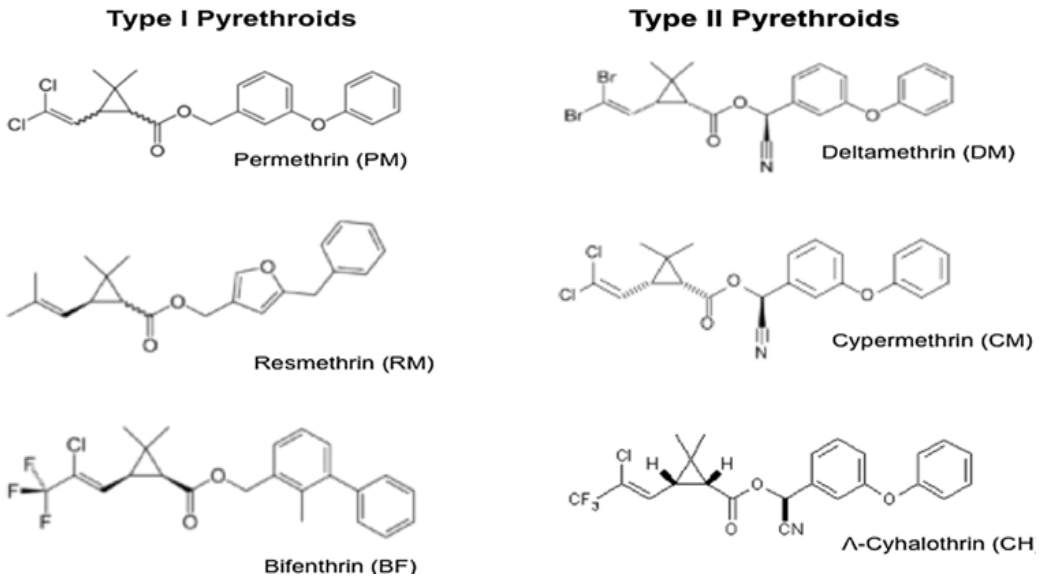

Fig. 17: Chemical Structure of Type I and Type II Pyrethroid Insecticides

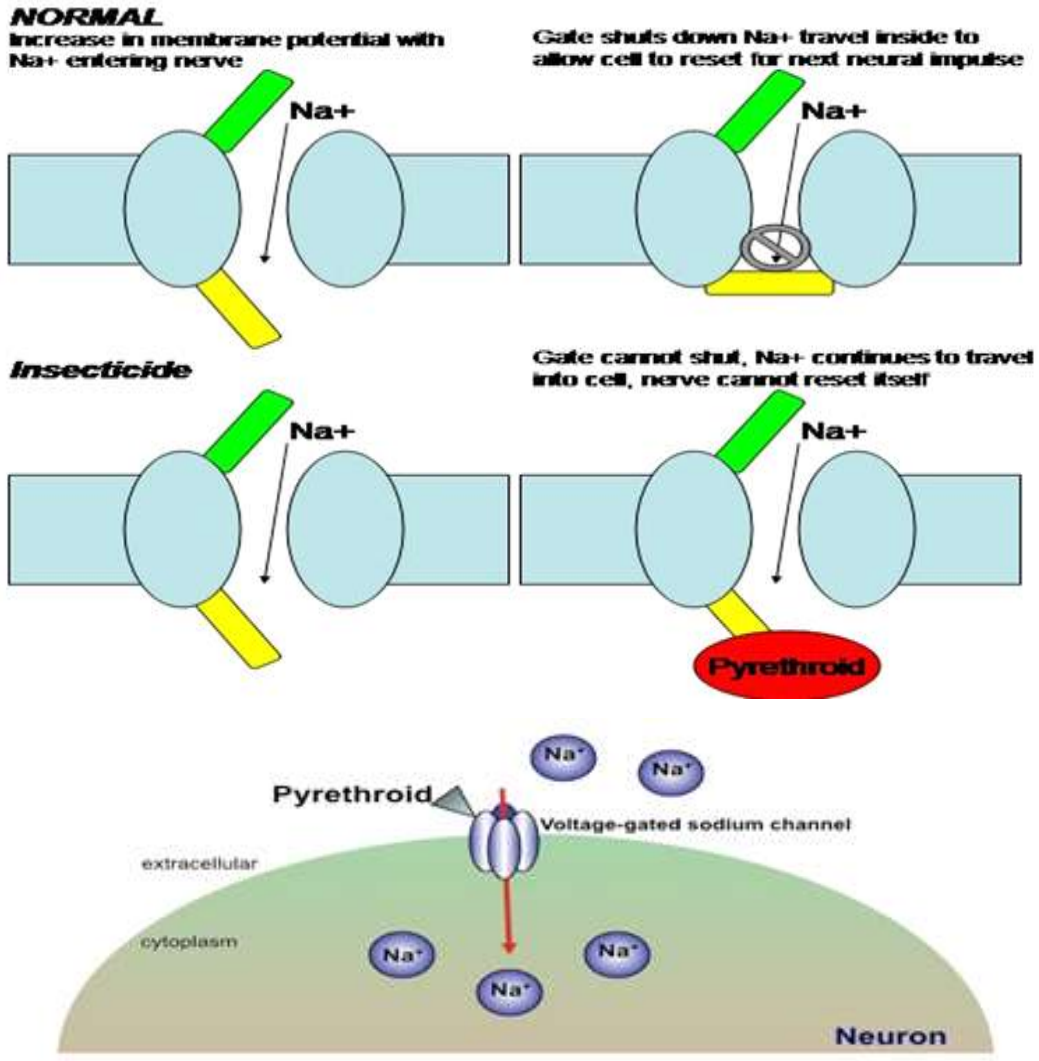

Fig. 18: Schematic diagram of mode of action of Pyrethroid Poisoning.

Organochlorine Insecticides

General Review

The organochlorine insecticides include the chlorinated ethane derivatives, such as DDT and its analogues; the cyclodienes, such as chlordane, aldrin, dieldrin, heptachlor, endrin, and toxaphene; the hexachlorocyclohexanes (HCH), such as lindane; and the caged structures mirex and chlordecone (Fig: 19). From the 1940s to the 1970s and 1980s, the organochlorine insecticides enjoyed wide use in agriculture, structure insect control, and malaria control programs. Their acute toxicity is moderate (less than that of organophosphates) [24]. *DDT was synthesized by the German chemist Othmar Zeidler in 1874*, but he failed to realize its value as an insecticide. It was the Swiss Paul Hermann Muller (1899–1965) who recognized its potential as an effective insecticide (Nobel Prize in Medicine in 1948). It is ironic that just 24 years later, in 1972, DDT was banned in the United States [25].

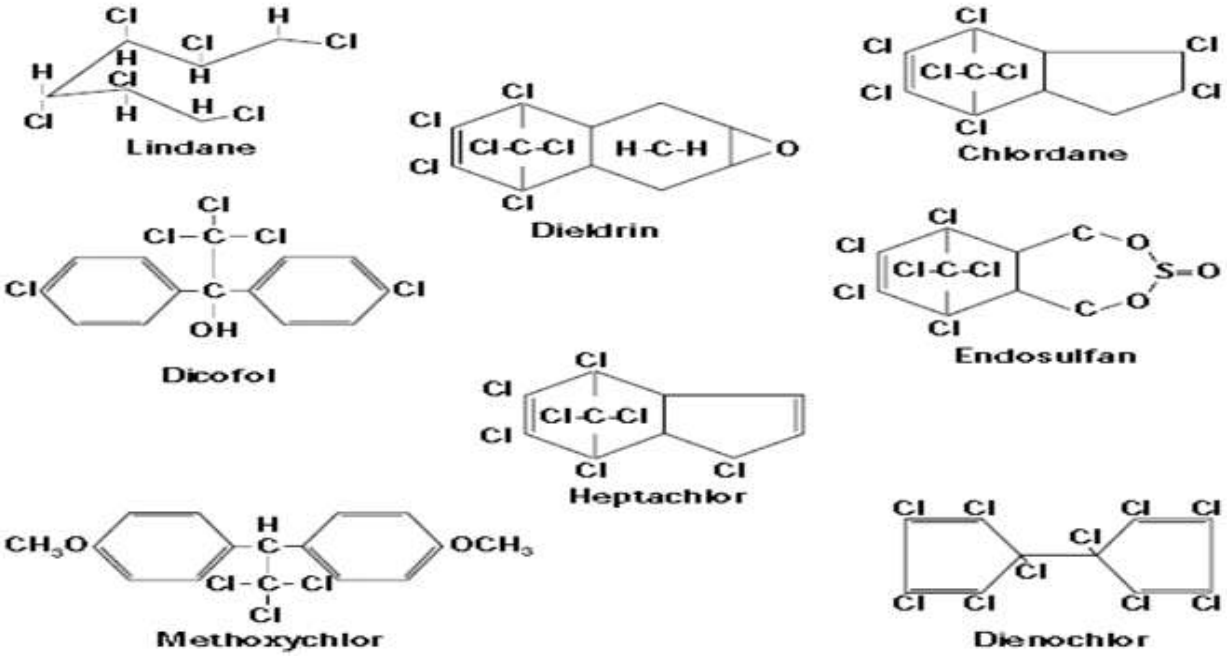

Fig. 19: Chemical Structure of Organochlorine Cyclodiene type Insecticides

Neurotoxic Mode of Action of Organochlorine Insecticides

The two main groups of organochlorine insecticides are the DDT-type compounds and the

Chlorinated cyclodienes (alicyclics). Their mechanism of action differs slightly:

The **DDT(1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane** (Fig:20) like compounds acts on the central nervous system by interfering with the movement of ions through axon neuronal membranes. DDT both delays the closing of the sodium ion channel and prevents the full opening of the potassium gates [36]. DDT has been shown to target a specific neuronal adenosine triphosphatase (ATPase) thought to be involved in the control of the rate of sodium, potassium, and calcium fluxes through the nerve membrane [26] (Fig: 21). This leakage causes repeated discharges in the neuron either spontaneously or after a single stimulus.

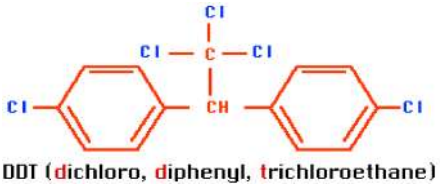

Fig. 20: Chemical structure of DDT

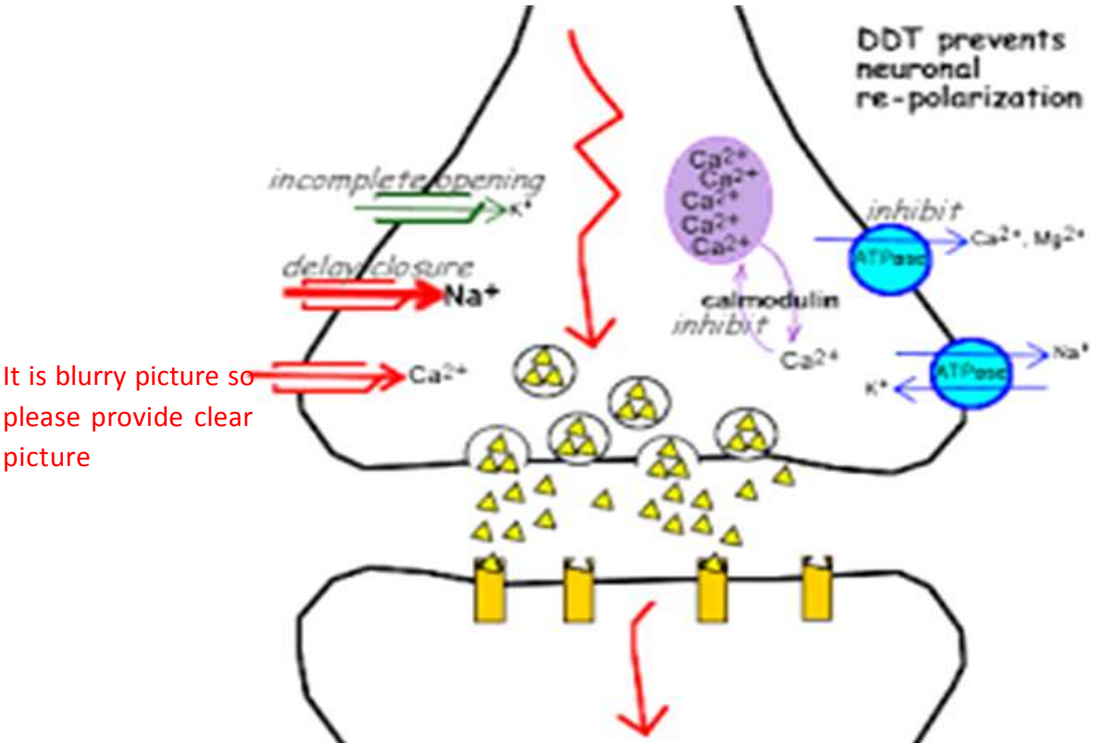

Fig. 21: Schematic diagram of mode of action of DDT Poisoning

Chlorinated Cyclodienes

After 2- to 8-hour exposure leads to depressed central nervous system (CNS) activity, followed by hyperexcitability, tremors, and then seizures. The mechanism action of these compounds to interfere with ã- amino butyric acid (GABA)–mediated neurotransmission. GABA is an important neurotransmitter in the mammalian CNS and in the neuromuscular junction. GABA receptors are members of the super family of ligand-gated ion channels that contain a chloride ionophore; by binding to these receptors, endogenous GABA causes the opening of chloride channels resulting in hyper polarization of the membrane. Lindane and cyclodienes bind to a specific site (the picrotoxin site) on the chloride channel, thereby blocking its opening and thus antagonizing the "inhibitory" action of GABA [27, 28, and 29]. Additional reported neurochemical effects of organochlorine insecticides include inhibition of Na+- K+, Ca2+, and Mg2+ - ATPases, and changes in neurotransmitter levels.

Neonicotinoid Insecticides

General Review

The neonicotinoids is one of the newest categories of insecticides. The neonicotinoid family includes acetamiprid, cloth ianidin, imidacloprid, nitenpyram, nithiazine, thiacloprid and thiamethoxam. (Fig. 22). The nicotinoids similar to and modelled after the natural nicotine. Imidacloprid is the most widely used insecticide in the world. Imidacloprid was the first in this chemical category to obtain registration in internationally. Compared to organophosphate and carbamate insecticides neonicotinoids cause less toxicity in human.

Neurotoxic Mode of Action of Neonicotinoid Insecticides

Neonicotinoids, like nicotine bind or fill up the nicotinic- acetylcholine receptors and blocking neural transmission (Fig: 23). In mammals, nicotinic acetylcholine receptors are located in cells of both the central nervous system and peripheral nervous systems. Nicotinic acetylcholine receptors are activated by the neurotransmitter acetylcholine. While low to moderate activation of these receptors causes nervous stimulation or high level over stimulates and block the AchR receptors causing paralysis and death. Acetyl cholinesterase breaks down acetylcholine to terminate signals from these receptors. However, acetyl cholinesterase cannot break down neonicotinoids and their binding is irreversible [30].

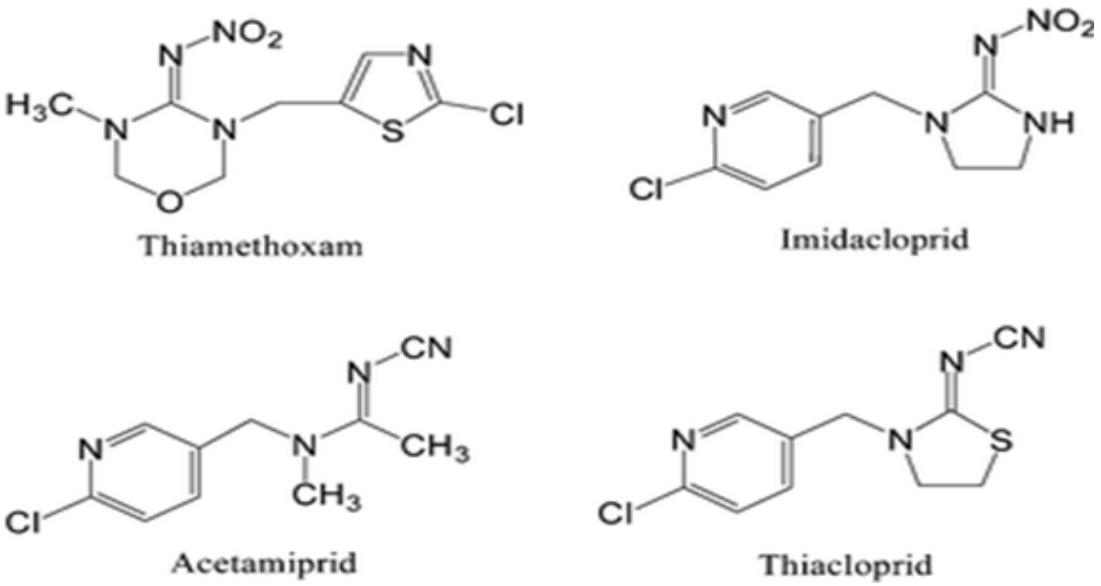

Fig. 22: Chemical structure of Neonicotinoid Insecticides

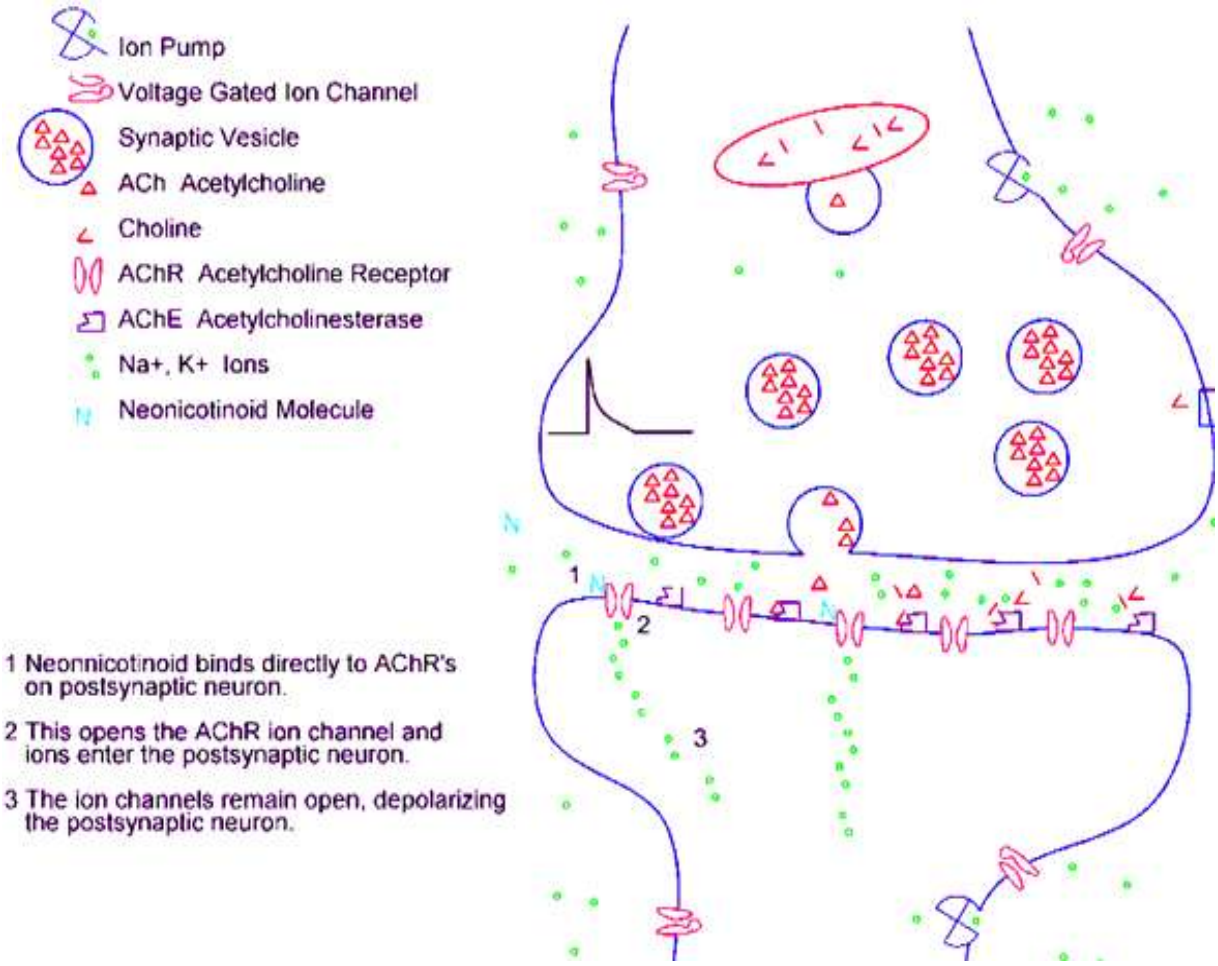

Fig. 23: Schematic diagram of Mode of Action of Neonicotinoid Poisoning

Conclusion

This section has covered only a few of the pesticides available today on the United States and world markets. An understanding of the basic chemical processes affected by pesticides has led to the discovery and production of new families of chemicals. Today's modern pesticide is generally safe to use if the directions on the label are followed. Many pesticides, particularly insecticides, are specifically designed to target the nervous systems of pests. For this reason, these substances can also be neurotoxic to non-target animals, including (in some cases) humans and other mammals. Understanding the underlying mechanisms in the inter-play between such environmental and genetic components is an important area of future research. There is, therefore an urgent need to reduce and, wherever possible, avoid human exposures to hazardous chemicals. In the case of agrochemicals, this will require us to fundamentally rethink and change our farming systems to eliminate our exposure to synthetic pesticides and protect the health not only of particularly highly exposed and/or vulnerable groups, such as agricultural workers and children, but also the general population and wild ecosystems.

Most studies of moderate pesticide exposure have found increased prevalence of neurologic symptoms and changes in neurobehavioral performance, reflecting cognitive and psychomotor dysfunction. Also the most studies have focused on organophosphate insecticides, but some found neurotoxic effects from other pesticides, including fungicides, fumigants, and organochlorine and carbamate insecticides. Future studies will need to improve assessment of pesticide exposure in individuals and consider the role of genetic susceptibility. Advances in instrumentation and an understanding of how adverse health effects are produced have resulted in the production of many environmentally friendly but effective pesticides.

- Organochlorine insecticides, pyrethoids as well as the new fiproles disrupt the sodium/ potassium/chloride channel systems that maintain the electrical charge of nerve cell membranes. When this is disrupted, nerves cannot properly transmit the electrical impulse. (NEURAL MEMBRANE DISRUPTION or *ION TRANSPORT DISRUPTION*).
- Organophosphate and carbamate insecticides block the acetylcholine-esterase enzyme which causes the receiving nerve to keep firing. This causes the affected animals to virtually twitch to death. (NEURAL SYNAPSE DISRUPTION or *ENZYME ACTIVITY DISRUPTION*).
- Neonicotinoids fill up the acetylcholine receptors, actually the nicotinic-acetylcholine receptors (which insects have), thereby blocking neural transmission. Affected insects simply stop activity especially feeding, grooming and protective behaviors. (NEURAL POST-SYNAPSE DISRUPTION or BLOCKAGE THE RECEPTORS).

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Review Article

Medical Negligence and Remedies: A Challenge to Medical Profession

Millo Tabin

Authors Affiliation: Professor, Faculty Incharge: Forensic Toxicology and DNA Lab, Department of Forensic Medicine and toxicology, All India Institute of Medical Sciences, New Delhi, India - 110029.

Reprints Requests: T. Millo, Professor, Department of Forensic Medicine and Toxicology, AIIMS, New Delhi, India.
E-mail: tabinmillo2003@rediffmail.com

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Abstract

Medical negligence litigation has become a common experience for the medical practitioners. It is important to know what constitutes a medical negligence and take preventive steps. The doctor has certain duties to his patient who comes to him for treatment for his illness. If there is any deficiency of duty or service due to commission or omission causing damage to the patient, it amounts to medical negligence. When there is a litigation of medical negligence, a legal procedure is followed to address the complainant. The doctor has to be aware of the medico-legal procedures and laws dealing with alleged medical negligence cases. It will help them to take precautions and follow safe medical practice.

Keywords: Medical Negligence; Litigation; Consent; Communication; Consumer Court; Civil Court; etc.

Introduction

Since ancient time, doctor and patient relationship was based on trust and faith. There were only few who mastered this profession with their knowledge, skill and experience. It was a noble profession dealing with the healing of sickness and doctors were considered next to God. But with time the situation has changed. With the advancement of allopathic medicine, there has been revolution in the management of human diseases.

Patient's have various options for treatment of their disease and the life expectancy of the people has increased due to better medical care and survival. There has also been change in the awareness of the public about medical treatment options and legal avenues due to better education and boom in the information technology. This has led to numerous litigations of medical negligence against the treating doctors.

The USA adopted four consumer rights in 1962 and UNO adopted guidelines for consumer protection in 1985. In 1986, the consumer protection Act (CPA) was passed in India. In IMA Vs P Shanta, the supreme court held that all medical services shall come under Sec. 2(1) of the CPA. Since then act there has been a sudden surge in the medical negligence cases lodged in the consumer court.

History of Healer Liability Suits [4]

Code of Hammurabi was the rule of law prepared by the king of Babylon (1792-1750 B.C). It described a scaled fee schedule for surgical services, which was linked to the outcome of the surgery, so if not met, resulted in severe penalties. It required documentation of diseases and therapies, including prescription benefits. The code fully explained patient's rights according of proclaimed King's code. It is clear that fees were fixed for treatment and penalties for the improper treatment. From there the concept of crime, tort and negligence were recorded.

There are various reasons for the increase in the litigation against the doctors which is a cause of concern:

1. The media has played a significant role in spreading the awareness of CPA Act, 1986 and also the rights of the patients. The trial by media is not uncommon.

2. The consumer activist have strengthened the consumer voice and encouraged the consumer to lodge cases. It has led to increase in the incidence of frivolous litigations.
3. There has also been increased and unrealistic public expectations from the doctors.
4. The doctor community also has a role in polluting the trust of the patient by too much commercialization of the medical services especially in the private sectors affecting the doctor patient relationship.
5. The patient has easy access to legal firms specialized in medical negligence case and the patient relative may be misguided by the money making lawyers who exploit the situation.
6. The practicing quacks has contributed to significant litigations and damage to medical practice.
6. It is also known that the medical doctors has been a soft target of litigations. Many a times the court and the administrations are unconcerned. There has been an increase in the incidence of hospital violence, especially in the emergency department.
7. There is a wrong public thinking that suing for medical mishaps is a way to obtain easy money or get their high medical bills waived off.
8. It is worth mentioning that the doctors are also not aware of the medico-legal issues in the practice and there is not enough medicolegal experts to guide them.

These increased litigations against doctors has also led to various consequences:

1. There is damage to the reputation of the medical professionals.
2. The doctors have started practicing defensive medicine. They have started advising more investigations than before. The diagnosis and management of the patient has been more on the basis of laboratory investigation than clinical diagnosis to avoid litigations.
3. The practice of defensive medicine has increased the cost of the health care and burdened the patient.
4. The doctors have started taking indemnity insurance to guard themselves against the medical litigations.
5. There is an increase hospital violence and violence against the doctors.
6. Medical litigations has led to the closure of many small medical establishments/nursing homes.
7. The affected doctors are taking early retirements and even changing profession.
8. In long term this may affect the popularity of the medical profession and fail to attract the best talents.
9. Now every patient today is a potential litigant and every doctor today is a soft target.

What is the Meaning of Negligence? [2]

As per Black's law dictionary Negligence means: the omission to do something which a reasonable man, guided by those ordinary considerations which ordinarily regulate human affairs, would do or the doing of something which a reasonable and prudent man would not do.

What is Professional Negligence?[2]

As quoted in Black's law dictionary (Reich Vs City of reading), Professional negligence is the negligence committed by a professional person. Professional is one engaged in one of the learned professions or in an occupation requiring a high level of training and proficiency. The following specific occupations have been accorded professional status: Architecture/ Engineers/ and quantity surveyors, Surveyors, Accountants, Solicitors, Advocates (Barrister), Medical practitioners and Insurance Brokers.

What is Medical Negligence?[2]

It is defined as absence or want of reasonable degree of skill, knowledge and care on the part of a medical practitioner in the treatment of a patient with whom a relationship of a professional attendant is established, leading to his /causing bodily injury/ damage or permanent disability or death/loss of life of the patient.

When does the liability of medical negligence arise?

To charge a doctor for Medical negligence, the following four fold test need to be applied:

1. *Duty*: Existence of a duty of care towards a patient.
2. *Dereliction of duty*: Failure of the doctor to provide reasonable degree of care and skill in the treatment of the patient.
3. *Damage*: Dereliction/breach of duty or care leading to damage/injury to the patient. The damage should have been foreseeable by a reasonable physician.

4. *Direct Causation:* The damage must have been directly caused from the dereliction of duty and not from any other cause or without which the injury would not have occurred.

What are the Damages to a Patient?

The damages to the patient could be in various forms like loss of earnings, expenses in the treatment of injury sustained, reduction in life expectancy due to the damage, reduction in the normal pleasure of life, pain and suffering (physical or mental), loss of potency, aggravating the pre- existing condition and death. The term damage means physical, mental or functional injury to the patient.

What is Reasonable Degree of Skill, Knowledge and Care?

As per Halsbury,s laws of England [2]" The law requires that the practitioner must bring to his task a reasonable degree of skill and knowledge and must exercise a reasonable degree of care. The law does not expect the very highest nor a very low degree of care and competence judged in the light of the particular circumstances of each case." Circumstance of the case in judging the cases of medical negligence, means the time, place, and the opportunity available to the practitioner when he undertakes the care of a patient. Depending on the these circumstances, different standards may become applicable while judging cases of alleged medical negligence.

The Bolam,s Test is the classic statement of the test of professional negligence. In Bolam,s test (John Hector Bolam Vs Friern hospital management committee, (1957) 1WLR 582, Justice McNairJ held : "But where you get a situation, which involves the use of some special skill or competence, then the test whether there has been negligence or not is not the test of the man on the top of a clampham omnibus, because he has not got this special skill. The test is the standard of the ordinary skilled man excercising and professing to have that special skill. A man need not possess the highest expert skill at the risk of being found negligent. It is well established law that it is sufficient if he excercises the ordinary skill of an of an ordinary competent man excercising that particular art".

It was mentioned by the supreme court in the Indian Medical Association (IMA) Vs P Shanta case that the practitioner must bring to his task a reasonable degree of skill and knowledge and must exercise reasonable degree of care. Neither the very highest nor a very low degree of care and competence judged in the light of particular circumstances of each case is what the law requires.

In a way it can be mentioned that the Medical practitioner are said to have committed medical negligence when he does something which a doctor of average prudence does not do or does not do something which a doctor of average prudence does, with similar qualifications, experience and facilities under similar circumstances.

What is Gross Negligence?

In Jacob Mathew Vs State of Punjab case (2005), the supreme court said that no criminal case should be filed against a doctor during the course of treatment of patient, unless the negligence is very gross, amounting to recklessness. The court did not define what is gross negligence. As per Black's law dictionary, "Gross negligence is defined as the intentional failure to perform a manifest duty in reckless disregard of the consequence". Ordinary negligence, is based on the fact that one ought to have known results of the acts, while gross negligence rests on the assumption that one knew results of his acts, but was recklessly or wantonly indifferent to results.

What is deviation from normal practice liability?

There is a usual and normal practice. The doctor has not adopted it. The course in fact adopted is one no professional man of ordinary skill would have taken had he been acting with ordinary care. It must be remembered that there may be more than one accepted normal practice or standards.

Types of Medical negligence

There are two types of negligence, namely civil and criminal negligence. As per Indian law, there is no proper definition for civil negligence. But criminal negligence is equated with Sec 304 A IPC which says "whoever causes death of any person by doing a rash and negligent act not amounting to culpable homicide shall be punished with imprisonment of up to 2 years, or with fine or with both" In such cases it has to be proved beyond reasonable doubt that there was a gross negligence, amounting to recklessness on the part of the doctor causing injury or death of the patient. This legal Section is applied in death due to road traffic accidents also. Regarding medical negligence various courts have commented that, a driver who is driving rashly and responsible for the accident and loss of life and a doctor treating to save the life a patient and acting in good faith

cannot be put in the same category. There needs to be a separate section to deal with medical negligence.

What is not negligence?

The people and law enforcing agencies (police and court) should know that not all cases of complains are medical negligence.

1. Mere occurrence of a complication

There are number of known complications associated with the treatment or surgery, which happens inspite of all precautions. It is generally communicated to the patient /relative in the informed consent.

2. Mortality Per se

The death of the patient itself should not be perceived as medical negligence. The patient might have died inspite of all the best medical care.

3. Failure of response to treatment

Many disease/infection may not respond to the standard line of treatment/Medicine due to various factors. It should not be considered as negligence, if the patient dies.

4. Bonafide error of Judgement [5]

The doctors are not perfect as God. They are also human being like all others and they may make errors unintentionally/inadvertently. Every errors should not be treated as medical negligence. In state of Maharashtra Vs Dr. Sou jayashree Ujwal Ingole (Criminal appeal no. 639 of 2017, SC), the supreme court of India clearly has said that error of judgement by doctor is not a criminal negligence and he cannot be tried under 304 A IPC for rash and negligent act.

5. Difference of opinion

The doctors may have difference of opinion regarding the treatment of patient according to their knowledge and experience and both may be correct in their own ways conforming with the acceptable practice of science. It should not be used to frame medical negligence.

6. Opting one of several acceptable options

Two doctors may follow two different options of treatment/management as per their preferred choice which is as per standard normal practice. The difference of methods used for treatment should not be considered as medical negligence.

7. Medical /surgical accidents

There are therapeutic misadventures in the course of treatment, which are unpredictable and occurs inspite of following all standard procedures and precautions. The doctor cannot be blamed for such accidents.

Standard of Proof/Level of Proof

It is the level of proof required in a legal action to convince the court that accuser's allegation is true. In civil trials the level of proof is based on balance of probability. It conveys that it is more likely than not or mathematically the probability of being guilty (doctor being negligent) is > 50% [7].

In civil negligence the burden of proving negligence lies more with the patient/complainant, whereas in criminal negligence the doctor has to prove his innocence as it becomes state Vs the doctor.

Res ipsa Loquitor (The truth speaks for itself)

In such situation burden of proof shifts to the doctor when alleged event cannot occur without negligence or events exclusively under respondent's control. The examples of Res ipsa loquitor are: Amputation of the wrong leg, enucleation of the wrong eye, Leaving instrument /sponge inside abdomen, operating on the wrong side of brain etc.

Legal Sections in India and Medical negligence[1]

In India the criminal negligence of the doctor may be prosecuted in a criminal court under Section 304 A IPC (Causing death by negligence) : Whoever causes the death of any person by doing any rash or negligent act not amounting to culpable homicide, shall be punished with imprisonment of either description for a term which may extend to two years, or with fine, or with both. It is a cognizable offence, bailable, non compoundable and triable by magistrate of the first class.

Investigation Procedure followed in Medical negligence cases

The police receives a alleged complaint of medical negligence from the patient party, when there is a death of the patient while receiving treatment against the treating doctor/hospital. The police takes the

body for the medico-legal autopsy. It is generally conducted by the board of doctors constituted by the head of the dept. of Forensic Medicine or the head of the hospital. The autopsy report is submitted to the investigating officer. The investigating officer request the state Govt. to constitute a board of doctors to examine the case. The state Govt. can constitute the board or may refer the case to State medical council. The board deliberates and opines on the medical negligence issue and submits the report to the investigating officer who then decide the legal course.

Various Forums to Approach against Medical Negligence

1. Civil court
2. Criminal court
3. Consumer court
4. Medical councils

The civil court and consumer court can award compensations and medical council of India[3] can issue warning or order penal erasure of his name to prevent him from practicing medicine. In consumer court the complaint has to made within 2 years from the cause of action and no fees is charged. Appeal can be made within one month from the date of the decision.

Landmark Judgments pertaining to medical negligence in India

1. Pt. Parmanand Katara Vs Union of India (1989)

It was held by the Supreme court that the doctor has to immediately start treatment of a patient under emergency. The outcome of the judgement was that the doctor has no right to chose a patient in case of emergency and medical duties of a doctor take precedence over legal duties.

2. IMA Vs VP Shanta (1995) 6 SCC 651

The judgment in this case clarified that i) All Medical services (private or government) will be included under the CPA, except those who offer free services to all patients at all times.

II) Services rendered by the doctors and hospital where it is partially free to certain poor patients is also included under 2(1) of CPA.

3. Dr. Suresh Gupta Vs Government of NCT Delhi (2004)

The supreme court stated that the negligence through lack of proper care, precaution and attention or inadvertence might create a civil liability, it does not create a criminal one. It could be termed criminal only when a) the doctor exhibits a gross lack of competence or inaction or wanton indifference to his patient's safety, which is found to have arisen from gross negligence. b) his negligence or incompetence shows such disregard for life and safety of his patient as to amount to a crime against the state c) Where a patient's death results merely from error of judgment or an accident, no criminal liability should be attached to it.

4. Jacob Mathew Vs State of Punjab (2005)

The patient was gasping in the ward. The doctor arrived in the ward within 20 minutes and connected the oxygen supply. It was found that the oxygen cylinder was empty. They looked for another oxygen cylinder. But by the time the cylinder arrived the patient died. The supreme court held that the doctors were not criminally liable and the Nonavailability of oxygen cylinders makes the hospital civilly liable. The court justified the use of word gross. It observed, negligence in the context of medical profession necessarily call for a treatment with a difference. To infer rashness or negligence on the part of profession, in particular a doctor, additional considerations apply. The word gross has to be used to denote criminal negligence of a doctor. It is true that the word gross has not been used in S. 304 A, IPC, yet it is settled that in criminal law, negligence must be of such a high degree as to be gross. The expression rash or negligent act as occurring in S.304A, IPC has to read as qualified by the word grossly. Mens rea has to be proved in criminal negligence, but not in civil negligence. For an act to amount to criminal negligence, the degree of negligence should be much higher i.e, gross or of a very high degree. Negligence which is neither gross nor of a higher degree may provide a ground of action in civil law but cannot form the basis for prosecution in criminal law. Bolam test, as a test of medical negligence was approved for application in India.

Supreme Court directed the Central Government to frame guidelines for prosecution of doctors U/S 304A IPC. Till then the court gave the following guidelines to be followed, to save the doctors from unnecessary harassment and undue pressure in performing their duties.

1. There is a need to protect doctors from frivolous and unjust prosecution
2. No criminal case should be filed against a doctor during the course of treatment of patient, unless the negligence is very gross, amounting to recklessness

3. For negligence to amount to an offence, the element of mens rea must be shown to exist
4. For an act to amount to criminal negligence, the degree of negligence should be much higher i.e. gross or of a very high degree
5. The investigating officer and the private complainant cannot always be supposed to have knowledge of medical science so as to determine whether the act of the accused medical professional amounts to rash or negligent act within the domain of criminal law under section 304-A IPC
6. The investigating officer should, before proceeding against the doctor accused of rash or negligent act or omission, obtain an independent and competent medical opinion preferably from a doctor in government services qualified in that branch of medical practice
7. A doctor accused of rashness or negligence, may not be arrested in a routine manner (simply because a charge has been leveled against him)
8. Finally a doctor may be arrested only if investigating officer believes that she/he would not be available for prosecution unless arrested.

5. Martin F. D, Souza Vs Mohd. Ishfaq (2009)

   The supreme court held that, even the consumer fora has to take an opinion from the competent doctor before taking decision against the negligent doctor to avoid harassment to doctors.

6. V. Krishna Rao Vs Nikhil Super Specialty Hospital (2010)

The supreme court held that expert opinion is not required for compensation in consumer forum. The reason was that there are two types of case, simple and complicated case. In simple cases like res ips loquitor or where negligence is obvious, there is no need for expert opinion. It tends to waste time. In complicated cases the complainant may be asked to approach the civil court for appropriate relief. As per Sec.3 of COPRA provides that the provisions of the act shall be in addition to and not in derogation of the provisions of any other law for the time being. However if any of the parties wants to bring in expert evidence on their own, the forum should consider the facts and circumstance of the case, and may allow the parties to adduce such evidences if it is appropriate to do so in the facts of the case. The discretion is left to judges.

iolation of Various Acts pertaining to medical field

a. PCPNDT Act, 1994 b) The transplantation of Human Organs and Tissues Act, 1994 c) MTP Act, 1971 d) Consumer Protection Act, 1986 e) Mental Health Act, 1987 f) Human Anatomy Act, 1948 g) Delhi Clinical Establishment Act, 2010.

Common Mishaps in Medical Practice

1. Mishaps in Anaesthetic practice

The common mishaps are: mistaken identity, incorrect positioning of the patient, faulty anesthetic equipments, electrocution or burns, fault with intravenous equipments, mishaps with drugs, monitoring of vital signs, human errors, mistake in intubation by the incompetent junior doctor etc

2. Mishaps in Surgical Practice

It consist of negligence due to anaesthesia and negligence primarily by surgeon

a. Acts of Omission

The common acts of omission are: failure to inspect, palpate or assess surgical condition properly, failure to decide whether surgery is required or not, failure to decide correct surgical path, delay in planning operation leading to complications, failure to use diagnostic techniques properly, failure to take informed consent, failure to carry out operation properly, failure to provide good postoperative care, failure to detect postoperative complications, failure to provide instructions and precautions to patient, failure in follow up of patient regularly

b. Acts of Commission

The common acts of commission are : Informed consent not taken, operation conducted on wrong patient or on wrong side, operation more extensively carried out than consented by the patient, leaving swabs / instruments/syringe in the body after surgery, use of infected instruments or unsterile operation theatre, unnecessary cutting of body tissues, applying plaster casts too tight or too light for a longer time than required, committing major blunder like cutting of big vessel or respiratory passage inadvertently, declaring the brain dead case dead without proper examination and making doubly sure, disregard or undignified management of dead patient etc.

c. Negligence by operating assistants
d. Corporate negligence during surgery

The common corporate negligence are: Faulty instruments, inadequate facilities in OT, recruiting unqualified staffs, shortage of oxygen cylinders, emergency drugs etc.

3. Mishaps in obstetric and gynecological practice

a. Common obstetrics litigations

The common obstetric litigations are: Brain damage in babies, perinatal deaths, maternal operative injuries, perineal tear, instruments/swabs retained in surgery, antenatal problems

Some common errors in care during labour are: Failure to diagnose the onset of labour , failure to induce and maintain proper uterine contractions, failure to monitor mother's exhaustion and well being, failure to monitor fetal heart rate, failure to detect fetal distress and to act promptly, Failure to act when the child is obstructed, failure to inform the patient and her relatives about the progress of labour and likely foreseable complications.

Some common errors in obstetric anaesthesia are : Delay in including anesthesia due to delay in arrival of anesthetist or lack of proper arrangements, inexperienced anesthetist or staff, lack of prior contact of mother with anesthetist, failure to inform mother about possible complication of anesthesia, failure to exercise by mother about the selection of anaesthesia of her choice within operative limits and safety, common errors and complications of anesthesia and resultant damage to child or mother.

Some common errors in obstetric operative procedures are: Failure to appreciate indications of assisted vaginal delivery like maternal distress, fetal distress and prolonged second stage of labour , attempting to do vaginal delivery when above signs are present, the delivery is being supervised by inexperienced junior doctor, there is delay in decision making to do assisted vaginal delivery, complications due to forceps applications, perineal trauma may be due to delay in repairing episiotomy or tear in vagina, unnoticed damage to anal sphincter or rectal mucosa, or retained swab.

b. Common Gynecological litigations

The common gynecological litigations are: Failed sterilization, ureteric damage, perforation of uterus, ectopic pregnancy undiagnosed, contraceptive failure/complications.

The common mishaps in gynecological surgical procedure are: Perforation of uterus and ureteric damage, inadvertently ovarian tissue may be removed while over treatment in pregnancies, ectopic pregnancy may be misdiagnosed as incomplete abortion or vice versa, impaired fertility or ovarian function due to surgery, interference in coital function as a result of alteration of vaginal anatomy.

The common mishaps in laparoscopic procedures are **:** Air embolism while performing diagnostic procedures, uterine perforation is a known complication of hysteroscopy, infection, pneumoperitonium, anesthetic complications.

The common errors in sterilization are: Failure in the form of undesired intrauterine pregnancy, ectopic pregnancy, injury at the time of operation. The failure of procedure may be due to woman pregnant at the time of operation, recanalization of tube, rings or diathermy coagulation are not properly applied, wrong structure is occluded.

c. Common antenatal mishaps

The common antenatal mishaps are: Failure to counsel about complications due to advanced maternal age, failure to refer to genetic tests where indicated like in advanced maternal age, previous history of abnormal fetus or family history of congenital malformation or genetic disorders, failure to diagnose pregnancy in various tests, failure to take proper history, do proper obstetrics examination and advise proper investigations, failure to monitor fetus growth correctly, failure to monitor patient's health properly like improper handling of pregnancy-induced hypertension etc., failure to do ultrasound on routine interval, failure to detect congenital anomaly and inform mother.

d. Mishaps/complications in neonatal care

Neonatal care is a very important aspect of completion of birth process. It is also a potential area where a lot of litigations can start with. If something goes wrong with the baby, it raises a lot of hue and cry Some common errors in neonatal care are :Failure to anticipate the need of pediatric help in delivery of high risk births, failure to attend congenital abnormalities that have already been detected in sonogram and to provide pediatric assistance according to that, Failure/delay to provide resuscitation in time leading to brain damage, failure/delay to providing suction properly leading to complications or death, failure to communicate well with family in case of still birth or abnormal or brain damaged baby, failure to provide reasonable care for preterm baby, inadequate infrastructural facilities to deal with any pediatric emergency,

carelessly declaring the preterm baby dead, undignified handling of dead baby etc.

Safeguards to Avoid Medical Negligence

a. Develop a good system in the hospital

Research repeatedly tell us that more often than not, errors are related to poor systems rather than people. It is essential to Change and improve the work culture of the place, slowly. Work Culture of the hospital simply put is the way we do things around here. Work Culture can be changed but the rewiring of practice takes time and concerted effort. It is not a course that one can attend and learn from, but a continuous cycle of learning and reinforcing good practice.

b. Better communication

Various studies have shown that the poor/ inconsiderate/uncompassionate communication [6] is at the core of why patients sue the doctor. So investing in a programme, which embeds a culture of transparency, openness and compassionate communication, makes both moral and financial sense. Compassion is not an add-on option and it has to be demonstrated in practice as much as felt. Hospitals need to have more of a facilitating attitude towards compassionate practices and create a guideline to support it. A compassionate attitude of staff in clinical practice is more important than all the fancy science, star profiles, flashing monitors and state of the art kit put together. Don't do a non-apology practice. An apology is not an admission of guilt but it is an acknowledgement of the pain they have been through. It builds a positive attitude showing concern for the patient's suffering.

- Talk to patient, explain and care. All patients should be fully heard and communication should be effective and complete
- Be polite and treat the patient with respect and dignity
- Do not try to conceal information if some complication occurs or something goes wrong. Do not lie to the patient (speak the truth in love)
- Must spend adequate time to explain in details to the patient about the treatment and the various option available
- Give complete instruction regarding dosage and side effects of medicines. Never assume patient knows it
- All information should be tangible, trustworthy open and accessible
- Do not argue over fees or charges. Settle it amicably. If patient does not pay, if possible forget it

c. Good documentation and record keeping

- Keep a copy of every document given to the patient, and insist for an acknowledgement
- Preserve a case summary including short history, investigation reports and treatment given
- If surgery/referral refused, take it in writing from the patient/relative that they are aware of the risk of such refusal
- The medical record has to preserved in the hospital for the standard duration and disposed off only after proper documentation
- Maintain confidentiality of the medical record. Do not show the medical records or share patients information without his permission

d. Always Obtain Informed and Valid Consent in writing.

Informed Consent: Disclosure of the nature of the proposed procedure**, i**ts reasonable alternatives**, m**aterial benefits, risk and uncertainties related to each alternative, possibility of alteration of the procedure**, o**pportunity to clear all doubts/queries**,** acceptance by the patient & relatives with understanding. In case of tubal ligation explain the patient about recanalization and failure rates of procedure.

Valid Consent: voluntary consent without any coercion, devoid of fraud and undue influence. Person should be competent to give consent ($\geq$ 18 yrs), properly informed consent and Procedure specific consent. *Consent from Spouse* is to be taken in **t**ermination of pregnancy**,** Sterilization, artificial insemination**,** donation of the sperms and any operation that can have a bearing on the sexual rights of the spouse.

e. Develop a suitable and effective grievance redressal mechanism for resolving patient complaints f) Do not overstate your qualification (A higher degree of case is expected from a specialist) g) Do not give blanket assurance or guarantee the results h) Keep only qualified assistants i) Do not give any diagnosis on telephone j) Do not prescribe medicine without examining the patient k) Doctor should be 100% sure before death is pronounced (Neonatal deaths)[6]. Respect human dignity and the dead should be handled with sensitively and respect. l) Update yourselves with the recent medical

knowledge and developments m) Conduct regular studies on the nature of medical negligence cases and patient complaints n) Develop a code of conduct for the doctors based on existing laws o) Identify measures for providing qualify medical services at the most affordable price p) Keep yourself updated about the laws in relation to medical practice (MTP Act, PNDT Act, CPA, Organ transplantation Act etc.) and negligence cases in the court q) Learn from each case r) Indemnity insurance s) Ethical clinical practice t) Work within your limits u) Liberal with necessary investigations and 2nd opinion v) Timely and proper referral if required w) Be sensitive to the patient's misery x) Have a good legal advisor y) Code of conduct for patients should be developed f) Promote medical professionalism. It can be done by leadership commitment, developing supportive institutional policies, program or model to guide graduated interventions like surveillance tools to capture allegations, process for reviewing allegations and interventions, organizing multi level training for staffs, develop resources to help unprofessional colleagues, victims (staff, patient, student, trainees, colleagues).

Above all, health and safety of the patient should always prevail over profit and personal convenience. It demands placing the interest of patient above us.

Conclusion

Medical negligence is an important issue to be dealt with in the medical practice. It can be safely avoided if we adopt adequate precautions. The individual as well as institutional or system lapses should be dealt with and promote a culture of medical professionalism [8]. The medical professionalism consists of professional excellence, humanism, accountability and altruism. This can be achieved by practicing medicine with good understanding of its ethical and legal aspects, good communication skills and clinical competence. It worth mentioning that the purpose of holding a professional liable for his act or omission, if negligent, is to make the life safer and to eliminate the possibility of recurrence of negligence in the future

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48/41-42, DSIDC, Pocket-II

Mayur Vihar Phase-I

Delhi - 110 091(India)

Phone: 91-11-22754205, 45796900, 22756995, Cell: +91-9821671871

E-mail: author@rfppl.co.in

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Review Article

Molecular Techniques used for Determination of Genetic Identity in Forensic Samples

Sharma N.[1], Dey A.[2], Balayan A.[3], Millo T.[4], Gupta S.K.[5]

Please provide the Designation of all Authors

Authors Affiliation: Department of Forensic Medicine and Toxicology, All India Institute for Medical Sciences, New Delhi.

Reprints Requests: Nidhi Sharma, Department of Forensic Medicine and Toxicology, All India Institute for Medical Sciences, New Delhi.

E-mail: nidhisharma306@gmail.com

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Abstract

Molecular techniques in forensics is gaining importance in medico-legal and criminalities identification after establishment of molecular methods such as DNA (Deoxyribose Nucleic acid) finger printing to identify individuals. The principles and techniques used for forensic means; modern DNA profiling procedures that are widely used in resolving various issues of inheritance or succession, identifying missing child, paternity dispute, murder and sexual assault,adoption maintenance of minor child and victim of mass disasters. Nuclear DNA evidences have been recovered from blood, semen, saliva, skin and hair roots. DNA base techniques such as PCR (Polymerase chain reaction), RFLP (Restriction fragment length polymorphism), VNTR (variable number of tandem repeats), STR (Short tandem repeats), Y-chromosome analysis and Mitochondrial analysis are being used for investigation. DNA-based evidence has become a significant supporting tool for law enforcement agencies/investigators to solve difficult crime cases and give right decision and judgment.

Keywords: Molecular Forensic; DNA Profiling; PCR-RFLP; STR- Genotyping; NGS; Microarray.

Introduction

Molecular forensic is method of extracting evidence from biological samples collected from the crime scene. Over the last 30 years such biological samples have revolutionized the forensic investigation i.e. Analysis of DNA. All biological materials contain DNA and all DNA carries identity of each individual. Dr Alec Jeffreys introduced DNA finger printing technique first time to identify individuals in 1984 at University of Leicester.[1,2] DNA based techniques such as PCR (Polymerase chain reaction), RFLP (Restriction fragment length polymorphism) have been used for investigation to analyse VNTR (variable number of tandem repeats), STR (Short tandem repeats),Y-chromosome and Mitochondrial analysis from biological samples. The focus of most criminal investigations is on linking the evidence after discovery from the crime scene to suspects; so genetic science has played an important role in this process [2]. Currently, forensic DNA profiling by PCR based STR markers have been found to be suitable for forensic application. A panel of multiple–allelic STR markers have been developed and standard protocol have been validated in the laboratories worldwide using capillary electrophoresis. The incorporation of these STRs into commercial kits have improved the application of DNA evidence with reproducible results from the less nucleated cells or even severely compromised materials. RFLP for VNTRs (long motifs) have been replaced due to labour intensive and statistical errors, chances of cross-contamination and inappropriate forensic samples. Furthermore, allelic profile can be generated from Mitochondrial DNA (recovered from both bone and teeth dating back to thousands of years) and applied in anthropological domain. DNA fingerprinting is one of the most significant advances in forensic science in the era of today's criminology[3,4]. Now in the

emergence of Next generation sequencing (NGS) more forensic data can be generated with faster analysis in future. In this article molecular methods applied in the analysis of genetic material (DNA) of forensic samples will be highlighted and discussed.

Development of DNA Fingerprinting

Sir Alec John Jeffreys, a British Geneticist born on 9th January 1950, first time developed techniques for DNA fingerprinting and DNA profiling in the field of forensic genetics in 1984. Dr. Jeffreys unexpectedly observed that DNA patterns of different people showed both similarities and differences while working in his laboratory at Leicester on samples of his technician's family. He realized that there are possibilities that DNA fingerprinting can be used for recognizing the variations in genetic code to identify individuals. He also found that some regions of DNA sequences were repeated again and again in a sample and he developed a technique to examine the length variation of these repeat sequences and created the test to perform human identity test. These repeated pattern of DNA are known as VNTRs (variable number of tandem repeats) and were detected by a technique called RFLP (Restriction fragment length polymorphism). In forensic genetics STRs (Short tandem repeats) known as microsatellites and minisatellites are generally performed to solve casework by using PCR (Polymerase Chain reaction) technique. Microsatellites and Minisatellites are together known as VNTRs[5,6]. Thus, DNA profiling based on typing of individuals, highly variable minisatellites in the human genome was also developed by Alec Jeffrey and his co-workers in 1985.

Biological Samples for Forensic DNA Profiling

All biological samples that possess nucleated cell can be considered for DNA profiling. The proper collection of samples from crime scene or for paternity testing and their proper storage and transportation to the laboratory for investigation is very important to minimize the contamination and degradation of DNA present in it. Ideally biological samples should be collected within 24 hours from the crime scene, after that degradation occurs as a result of enzymatic action of cells. To avoid the contamination, one should wear gloves all the time, avoid coughing and sneezing during the collection of samples. As a general rule samples of fluids should be refrigerated and anything else should be kept dry in paper envelopes. Samples should be clearly labelled with name, date and time of collection and transported safely in correct manner.

The biological samples that are present at crime scene or on victim's body and for paternity disputes, such as blood and semen (liquid / dry deposited on support), biological secretions in case of sexual acts (saliva, semen, mixture of secretions), buccal swab, hard tissues (bone, teeth), hairs with follicles, skin cells, brain cells, muscles, tissue, in some cases fingernails should be collected. Eventually blood, saliva and semen are the main sources of DNA profiling in forensic genetic testing[7].

Isolation of DNA from Forensic Biological Samples

Forensic evidence when sent to laboratory for profiling, the genetic material (DNA) is extracted from the samples by organic method (phenol-chloroform method). Briefly to the sample an equal volume of forensic buffer should be added, incubated at -20°C for 2 hours and then incubated at 60°C for 10 minutes. The samples are washed with PBS, followed by centrifugation and then refrigerated. Now forensic buffer, 20% SDS and Proteinase K (20 mg/ml) are added to the washed sample and incubated for 16 hours at 37°C. After incubation, phenol extraction is performed to remove cellular debris by separating aqueous layer using centrifuge. The DNA is precipitated by adding 3 M Sodium Acetate and Absolute Alcohol, and then DNA is dried to dissolve it in T.E. buffer for further use [8,9].

Quantification of Isolated DNA

DNA quantification can be performed by various methods. The commonly used commercial method is measuring absorbance of DNA samples at 260 nm and 280nm on spectrophotometer. Spectrophotometry is based on the interaction of substance with incoming radiation.The DNA purity/quality can be computed from the ratio absorbance of DNA samples at 260 nm and 280nm, if absorbance quotient (Q) is 1.7 to 2 the DNA is considered to be pure. This technique is not species specific so if there is bacterial or fungal DNA contamination it can affect the profiling results. It is important one should maintain sterility during DNA extraction and quantification to avoid contamination [10].

VNTRs Analysis based on RFLP

In this technique high molecular weight targeted DNA is digested with restriction enzyme which has recognition sites at both ends of hyper variable regions. The fragmented DNA after digestion are arranged in specific order by the number of repeats

of sequence, these fragments are separated (according to size) by agarose gel or by polyacrylamide gel electrophoresis and detected by using labelled VNTR probes. A unique pattern of DNA fragments of multiple VNTR loci on gel is compared with known and unknown origin of DNA samples and analysed on the basis of similarities and dissimilarities of patterns. RFLP analysis of VNTR loci is a good method to resolve the paternity disputes where DNA can be extracted from blood samples. However, this technique is not performed on other forensic investigations because high molecular weight DNA are required. Therefore, analysis of VNTR is limited to investigation where large amount of DNA is recovered. This method has been replaced due to statistical errors and chances of cross contamination and now PCR based STR profiling is done worldwide for forensic investigation[11,12].

STR Analysis based on PCR

Short tandem repeats (STR) loci are a class of polymorphic markers that are present throughout the human genome and consists of tandemly repeated sequence (1 - 6 base pairs) in length[13]. Their abundance, hyper-variability and amenability to amplification by Polymerase chain reaction (PCR) makes them ideal markers for use in the identification of individuals. PCR is an in-vitro method for amplifying specific DNA sequence. Starting with trace amounts of a particular nucleic acid sequence of any source, PCR enzymatically generates millions of exact copies, thereby making genetic analysis of tiny samples a relatively simple process. PCR was invented by Kary& Mullis of Cetus Corporation in 1983 and is widely used technique in molecular biology with direct application in genetic research, medical diagnostics, forensic science & paternity testing. This process requires pairs of primers to amplify target DNA sequence that hybridize (Stick) to the target DNA (3 prime end to 5 prime end), four dNTPs (deoxyribonucleate triphosphates), heat stable taq DNA polymerase and DNA template (extracted DNA). The PCR process consisted of three step cycles: denaturing step, annealing step and extension step.

1. *Denaturing step*: In this process double stranded targeted DNA molecules are denatured (separated by heating) into single strands by incubating them at high temperature (usually 94°C for 60 seconds).
2. *Annealing step*: In this step temperature is lowered down to allow the primers to specifically bind (anneal) to their complementary target (flanking) sequence. This is usually done at 45°C-65°C depending upon GC content of primers.
3. *Extension reaction*: In this step usually, temperature is raised to 72°C during which the annealed primers are extended on DNA template by a thermostable DNA Taq Polymerase to allow the synthesis of DNA region.

The above steps comprise as one cycle (denaturing DNA to synthesis). This process is repeated for about 30-40 cycle on thermocycler (PCR machine). In this process the completion of each cycle doubles the number of DNA molecules[14].

The DNA regions with repeat units which is 2-6 base pairs in length are STRs, polymorphic STR loci can be copied simultaneously by multiplex PCR. Currently, in forensic investigation and paternity testing multiplex STR typing or profiling are being used to study 15 STR loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, FGA) and one gender marker Amelogenin by Capillary Electrophoresis (CE) on Genetic Analyzer[15].

PCR Amplification for 15 STR Loci

PCR is performed by using commercially developed STR multiplex kit for autosomal and Y-chromosome markers. It amplifies specific product at the X and Y- chromosomes which are 107 bp and 113 bp long (each pair of primers fluorescently labelled to distinguish profiles). The characteristics of 15 loci are shown in **Table 1.** For PCR amplification of STRs; in a PCR tube 10 µl of Master mix is transferred, which contains reaction buffer and taq polymerase, 5 µl set of primers and extracted DNA template (1-5 ng) as recommended by manufacturer. After mixing the reaction is run in thermocycler machine with the amplification condition such as initial denaturation at 95°C for 11min; 28 cycle of 94°C for 1min (denaturation), 59°C for 1min (annealing), 72°C for 1min and final extension of 60°C for 60 min. The PCR product is taken out and kept in the deep freezer (-20°C) till it can be used for genotyping in Genetic analyser[16,17,18].

STR Profiling (Genotyping)

To analyse the profile the PCR product (1 µl) is mixed with commercially available Hidi Formamide (8.7 µl) and GeneScan 500 LIZ (0.3 µl). it is denatured at 95°C and chilled at deep freezer. The sample is loaded into each well containing PCR product along with one standard and one negative control in micro-well plate and run in the Analyzer using Polymer 4 with capillary to generate the profiles. Fragments are separated according to their length by electrophoresis.

Table 1:

16 Loci	Locus (Genetic Markers)	Chromosome location	Repeat motifs	Allelic range	Primer labelled
1	D8S1179,	8q	TCTA	8-19	6-FAM
2	D21S11	21q11-21	TCTA	24-38	6-FAM
3	D7S820	7q11.21-22	GATA	6-15	6-FAM
4	CSF1PO	5q33.3-34	AGAT	6-15	6-FAM
5	D3S1358	3p	TCTA	12-19	VIC
6	TH01	11p15.5	AATG	4-13.3	VIC
7	D13S317	13q22-31	TATC	8-15	VIC
8	D16S539	16q24-qter	GATA	5-15	VIC
9	D2S1338	2q35-37.1	TGCC	15-28	VIC
10	D19S433	19q12-13.1	AAGG	9-17.2	NED
11	vWA	12p12-pter	TCTA	11-24	NED
12	TPOX	12p23-pter	AATG	6-13	NED
13	D18S51	18q21.3	AGAA	7-27	NED
14	D5S818	5q23.3-32	AGAT	7-16	PET
15	FGA	4q28	TTTC	17-51.2	PET
16	Amelogenin	X: p22.1-22.3, Y: p11.2	Gender markers	X, Y	PET

The data is analysed with software to assess the quality of amplification and fragment length of each STR marker that is recorded as series of number (number of repeats) assigned to specific alleles at every chromosomal locus [19].

Analysis for DNA profiling in Crime/Casework/ Paternity Disputes

The DNA profiling generated as fragment length or STRs by genetic analyser has to be compared with the Profile of suspect with the DNA profile of evidence. The samples collected from suspects including victim and other person present at the time of crime are referred as Reference Samples. If DNA profiles of reference and evidence are found to be identical, then it is considered as inclusion (match); if they are not identical it is considered as exclusion. In terms of Inclusion it is important that the pair of profiles are perfectly similar at each and every locus with the profile of suspect and profile of evidence. If pair of profiles are different or does not match in certain locus, it should be reported as partial match or exclusion. Similarly, in Paternity case matching of DNA profiles of child, mother and disputed fathers, the child (either son or daughter) will inherit half of their autosomal alleles from each parent and the son will inherit a Y-chromosomal allele from the father and daughter will inherit X-chromosomal alleles from the father. The male child will match the biological father at all Y- chromosome loci, whereas the female child will match the biological father at all X-chromosome loci. Statistics will help to determine if an alleged father should be included or excluded as the father of the child, because no known phenotype are associated with STR loci, so only genotype of parents and child should be considered[20,21,22].

Furthermore, the statistical calculation is used in forensic science to assess the strength of evidence and the probability that there could be random match to some other person. In paternity cases, likelihood ratios and combined probability of exclusion are two calculations that can be performed to evaluate the certainty of the evidence [23,24]. Therefore, DNA analysis of any physical evidence is a very strong form of technology which is exceptionally accurate – DNA evidence doesn't lie!

Automation in STR Analysis

Human identity testing has been widespread by using DNA typing methods. During the last decade tremendous growth in the use of DNA evidence in crime scene investigations, paternity testing, unidentified body and missing persons have been reported.

As demonstrated above many molecular techniques have been used earlier and even currently, depending on PCR based capillary electrophoresis (CE) on Genetic Analyzer. Now various commercial kits with semi-automated and automated DNA extraction are available to generate STR profiles associated with biological evidence to avoid cross contamination. However, the procedure is still labour intensive and time consuming in handling the biological samples of decomposed unidentified body. Such constraints for the STR typing limit expeditious processing of results. To provide expeditious STR profiling numerous Rapid DNA instruments have been developed to perform DNA extraction, PCR, STR profiling and interpretative result in less time. STR profiling by using 24 loci, 10 mini STR loci and global filer express kit with allelic ladder are available[25,26].

Next Generation Sequencing for Forensic DNA Analysis

The next generation sequencing is the high throughput sequencing technique for DNA and RNA. This technique is more quick and cost-effective than Sanger sequencing and has revolutionized the study of genomics and molecular biology. STR analysis is likely to remain the most important and commonly used genetic technique in forensic science for the foreseeable future. When NGS technology was firstly introduced to genomics, it was not suitable for STR testing because the read length was generally too short. With technological advances, the average read length has been continually increasing. Since alleles with similar length can be easily distinguished using NGS technology and digital read count could significantly facilitate the identification of mixed DNA samples and analysis of complex paternity cases, some researchers have recently started using NGS technology for STR testing. To process the forensic NGS data, various workers developed software such as STRait Razor, software that can analyse the NGS data for 44 STRs, including 23 autosomal and 21Y chromosome STRs. Illumina's MiSeq system establishes reference allele database to detect single source and mixed DNA samples and observed that most locus genotyping results were stable and reliable[27].

NGS technologies are going to be crucial for human DNA genotyping in cases like mass disasters or other events where forensic specimens and samples are compromised and degraded. With the use of NGS it will be possible to achieve the simultaneous analysis of the standard autosomal DNA (STRs and SNPs), mitochondrial DNA, and X and Y chromosomal markers.

NGS technology has many potential advantages for STR analysis. These include high throughput, low cost, simultaneous detection of large numbers of STR loci on both autosomes and sex chromosomes and also the ability to distinguish alleles with similar length or digital read count. NGS technology would therefore significantly facilitate the identification of mixed DNA samples and analysis of complex paternity cases, and ultimately greatly increase the efficiency and cost-effectiveness of legal cases[28,29].

Application of Microarray in Forensic

DNA microarrays have provided to be a new and powerful tool to perform important molecular biology and clinical diagnostic assays. "Microarray" refers to a microchip-based testing platform that allows high-volume, automated analysis of many pieces of DNA at once. A DNA microarray is an ordered arrangement of oligonucleotides attached to a solid support used to analyse nucleic acid samples via hybridization.

The term "DNA microarrays" was first used in an assay that examined the expression of multiple genes in parallel. The development of this technique was originally derived from Southern Blotting, developed by Professor Ed Southern of the University of Oxford in which fragments of DNA were relocated from an agarose gel to a cellulose nitrate filter where they were hybridized to radioactive RNA probes. Following the hybridization, autoradiography was employed to confirm the presence of the labelled DNA, based on the sequences of the RNA probes. From this protocol the field of DNA microarrays has exponentially grown due to the fact that it can be implemented in a large number of scientific fields. Beginning with Southern's methodology, the use of DNA microarrays has become a standard tool for molecular biology research and clinical diagnostics. DNA microarrays have generally been used to detect bacterial pathogens commonly found in water and food, immunological assays, PCR-based assays, electrochemical assays and array-based biosensors.

The field of molecular forensic has implemented DNA microarrays for SNP genotyping. Analysis of SNPs within the entire genome, by investigating short sequences of DNA that include a single base pair change, can be used to create a unique profile of SNPs for an individual. The use of SNPs in forensic is common because they occur frequently, with estimates of 1 for every 1,000 base pairs and over three million are present in the genome. An array-based format not only can test thousands of SNPs at once but is highly successful when handling degraded or minute amounts of DNA such as in crime scene investigations[30]. The field of forensics often must solve problems requiring parental identification. Individual identification using microarray platforms designed to genotype SNPs provide a viable method of such testing. The use of DNA microarrays as an efficient assay to sequence a genome through multiple hybridization experiments in parallel has been previously reported. For Chromosomal Micro Array (CMA) a chip uses labels or probes that bond to specific chromosome regions. Computer analysis is used to compare a patient's genetic material to that of a reference sample. The basic idea behind DNA microarray technology has been to immobilize known DNA sequences referred to as probes in micrometre-sized spots on a solid surface (microarray) and specifically hybridize a complementary sequence of the analyte DNA or a target.

A fluorescent labelled reporter facilitates fluorescence detection of the presence or absence of a particular target or gene in the sample. By using laser scanning and fluorescence detection devices such as CCD cameras, different target hybridization patterns can be read on the microarray and the results quantitatively analysed[31].

Conclusion

In the era of new generation technologies, in our opinion the most popular methods are those that are commercially available in an easy to perform either in the kit format or not technically demanding. Improved next generation sequencing (NGS) and DNA Microarray are all being applied for DNA profiling for forensic purposes in future. It is inevitable that these techniques will become the standard technology globally for DNA base profiling. With passage of time, more developed techniques are being used, specifically suited for various investigative purposes such as methylation- specific PCR.

DNA profiling is a tool that is not only used to apprehend the guilty but also to exonerate the innocent. DNA evidence thus unravels the truth-it never lies. The Passage of time does not affect it and neither does is change. Thus, the use of DNA profiling and Molecular Forensics have opened a new era in Forensic investigations. It is important to remember that DNA evidence is not the only form of evidence in a case and that other supporting evidence will still be needed by a court of law to convict a person of a crime.

Conflict of Interest

None

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Case Study

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Postmortem Detection of Child abuse at a Premier Hospital: Two Case Reports

Mahesh Kumar[1], Mansi Kumar[2], Abhishek Yadav[3], Kulbhushan Prasad[4]

Authors Affiliation: [1]Assistant Professor, Department of Forensic Medicine and Toxicology, Lady Hardinge Medical College New Delhi India. [2]Assistant Professor, Department of Obstetrics & Gynaecology, Rama Medical College Hospital and Research Centre, Hapur Uttar Pradesh, India. [3,4]Assistant Professor, Department of Forensic Medicine and Toxicology, All India Institute of Medical Sciences (AIIMS), New Delhi, India.

Reprints Requests: Mahesh Kumar, Assistant Professor, Dept. Of Forensic Medicine, Lady Hardinge Medical College New Delhi India.

E-mail: mahesh25881@gmail.com

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Abstract

Physical Child abuse also known as Battered baby Syndrome (BBS) or Non Accidental Injury (NAI) occurs when the child suffers repetitive physical injuries by a parent or guardian. It can cause severe injuries to the children and even death. We report two autopsy cases in which both the children were brought dead in the All India Institute of Medical Sciences (AIIMS), New Delhi by the guardians with no complaint of foul play. During autopsy the characteristic findings of physical abuse were found and were reported to the investigation officer. It led to the arrest of the guardians and legal proceedings were initiated. The western countries have developed a centralized reporting system for child abuse but the cases of physical and mental abuse of children mainly at their home from parents or guardian are highly under-reported in India. The authors intend to highlight the importance of interpreting such injuries not only during autopsy but also in clinical setups so that perpetrators of child abuse are not spared. The authors have given recommendations about the steps which could be taken to detect child abuse at an early stage and save the life of a child.

Keywords: Physical Child Abuse; Battered Baby Syndrome; Non Accidental Injury; Child Neglect; Torn Frenulum.

Introduction

Child maltreatment is the abuse and neglect that occurs to children under 18 years of age, which includes all types of physical and/or emotional ill-treatment, sexual abuse, neglect, negligence and commercial or other exploitation, which results in actual or potential harm to the child's health, survival, development or dignity in the context of a relationship of responsibility, trust or power[1]. Physical Child abuse is also known as Battered baby Syndrome (BBS) or Non Accidental Injury (NAI) and occurs when the child suffers repetitive physical injuries by a parent or guardian[2]. The western countries have developed a centralized reporting system for such cases[3,4] with many studies and cases also being reported in Medical Literature [5-11]. In India, the protection of children is ensured through 'The Juvenile Justice (Care And Protection Of Children) Act, 2015'[12] and 'The protection of children from sexual offences act, 2012'[13] but the cases of physical and mental abuse of children mainly at their home from parents or guardian are highly under-reported.

We report two cases in which both the children were brought dead in the All India Institute of Medical Sciences (AIIMS), New Delhi by the guardians. Police did not report any foul play as there were no complainants. During autopsy the characteristic findings of physical abuse were found and were reported to the investigation officer. Subsequently the guardians were immediately arrested and legal proceedings were initiated. The authors intend to highlight the importance of interpreting such injuries not only during autopsy but also in clinical setups so that perpetrators of child abuse are not spared.

Case 1

An eight year old male child, who was living with her step mother, was brought to emergency department, Trauma centre, AIIMS, New Delhi, by his step mother with alleged history of fall from chair. The child was found brought dead and a medicolegal case was registered. The mother and father of this child had been divorced and married to different persons again and presently this child was staying with his step mother.

Autopsy examination revealed multiple old and fresh injuries all over the body in the form of Patterned abrasions, contusions and laceration (Image-1,2,3). Infected dermatitis was present over face, lips, forearms, ears and neck (Image-2). Burn injury was present over gluteal region (Image-4) and penis suggesting cigarette butt burns (Image-5). Multiple bruises were present over the forehead. Subdural haemorrhage was present over both the parietals and occipital regions of the brain. During the course of investigation, the investigating officer (IO) produced an electric iron (Image-6) that was allegedly used to burn the gluteal region of child for correlating the burn marks.

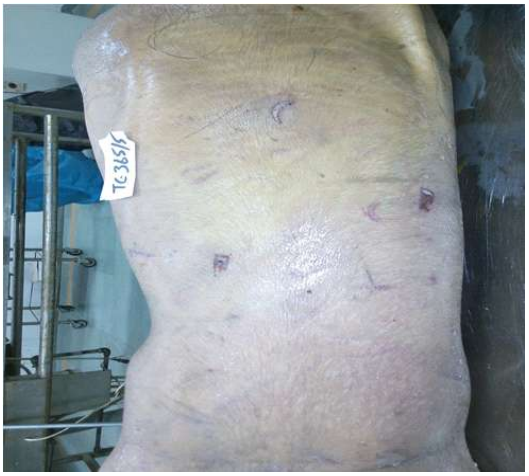

Image 1: Multiple Imprint abrasion, contusion and Laceration over the back of child

Image 2: Patterned abrasion and Infected dermatitis over right forearm

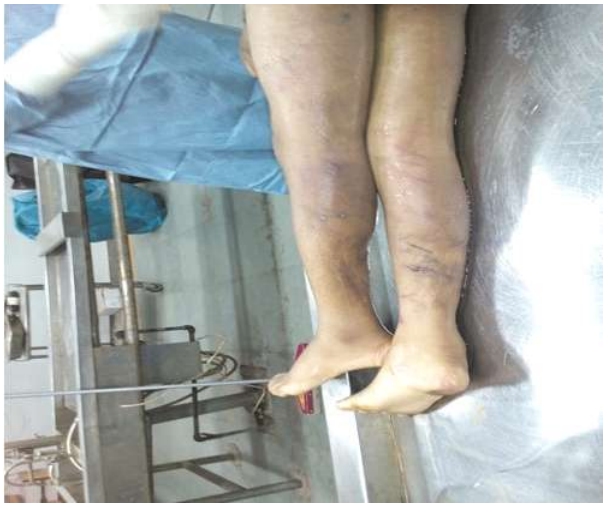

Image 3: Patterned abrasions and contusions over lower limbs in various stages of healing

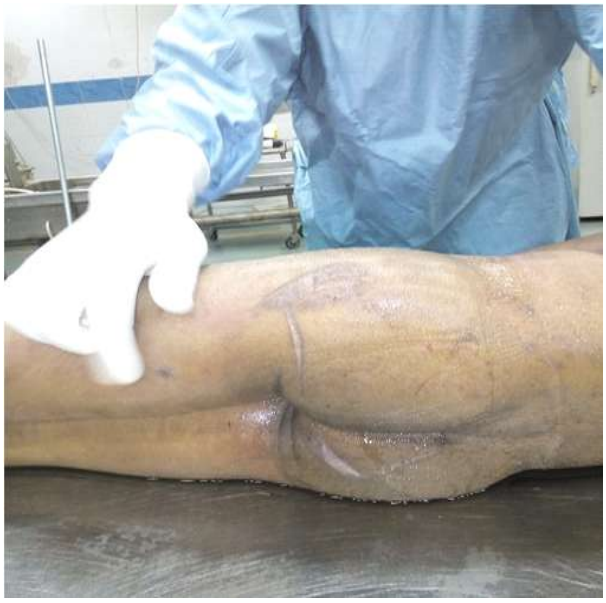

Image 4: Burn marks in different stages of healing over gluteal region.

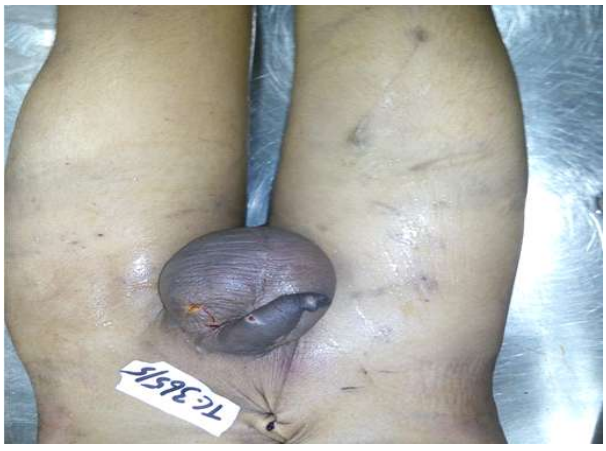

Image 5: Burn marks, abrasions and contusions in different stages of healing over genitalia and right thigh

Image 6: Electric iron used to inflict burn marks on child as per Investigating Officer

Case 2

A three year old male child, who was brought to emergency department AIIMS, New Delhi with alleged history of fever and unconsciousness. The child was found brought dead and a medicolegal case was registered. The IO gave the history that the child was sold by his biological parents to a lady in Jharkhand State, who came to Delhi and started working as a household maid through a couple working as placement agent. She was not allowed to bring the child along with her and usually handed him over to the above mentioned couple. This lady went missing for about a month and the child was left with this agent couple who brought the child to emergency department of AIIMS.

Autopsy examination revealed multiple old and fresh injuries all over the body in the form of Patterned abrasions and contusions (Image-7A&B). Infected dermatitis was present over face, lips, ears and neck (Image-7, 8). The left ear had Cauliflower appearance indicative of repeated slapping (Image-8). On radiological examination recent fracture of lower end of shaft of left Humerus and old fracture of shaft of left radius bone were found. Liver was lacerated, and spleen and kidney were showing hematoma in the parenchyma. Diffuse subdural haemorrhage was present over right parieto-occipetal region of brain (Image-10).

Image 7: Multiple Patterned abrasion, contusion and Laceration over the body

Image 8: Cauliflower appearance over left ear

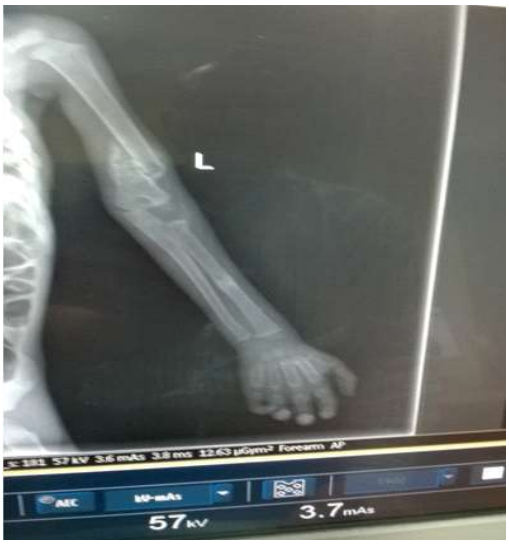

Image 9: Recent fracture of left Humerus and old fracture of Radius bone

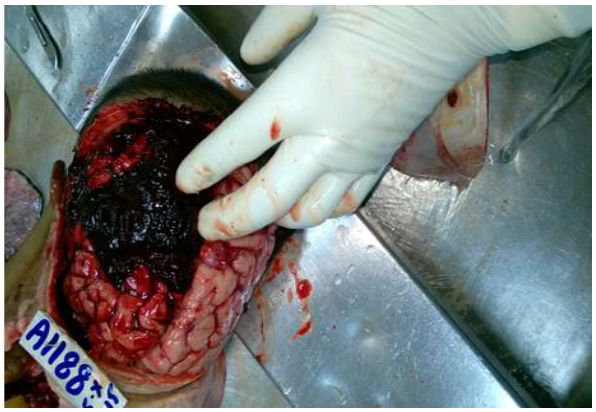

Image 10: Subdural haemorrhages over cerebrum

Discussion

The child abuse can be diagnosed clinically by the following classical sign and symptoms, in **different stages of healing**:[2,14,15]

1. Bruises may be patterned, particularly on back, buttocks, genitals, ears and back of hands.
2. Multiple abrasions and lacerations.
3. Fractures in long bones.
4. Burns reflecting the pattern of object or method of injury.
5. Torn Frenulum-considered as a reliable indicator of child abuse.
6. Eye injuries such as bleeding into vitreous humour, dislocation of lens, retinal detachment, and retinal haemorrhages.
7. Dislocation and injuries of teeth and gums.
8. Bite marks.
9. Scars.
10. Pulling of scalp hair.

The head injury is the most common cause of death as evident in both of the cases. Both the cases had multiple injuries at different stages of healing. Skin infection was present indicating the neglect of children. In case no 2, the child had Intra-abdominal injuries also, which are the second most common cause of death in battered children[2,14]. His whole body X-ray revealed fractures of long bones of different age. In cases of suspected child abuse, radiological investigations are vital in identifying bodily fractures and head injury[10]. The child abuse is more prevalent in males and children are more likely to cause severe injuries as seen in our cases[11]. The burn injuries on the penis and gluteal region of child in case no1 by cigarette and electric iron show the brutality the child was subjected. We can clearly infer by the autopsy findings in both the cases that the children were subjected to physical abuse, which was also confirmed by the police investigations into the cases.

In united Kingdom, National trauma audit is performed by the Trauma Audit Research Network (TARN), which includes data from 96% of acute hospitals in England and Wales reported severe injury (ISS >15) due to NAI in 9.7% of children below 2 years with Mortality rate of 7.8% and in 2.1% of children above 2 years with Mortality rate of 27.6%[4]. This indicates the urgent need of a similar Trauma registry network in India for children. In the reported cases the culprits would have gone scot free with dead children getting no justice if the autopsy surgeons had not diagnosed child abuse.

Recommendations

1. The Emergency doctors, Paediatricians and doctors in trauma management like Orthopedician, Surgeons, Neuro-surgeons etc should trained and sensitized to diagnose the cases of child abuse properly so that it can be identified at an early stage and the life of the child could be saved. But care should also be taken to prevent any misdiagnosis which could cause the harassment of innocent parents or guardians.
2. Private family practitioners should be given training and professional to interpret the sign and symptoms of child abuse and report it to the police or child welfare committee.
3. Whole body X-ray should be mandated in all the autopsy cases of children dying with no clear history.
4. A Paediatric Trauma registry network should be started by Government of India which could connect with the major district hospitals all across the country.
5. The same can be implemented by starting initially at the central govt hospitals and then gradually involving hospitals at state level, then district level and so on.

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Case Report

Kidnapping, Rape and Murder of a Minor Girl

Arijit Dey[1], Swati Tyagi[2], Ashok Chanana[3], Gurmanjit Rai Mann[4], Neha Sharma[5]

Authors Affiliation: [1,2,5]SeniorResident, Dept. of Forensic Medicine & Toxicology, AIIMS, New Delhi. [3]Associate Professor & Head, Dept. of Forensic Medicine & Toxicology, Government Medical College, Amritsar. [4]Professor & Head, Dept. of Forensic Medicine & Toxicology, SRMS Bareilly.

Reprints Requests: Arijit Dey, Senior Resident, Dept. of Forensic Medicine & Toxicology, AIIMS, New Delhi.

E-mail: arijit.forensic@gmail.com

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Abstract

Physical and sexual assault with or without homicide occurs throughout world regardless of race, age,nationality, financial status etc. Women and childrenare the most vulnerable group as they may not be able to defend themselves.Sexual cases are generally not reported because of social stigma and whatever we see is the tip of iceberg.This case deals with the story of a minor [14yrs]girl who was found lying by the side of road in an unconscious condition with multiple injuries. Inquest report was prepared as a case of road traffic accident but later on she was identified and then relative of injured complaint to police against certain suspects that she was kidnapped from her house and was allegedly raped and later on died and the postmortem examination was conducted inDepartment of Forensic Medicine and Toxicology,Government Medical College, Amritsar.

Keywords: Child Sexual ABuse; Kidnapping; Intracranial Haemorrhage; Spermatozoa; Delayed Investigation.

Introduction

Child Sexual Abuse typically includes unwanted and inappropriate sexual solicitation of, or exposure to, a child by an older person; genital touching or fondling; or penetration in terms of oral, anal or vaginal intercourse or attempted intercourse [1]. Sexual offences in India are covered under different sections of Indian Penal Code and it does not distinguish between adult and child victims [2]. However, the Protection of Children from Sexual Offences Act (POCSO), 2012, which has been recently drafted to strengthen the legal provisions for the protection of children from sexual abuse and exploitation, defines a child as any person below the age of 18 years and provides protection to all children under the age of 18 years of the offence of sexual assault, sexual harassment, and pornography [2].According to a study published by the Ministry of Women and Child Development in 2007, India has the world's largest number of sexually abused children; children below 16 years are raped every 155th minute and below 10 years are raped every 13th hour and there is severe under-reporting of such crimes[3]. Most of rape cases in India, as elsewhere in the world, are never reported due to the social stigma [4,5]. Children are more frequently raped than adults as they cannot offer much resistance, and due to false belief that venereal diseases are cured by sexual intercourse with a virgin [6].

History A 14 year female child of moderate built and moderatenourishment,wearing a yellowish floral designed salwar and Kameej, was found in early hours of the day lying by the side of road in Amritsar, in an unconscious condition and with multiple injuries. Then a passerby noticed her and she was admitted in a nearby hospital as an unknown patient and was treated for her condition. Later on she was identified as a class 9 student of local school and her parents were informed. She remained unconscious in the hospital for 3 days,later tracheostomy was done as the condition of the patient was deteriorating and later on she succumbed to her injuries, 5 days after admission. Her postmortem was

conducted inDepartment of Forensic Medicine and Toxicology, Government Medical College, Amritsar.

Postmortem Findings

External Finding—Eyesand mouth were closed and pupil dilated.Face was swollenon left side.Tracheostomy mark was present on front and centre of neck. Rigor mortis was present in whole body except eyelids, neck and jaw. Post mortem staining was present on the back except the area of contact flattening, and was fixed.The following injuries were present:

1. Diffuse swelling of scalp present. On dissection scalp was oedematous. Subgalealhaematoma was present in an area of 8 cmX 7cm along with subdural and subarachnoid haemorrhage present diffusely in brain measuring about 200 cc. Brain is oedematous, weight of 1109 gm.
2. Multiple dark coloured abrasions, 26 in number with scabs falling from the periphery and varying in size 1 cm X0.8cm to 7 cmX3 cm were present on both the knees, back of right and left hand,back of both upper limbs and various sites over face. Some of the abrasions were infected and purulent.

Genital Finding—Labiamajora were lax and gaping. Labia minora were thick, large gaping and cutaneous. Vestibule was gaping.Introitus was dilated.

After conduction of post-mortem examination, routine viscera was collected, swabs were taken from vaginal introitus and posterior fornix, slides were also prepared which were sent to Forensic Science Laboratory for Chemical Analysis and for presence of spermatozoa.

Opinion: All injuries were of antemortem origin.Cause of death in this case was declared as compression of brain due to Intracranial Hemorrhage as a result of trauma, which was sufficient to cause death in ordinary course of nature.

Proceedings in Witness Box

Summons was received in this case by the autopsy surgeon to appear as expert medical witness in a complaint case in the court of Judicial Magistrate at sub-divisional level of District Magistrate. After tendering evidence in court of law by expert medical witness the case was transferred to higher competent court i.e. court of Additional Session Judge for trial under section376/302**.** While tendering his evidence he was questioned by prosecution regarding rape in this case but till date. The autopsy surgeon didn't receive any report of Chemical Examiner in this case i.e.it was neither sent by chemical examiner nor sent by investigating officer. On examination of judicial file it was found that report of chemical examiner was attached there. The report of chemical examiner reported the presence of spermatozoa in slide and swabs, which were prepared during postmortem examination. Then opinion of vaginal intercourse was furnished by autopsy surgeonin the court itself.

Discussion

It is observed from above stated finding and proceedings in witness box that the case was of rape and murder of a minor. Although no age is safe from rape, Malhotra and Sood reported that majority (76.9%) of victims in their study were adolescents [7], as is being reported in this case. In studies conducted in the United States, it was observed that females are more likely to suffer from child sexual abuse and 12-40% of females and 4-16.5% of males have experienced at least one instance of sexual abuse in their childhood or adolescence [8,9].

In this case, the Police didnot take any cognizance and the investigative agency turned a deaf ear to the best efforts by the parents/relative of deceased to lodge a FIR under relevant section of law. Thus, they had to knock the door of Court hoping that they will be able to get justice. Keeping their hopes alive the Court did not disappoint them and fair trial started in this case. The carelessness of investigating agency can be gauged from the fact that no FIR under any relevant section of 376 I.P.C./Prevention of Children against Sexual Offence (POCSO)was lodged. Moreover,the report of chemical examiner which is usually received by autopsy surgeon within 3-4 months after its submission to laboratory of chemical examiner was never submitted to the doctor by police, even after passage of 16 months after conduction of of postmortem examination and the autopsy surgeon saw it first in witness box in court itself.

To the belief of the authors of this article, the present case was more heinous than "Nirbhaya Delhi Rape Murder Case", (which led to change of the then prevalent rape law, and the Criminal Law (Amendment) Act 2013 was passed) [10] as the deceased here was aminor who was first kidnapped and brutally assaulted sexually and physically and then left on roadside to succumb to the injuries.No treatment aid was given by the Government agency and no FIR was lodged by the police at any stage.Post Mortem examination was conducted U/S 174

Criminal Penal Code. Police did not submit the viscera to the laboratory for chemical examination in the optimum time. No report of viscera or the collected swab and smear was submitted to the autopsy surgeon by police. Doctor opined regarding vaginal intercourse after going through the judicial file and media was covering the Court proceedings.

Conclusion

Sexual abuse leaves a permanent scar on the mind and body of the victim and puts the family under shame and humiliation even though they are not at mistake. So,more efforts must be made to provide justice to all victims of sexual assault, an active legislative and judicial actions, comprehensive quick approaches of investigative officers and healthcare providers, and rehabilitation is of very much need in a case of sexual assault.Also, spreading awareness encouraging early reporting, harsher punishment to the criminals and prompt care and protection of these innocent victims can reduce this heinous crime from the society.

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