Indian Journal of Forensic Medicine and Pathology

Indexing Information: NLM catalogue & Locator Plus, USA; Google Scholar; Index Copernicus, Poland; EBSCO Publishing`s Electronic Databases, USA; Academic Search Complete, USA; Academic Search Research & Development, USA; ProQuest, USA; Genamics JournalSeek USA.

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Indian Journal of Forensic Medicine and Pathology

October - December 2016
Volume 9 Number 4

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excopernicus.com/Indian+Journal+of+Forensic+Medicine+and+Pathology,p24785404,3.html
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Indian Journal of Forensic Medicine and Pathology [IJFMP]
ISSN:
0974-3383, 0974-3391
ICV 2015: 75.15
Area: Technical science
Print version: yes
Electronic version: yes
Previous evaluation ICV
2011: 4.71
2010: 4.26
2009: 3.13
Desktop

Original Article
Received on 25.10.2016, **Accepted on** 08.11.2016

Indian Journal of Forensic Medicine and Pathology
Volume 9 Number 4, October - December 2016
DOI: http://dx.doi.org/10.21088/ijfmp.0974.3383.9416.1

The Effect of Room Temperature Exposure on Sweat Spot-Derived DNA Samples Through Analysis of 143-bp mtDNA D-Loop HVS-1 (nt 16268-16410) And 126-bp HVS-2 (nt 34-159)

Yudianto Ahmad*, Koesbardiati Toetik, Putri Ni Putu P.E.***, Gunawan Nola M.******

Abstract

Context: Identification is an unavoidable process in forensic setting. DNA analysis is one of several scientifically recognized methods to identify somebody. As an alternative to the largely used DNA specimen, the last object frequently used by the perpetrator or victim can be used as well. Clothes or apparels stick to the outer skin; thus the epithelial cells of the skin can be expected to attach to them. One factor that may affect the quality of DNA is the duration of exposure. From the two DNA analysis methods, mitochondrial DNA (mtDNA) has better durability than nuclear DNA due to its relatively larger amount. Hence, mtDNA has some greater chance of success in the amplification process. *Aims:* From this study, we would like to understand of the effect of the duration of exposure to room temperature on the quality of DNA derived from sweat spots in Indonesia remaining that the data remains unavailable to date. *Setting and Design:* This is an analytical experimental research with time-series design. *Material and Method:* Sweat-spotted clothes that had been worn were subsequently exposed to room temperature for 0, 1, 7, 14 and 20 days. *Statistical analysis used:* nil. *Results:* Results showed that longer exposure markedly decreased the concentration of DNA from day 1 to 20 at $p < 0.005$. *Conclusions:* Longer duration of exposure to room temperature significantly decreased the quality of DNA derived from sweat spots on clothes. Visualization of PCR detection results show positive (+) detection only on day 0 of exposure on both 143-bp mtDNA HVS 1 and 126-bp mtDNA HVS 2.

Keywords: Sweat Spot; DNA Quality; Mitochondrial DNA; Identification.

Introduction

Personal identity is of paramount importance in order to obtain the fairest justice. According to Interpol Disaster Victim Identification (DVI) guide, there are two main groups of methods of identification; primary and secondary. Primary means of identification include friction ridge analysis, comparative dental analysis and DNA (Deoxyribonucleic Acid) analysis and secondary means of identification include personal description, medical findings, tattoos, as well as property and clothing found on the body [3].

Identification is frequently impeded by materials poor condition; thus, an alternative is pursued through the use of mini-primer set, in which the region of mitochondrial DNA amplification for use as the amplification product is reduced. The fact that there is a relationship between the size of a locus and the success of the amplification of degraded DNA obtained from the crime scene and in a mass disaster corroborates this situation. Mitochondrial DNA is used since it can determine density, thus it hastens the process of identification, especially in mass disaster cases with a large number of victims [1].

To date, however, there is not any specific research yet on successful use of the mini-primer sets as an

Authors Affiliation: *Master of Forensic Science Program – Program Director of Master of Forensic Science Program, Postgraduate School, Universitas Airlangga, Surabaya, East Java, Indonesia. Department of Forensic Medicine and Medicolegal Study – Program Director of Forensic Medicine and Medicolegal Study Residency Program, ****Department of Forensic Medicine and Medicolegal Study – Resident in Forensic Medicine and Medicolegal Study, Faculty of Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia. **Master of Forensic Science Program – Lecturer of Master of Forensic Science Program, ***Master of Forensic Science Program – Student of Master of Forensic Science Program, Postgraduate School, Universitas Airlangga, Surabaya, East Java, Indonesia.

Reprints Requests: Ahmad Yudianto, Master of Forensic Science Programme, Postgraduate School, Universitas Airlangga, Kampus B Universitas Airlangga, Jl. Airlangga no. 4 – 6 Surabaya 60286, East Java, Indonesia.
E-mail: yudi4n6sby@yahoo.co.id

alternative to forensic DNA identification using degraded DNA, especially mitochondrial DNA. It is necessary to determine the loci potentially useful for examination of degraded DNA. This mapping allows determination of the DNA loci that can be used for forensic DNA examination, either for the purpose of individual identification of a mass disaster's victims or revealing some criminal cases with attempts of evidences removal on victims and suspects.

In mitochondrial DNA, there are protein non-coding regions, called D-Loop, at nucleotide (nt) 16 024-576. This region is also called the control region since it is a segment that contains the elements of replication origin, transcription initiation and regulators. In D-Loop, there are segments called hypervariable segment I (HVS I) located at nt 16024-16383 (360 bp) and HVS 2 located at nt 57-372 (316 bp) [8,9].

A mechanical abuse will always leave a biological trace evidence or an object originating from the body of living things/humans. Biological evidence includes blood or blood spot on clothes or fabrics as well as sweat spot on worn clothes. So far, the effect of exposure to room temperature on the quality of DNA samples derived from sweat spot on clothes identified through mitochondrial DNA analysis has not been known.

A previous study by Yudianto (2006) showed that sweat spots could be an alternative to identification assays since perpetrators often ignore these spot. The length of exposure to the spots was in accordance with the statutory number of days for investigation process in the Criminal Code, which is 20 days. Thus, this study used the intervals of exposure length according to the repetition of day in the week (day 0, day 1, day 7, day 14 and day 20). The room temperature used was in accordance with the standard urban area, 29°C-32°C.

Material and Method

This study was of analytical experimental to analyze the feasibility of degraded mtDNA to be used in DNA assay). This study used a time-series design and samples were derived from clothes with sweat spots taken from respondents by means of the ethical feasibility test. Calculation of sample size indicated that 15 respondents were required in this study.

Sample Handling

The initial stage of the study was the preparation of the DNA templates for the Polymerase Chain Reaction (PCR) process. DNA templates for amplification were derived from the lytic human epithelial cells from clothes with sweat spots. They were cut, put into a conical tube, mixed with water free, and centrifuged at 10,000 G for 10 minutes. The pellet was taken and mixed with 1 ml of DNAZol and centrifuged at 10,000 G for 10 minutes at 4°C. Subsequently, 0.5 ml absolute ethanol (100%) was added to the viscous supernatant and centrifuged at 4,000 G for 1-2 min at 4°C. The pellet was washed with 8-10 ml of 75% ethanol. The DNA-containing pellet was dissolved with 0.2-0.3 ml of 8 mM NaOH solution and subjected to vortex sufficiently and stored at -20°C.

PCR Amplification

Materials for PCR amplification consisted of dNTP dNTP (ATP,CTP,TTP GTP), MgCl2 dan Taq Polimerase, Nuclease free water, and mtDNA primers HVS1-143 bp (nt 16268-16410) (*AFDI, primer)*: 5'CACTAGGATACCAACAAACC 3' and 5' GAGGATGGTCAA GGGAC 3' , HVS 2- 126 bp (nt 34-159) (*AFDIL. primer*) : 5' GGG AGC TCT CCA TGC ATT TGG TA 3' and 5' AAA TAA TAG GAT GAG GCA GGA ATC 3'.

- PCR amplification of mtDNA for HVS-1 143bp (Optimation PCR): Initial denaturation 96^0C - 4 minute, [35X : Subsequent denaturation 94^0C -1 minute, Annealing 61^0C - 1 minute, Extension 71^0C - 1 minute], elongation 72^0C - 5 minute.
- PCR amplification of mtDNA HVS 2- 126 bp (nt 34-159): Initial denaturation 95^0C - 3 minute, [30X : Denaturation 94^0C - 1 minute, Annealing 56^0C - 1 minute, Extension 72^0C - 1 minute], final Extension 72^0C - 3 minute.

Positive PCR results: purification, DNA quantity, preparing/labeling, purifying extension product/ precipitation and subsequently sequenced in the stage of purification and visualization (DNA Quantity), preparing/labeling, purifying extension product.

Sequence Analysis

Each nucleotide produced a peak with some different color on the electrophoregram, in which nucleotide G was black, nucleotide C was green and nucleotide T was red. Analysis of nucleotide sequencing results for determination of degradation to the nucleotide was carried out by labeling it with the letter 'N' on the sequencing results.

Result

There are two things to be analysed in this study. Firstly, we analysed the effect of the duration of exposure to room temperature on DNA concentration derived from sweat spots. The study began with exposing the sweat spot samples to room temperature (29.5°C-30°C). The length of exposure used in this study is 0, 1, 7, 14 and 20 days consecutively. DNA samples were extracted using DNAzol method. The DNA concentrations obtained from the extraction were measured using an UV-visible spectrophotometer and the results are shown on Table 1. From Table 1 we can clearly see that the longer the exposure to room temperature, the lower the concentrations of DNA were.

Secondly, we analysed the effect of exposure duration to room temperature on DNA derived from sweat spots through detection of 143-bp mtDNA HVS 1- (nt 16268-16410) and 126-bp HVS 2 (nt 34-159). To determine the effect of exposure to temperature and the extended exposure duration on bone DNA, the loci of 143-bp (nt 16268-16410) and 126-bp (nt 34-159) mtDNA amplicon products were examined. Results of PCR amplification by means of 2% agarose gel electrophoresis can be seen on figure 1 and 2. Both figures of PCR results show that DNA was detected only on day 0. Results of sequencing (Figure 3) indicated that there were DNA fragments detected. The reading of DNA sequencing after exposure to high temperature found a large number of the symbol 'N'. According to International Union of Pure and Applied Chemistry (IUPAC), the symbol 'N' is a method to mention the presence of ambiguities [5].

Table 1. Mean of DNA concentration derived from sweat spots Extended room-temperature exposure significantly reduced the DNA concentration from sweat spots (sig. (p) = 0.000; sig. limit p <0.05)

Sample	Quantity (x ± SD) (µg/ml)
Day 0	150.16 ± 5.71
Day 1	10.12 ± 5.46
Day 7	1.05 ± 0.72
Day 14	0.94 ± 0.61
Day 20	0.16 ± 0.31

x: mean of DNA concentration; SD: Standard deviation

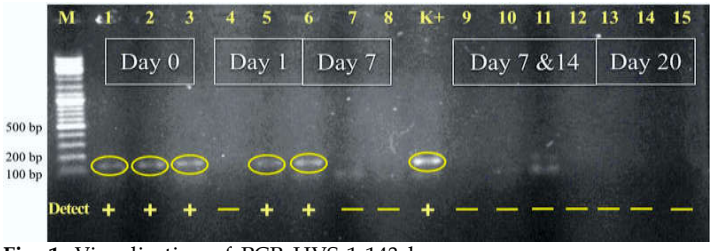

Fig. 1: Visualisation of PCR HVS 1-143 bp.
M is marker ladder of 100 bp; 1 to 15 represents the research samples; K+ is positive control; Detection + at mtDNA HVS 1-143 bp.

Fig. 2: Visualisation of PCR HVS 2-126 bp.
K+ is positive control.

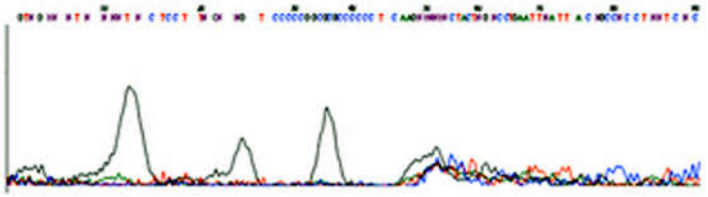

Fig. 3: Sequencing of HVS 2-126 bp

Discussion

Analysis of the Effect of the Duration of Exposure to Room Temperature on DNA Concentration Derived From Sweat Spots

The concentration of DNA constitutes an important factor in DNA analysis, particularly in regards of the success of DNA sample amplification. One nanogram reduction of DNA concentration potentially decreases STR detection capability up to 95% [1]. DNA integrity is important for forensic DNA assay. This implies that, despite the relatively high DNA concentrations in the assays, the high level of fragmented or degraded DNA will be less meaningful.

DNA degradation can be produced by both endogenous and exogenous factors. Endogenous factors originating in the cells are known as spontaneous degradation. Exogenous factors derive from the environment. Exogenous factors, such as humidity and room temperature, greatly affect DNA conditions. Another exogenous

factor is the presence of contaminants, such as bacteria. Tissues degraded by bacteria may subject to autolysis and spontaneous depurination [1,10,12]. Abnormal exposure to chemical agents, pH, temperature, or other exposures may lead to DNA degradation. Extended exposure to room temperature resulting in damaged conjugation bonds and detached nitrogen bases will lead to DNA fragmentation, even degradation.

Analysis Of The Effect Of Exposure Duration To Room Temperature On DNA Derived From Sweat Spots Through Detection Of 143-bp mtDNA HVS 1- [nt 16268-16410] And 126-bp HVS 2 [nt 34-159]

Undetected DNA in the visualization of PCR on these samples was due to the quality of DNA. The quality of the DNA includes DNA concentration, purity and condition. DNA quality for analysis should be good and, if degraded, the degradation should be as minimal as possible. Severely degraded DNA prevents primer from annealing the target DNA to be replicated. Thus, good quality of DNA is a fundamental prerequisite to the success of PCR reaction as a whole. According to Butler [1], sensitivity of PCR is a function of the number of cycles and concentrations and the integrity of DNA.

Severely degraded DNA prevents primer from annealing the target DNA to be replicated [5,6]. According to Muladno, obtaining adequate visualization requires adequate DNA purity and adequate DNA concentration, so that DNA can be used as material for DNA assays for identification and paternity tests [6].

DNA degradation is among the causes of failed DNA detection in DNA analysis using PCR method in this study. This is consistent with the notion of Yudianto (2010) concerning several possible causes for the failure of DNA detection, such as the minimum amount of target DNA, DNA degradation preventing primers from annealing, lacking DNA polymerase and PCR cycles and the presence of PCR inhibitors.

PCR cycles and insufficient DNA polymerase can be controlled by previous optimization. Failed amplification process is characterized by the absence of bands on the electrophoresis results. It can be overcome by the use of PCR master mix, which contains Mg^{2+}, taq DNA polymerase, dNTPs in quantities tailored to the requirement for optimal PCR reaction and has a proven reliability in PCR reaction, while inadequate cycles in PCR reactions have been overcome by PCR optimization of the primers used [1,12].

Exogenous DNA degradation caused by such exposures as X-rays, chemical agents, spontaneous instability, or extremely high temperature will result in various types of degradation, such as degraded double strands or single strands, base damage, sugar damage, and even DNA-DNA crosslink and DNA-protein crosslink. Those environmental factors lead to DNA degradation. Degradation can be fast or slow and it depends on the affecting factors and duration of exposure.

Conclusion

Duration of exposure to room temperature had an effect on the quality of DNA derived from sweat spots on clothes. It significantly decreases the DNA concentration at p <0.005. Visualization of PCR detection results show positive (+) detection only on day 0 of exposure on 143-bp mtDNA HVS 1 and 126-bp mtDNA HVS 2.

Key Messages

Sweat spot on clothes can be useful for DNA specimen in mtDNA analysis for identification purpose at an acceptable level. However, room temperature remarkably alters its availability to be a DNA specimen as well as several other factors.

Acknowledgement

This research received financial support from Postgraduate School, Universitas Airlangga, Surabaya, Indonesia.

Conflict of Interest

None disclosed.

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Erratum

Article Titled **"Pathological Findings of Liver in Autopsy with Emphasis on Incidentally Detected Lesions"**

Sapna Patel*, Rajalaxmi B.R.*, Manjunath G.V.**

Publsihed in

Indian Journal of Forensic Medicine and Pathology

Volume 9 Number 3, July - September 2016

DOI: http://dx.doi.org/10.21088/ijfmp.0974.3383.9316.13

Authors Affiliation and Figure legends in said article has been replaced by the author and now to be read as

Authors Affiliation: *Assistant Professor, **Professor and Head, Department of Pathology, JSS Medical College, JSS University, Mysuru, Karnataka, India.

Figure 1C: Liver displaying features of large bile duct obstruction. H&E, x100

Figure 1D: Liver displaying features of von Meyenburg complex. H&E, x100

Figure 2A: Nests and trabeculae of neuroendocrine cells amidst hepatocytes. H&E, x100)

Figure 2B: Specimen of liver showing multiple grey white nodules

Mistake is regretted.

Editor-in-Chief

Original Article
Received on 24.08.2016, **Accepted on** 02.09.2016

Indian Journal of Forensic Medicine and Pathology
Volume 9 Number 4, October - December 2016
DOI: http://dx.doi.org/10.21088/ijfmp.0974.3383.9416.2

Study of Fly Species Composition in Human Decomposed Bodies A Prospective Study for 2 Years

Shashikanth Naik C.R.*, Lohith Kumar, S. Venkata Raghava***, Abhishek Yadav****, Kulbhushan Prasad******

Abstract

Dead bodies are attracted by numerous species of arthropods, primarily flies, beetles, mites, isopods opiliones, nematodes and their larvae. These arthropods feed, live and breed in and on the dead bodies, depending on their biological preference and on the state of decomposition. At the very initial stages of the decay, just a few moments after the death, no odour is perceptible by a human being, but arthropods do smell it and locate their target in minutes following the death, in optimal environmental conditions of humidity, temperature, sun exposure, and corpse localization. Here in this study on 51 decomposed cases, we have collected eggs, larvae, pupae and reared till flies and these flies were identified and studied.

Keywords*:* Arthropods; Decomposition; Larvae; Pupae**.**

Introduction

Insects have existed on the earth for more than 250 million years; comparatively humans have existed for about 300,000 years. Such an enormous amount of time has allowed insects to attain wide diversity in both form and development. There are currently about 700,000 described species and it is estimated that there may be more than 10 million species of insects yet to be described [1].

Forensic entomology is the branch of forensic science in which information about insects is used to draw conclusions when investigating legal cases relating to both humans and wildlife, although on occasion the term may be expanded to include other arthropods. Insects can be used in the investigation of a crime scene both on land and in water [2].

Forensic entomology is not a new science. The first recorded case of forensic entomology was in China in 1235AD [8]. Chinese lawyer and death investigator Song Ci in the medico-legal text book (Xiyuan jilu**;** one possible translation: "Collected writings on the washing away of wrongs") describes the case of a stabbing near a rice field. The day after the murder, the investigator told all workers to lay down their working tools (i.e., sickles) on the floor. Invisible traces of blood drew blow flies to a single sickle. So he confronted, the tool's owner confessed to his crime and "knocked his head on the floor". It took nearly eight hundred years until the next Chinese book on forensic entomology was published [3].

In last 20 years, forensic entomology has become more and more common in police investigations. In 1996, the American Board of Forensic Entomology, a certification Board for Forensic Entomologists was developed, similar to the Board for Certification available for Forensic Odontologists and Forensic Anthropologists [4].

Insect specimens, such as blow fly larvae (maggots) or adults, must be considered as physical evidence just as blood stains, fingerprints, hairs, fibres, or any other biological material [5].

Objectives of the Study

1. To identify the various species of insects infesting the dead body and to determine their life cycle

Authors Affiliation: *Assistant Professor, Dept of Forensic Medicine, St John's Medical College. **Assistant Professor, Dept of Forensic Medicine, Shivamogga institute of Medical Sciences. ***Associate Professor, Dept of Forensic Medicine, Bangalore Medical College & Research Institute. ****Assistant Professor, Dept of Forensic Medicine, AIIMS, Delhi.

Reprints Requests: Abhishek Yadav, Assistant Professor, Dept. of Forensic Medicine, AIIMS, New Delhi-110029.
E-mail: drayad_in@yahoo.com

and growth characteristics.

Material and Methods

Source of Data

Medico legal autopsies of decomposed bodies conducted at the Department of Forensic Medicine, Victoria hospital, Bangalore Medical College and Research Institute, Bangalore for a period of 24 months from Nov-2012 to Oct-2014 were examined to obtain insect samples.

Collection of Data

1. *Study design:* Cross sectional study
   a. *Study period:* Nov 2012- Oct 2014. (24 months)
   b. *Sample size:* 51 Medico legal autopsies of decomposed bodies

Inclusion Criteria

a. All decomposed bodies with clear entomological evidence.
b. Exhumed bodies with clear entomological evidence

Exclusion Criteria

a. Bodies with early decomposition without entomological evidence.
b. Body stored in cold storage after arrival to mortuary.
c. Body preserved by other means during transportation.

Methodology

The decomposed bodies subjected to autopsy as per the request by the police department were examined for the clear entomological evidence. Only those bodies with the presence of any stage or stages of insects were utilized for the study. Background history of the decomposed body was filled in the proforma and the respective post mortem number was assigned for the individual cases. Samples of eggs and larvae were collected from different areas of the body and clothings. Fifty per cent of the samples collected from each body were preserved and remaining 50% of the sample was used for rearing. Beef liver served as food. The pupae were taken to the Department of Entomology, University of Agricultural Sciences, Bangalore along with the preserved samples in each case. Pupae were reared till adult emergence in the Entomology department.

For the identification of Calliphorid genera and species, the work of Senior White (1940) was used and the species were also got identified by Dr Meenakshi Bharti. The species of Sarcophagidae were identified using the work of Nandi (2002) and those of Muscidae by the work of van Embden (1965). All the identification was confirmed by Dr C. A. Viraktamath.

Photographs were taken using Leica M205 C microscope. Multiple images were taken at different depths and were combined using Combine ZM software.

Results

Out of 51 cases, 82.3% (42/51) cases were male and 17.7% (9/51) were female. Majority of the cases were in the age group of 31 to 50 yrs i.e. 56.8% (29/51) cases.

Out of 51 cases of decomposed bodies, *C. rufifacies* infested maximum number of cases i.e. 45% (23/51), followed by *C. megacephala* in 27.5% (14/51) cases, while mixed infestation was seen in 13.7% (7/51) cases, 11.7% (6/51) cases were infested by Sarcophagidae (*P. ruficornis)* and only one case (1.9%) was by *C. nigripes*.

In 7 cases of mixed infestation of flies 57.14% (4/7) had *C. rufifacies* and *C. megacephala* infestation, 14.28% (1/7) cases had *C. megacephala* and *P. ruficornis* infestation, 14.28% (1/7) cases had *C. rufifacies* and Muscidae infestation and 14.28% (1/7) cases had three species of flies infesting namely *C. rufifacies*, *C. megacephala* and *P. ruficornis.*

Among all the cases *C. rufifacies* infested 56.8% (29/51) cases, *C. megacephala* 39.2% (20/51) cases followed by Sarcophagidae (*P. ruficornis),* 15.6% (8/51) cases and 1.9% (1/51) cases each by *C. nigripes* and Muscidae.

Both *C. rufifacies* and *C.megacephala* infested more in bodies found outdoor i.e. 55.2% (16/29) and 60% (12/20) cases, respectively, followed by 44.8% (13/29) and 40% (8/20) cases found indoor, respectively. Sarcophagidae (*P. ruficornis*) dominated infesting 87.5% (7/8) indoor cases.

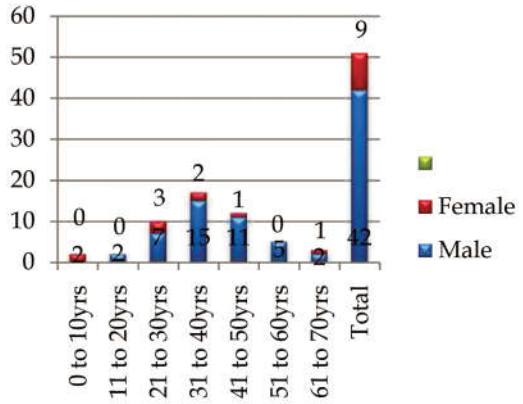
C. nigripes and Muscidae were found only in indoor cases.

As most of the bodies were in bloated stage,

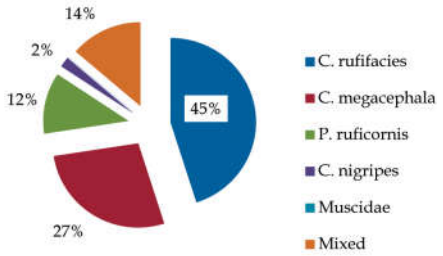
majority of the *C. rufifacies* species i.e. 57.1% (12/21) were in 3rd instar larvae at the time of collection and majority of the *C. megacephala* species i.e. 44.4% (8/18) were in 2nd instar larvae at the time of collection.

C. rufifacies is the dominating species and it was found infesting both male and female bodies 82.7% (24/29) cases and 17.2% (5/29) cases, respectively, followed by *C. megacephala* 85% (17/20) cases and 15% (3/20) cases, and Sarcophagidae (*P. ruficornis*) infesting 87.5% (7/8) and 12.5% (1/8) cases, respectively.

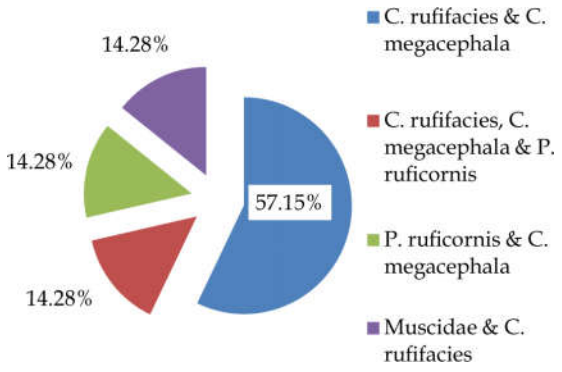
C. nigripes and Muscidae infestation were observed only in 1 male body each, of the total cases.



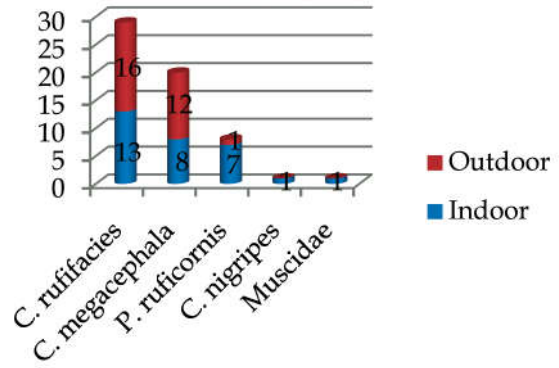
Graph 1: Age and sex - wise distribution of the cases



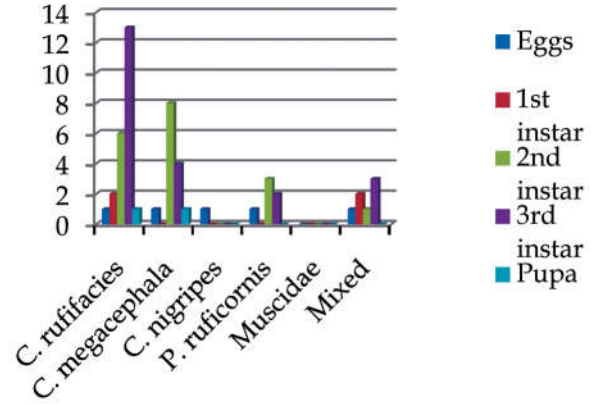
Graph 2: Species composition of flies infesting bodies



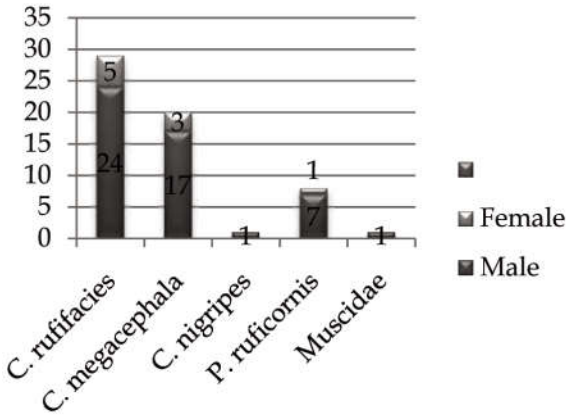
Graph 3: Species composition of flies in mixed infestation of bodies



Graph 4: Species composition of the flies and location of body



Graph 5: Distribution of fly species based on their stages of



Graph 6: Relative prevalence of fly species and sex of the development study subjects

Discussion

Morphology and and preparation of identification key

The larvae and adults of each species of flies identified were carefully observed under stereobionocular microscope and its morphological features were examined and compared to develop an identification key for species found in this study.

Both larval and adult stages could be differentiated by the following set of characters.

Larvae

a. Mandibular hooks
b. Processes of the body
c. Caudal spiracles
d. Body colouration

Adults

a. Total body length
b. Body colour
c. Colour of prothoracic spiracle
d. Stripes on the mesothorax
e. Colour of the male genital capsule
f. Distance between compound eyes in male
g. Relative size of the facets of compound eyes in upper and lower region of compound eyes

Based on these characters a dichotomous key for the identification of the families and species of the flies reared from human cadavers were prepared.

Identification Features of Larvae and Adult Flies

Larvae

a. Mandibular hooks: Only one hook was found in Muscidae larvae compared to two mouth hooks in both the larvae of Calliphoridae and Sarcophagidae (Photo 4A and 4B).
b. Processes of the body: Larvae of Muscidae, Sarcophagidae and *Chrysomya nigripes* and *Chrysomya megacephala* were smooth without body processes. Larvae of *Chrysomya rufifacies* were having series of short processes on the body (Photo 1, 2, 3).
c. Caudal spiracles: Larvae of Calliphoridae (all the three species) had caudal spiracles in deep cavity whereas the caudal spiracles of Muscidae and Sarcophagidae were completely exposed (Photo 5 and 6).
d. Body colouration: The larvae of *Parasarcophaga ruficornis* had dorsal sclerites of the body dark brown. In the remaining species of both Calliphoridae and Muscidae larvae were creamy white (Photo 1, 2, 3)

Adult flies

a. Total body length: Length of adult flies is given for each species.

Calliphoridae- *Chrysomya megacephala* 8-10 mm

Chrysomya rufifacies 7 mm

Chrysomya nigripes 5-6 mm

Sarcophagidae- *Parasarcophaga ruficornis* 11-14 mm

Muscidae – 7 mm.

b. Body colour: All the three species of Calliphoridae were metallic blue or green in colour. Species of Sarcophagidae and Muscidae were non metallic grey or black with stripes on mesothorax (Photo 9)
c. Colour of prothoracic spiracle: Both *Chrysomya nigripes* and *Chrysomya rufifacies* have white prothoracic spiracle (Photo 8A, 8C and 10A, 10C), *Chrysomya megacephala*, Muscidae and Sarcophagidae have brown prothoracic spiracles (Photo 7A and 11A).
d. Dorsal stripes on mesothorax: Species of Calliphoridae have black transverse stripes and short longitudinal stripes only in the anterior half. *Parasarcophaga* (Sarcophagidae) have three longitudinal stripes extending entire length of mesothorax. Muscidae has four longitudinal stripes extending entire length of mesothorax (Photo 9A and 9B).
e. Colour of male genital capsule: It is black in colour in the three species of Calliphoridae and one species of Muscidae. It is reddish brown in *Parasarcophaga* (Sarcophagidae) (Photo 11A).
f. Distance between compound eyes in male flies: In case of Muscidae, Sarcophagidae and *Chrysomya nigripes* (Calliphoridae) compound eyes are well separated (Photo 10B and 11B). In case of *Chrysomya rufifacies* and *Chrysomya megacephala* eyes are close together (Photo 7B and 8B).
g. Relative size of facets of upper and lower portion of compound eyes in male flies: The facets of compound eyes are more or less of same size or gradually reduced in size downwards in Muscidae, Sarcophagidae, *Chrysomya rufifacies* and *Chrysomya nigripes*. In case of *Chrysomya megacephala* facets in the upper 2/3rd of the portion were larger compared to the lower 1/3rd portion and the transition is abrupt (Photo 7B).

Key to larvae found in human cadaver

1. With one mouth hook (Photo 4B)..... **Muscidae**
- With two mouth hooks (Photo 4A)..... 2
2. Larvae with caudal spiracles exposed (Photo 5)..... **Sarcophagidae**
- Larva with caudal spiracles concealed in deep cavity (Photo 6)..... **Calliphoridae**..... 3
3. Larva with short projecting processes on body (Photo 2B and 2C)..... ***Chrysomya rufifacies*** **(Macquart)**
- Larva smooth without projecting processes on body (Photo 1)..... ***Chrysomya megacephala*** **(Fabricius) &** ***Chrysomya nigripes*** **Aubertin**

Key to adult flies reared from human cadaver

1. Flies metallic blue or green (Photo 7A & 7B; Photo 8A & 8C)..... **Calliphoridae**..... 2
- Flies non metallic (Photo 9A & 9B)..... 4
2. Flies not more than 6 mm in length, eyes widely separated both in male and female (Photo 10)..... ***Chrysomya nigripes*** **Aubertin**
- Flies more than 6 mm in length, eyes close together in male, widely separated in female (Photo 7 & 8)..... 3
3. Compound eyes of males with large facets in upper two thirds and smaller ones in lower one third (Photo 7B), prothoracic spiracle brown in both sexes (Photo 7A & 7C)..... ***Chrysomya megacephala*** **(Fabricius)**
- Compound eyes of both sexes with uniformly sized facets of eye (Photo 8B & 8D): prothoracic spiracle white in both sexes (Photo 8A & 8C)..... ***Chrysomya rufifacies*** **(Macquart)**
4. Thorax with three black stripes (Photo 9A); large flies measuring more than 11 mm; male with genital capsule reddish brown (Photo 11A)..... **Sarcophagidae**..... ***Parasarcophaga*** **(*Liopygia*)** ***ruficornis*** **(Fabricius)**
- Thorax with four black stripes (Photo 9B); smaller flies not measuring not more than 8 mm; male with genital capsule dark brown..... **Muscidae**

Photo 1: Larvae of *Chrysomya megacephala* (Fabricius). 1st Instar (A), 2nd Instar (B), 3rd Instar (C) larvae, lateral view.

Photo 2: Larvae of *Chrysomya rufifacies* (Macquart). 1st Instar (A), 2nd Instar (B), 3rd Instar (C) larvae, lateral view Please note the projecting processes (B and C)

Photo 3: Larvae of *Parasarcophaga ruficornis* (Fabricius). 1st Instar (A), 2nd Instar (B), 3rd Instar (C) larvae, lateral view. Note dark brown dorsal abdominal sclerites (C).

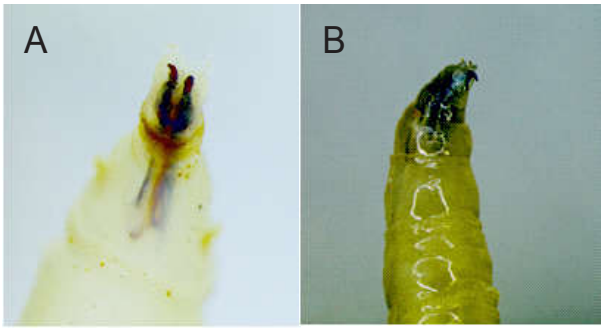


Photo 4: Larvae of Sarcophagidae and Muscidae. A - Sarcophagid larvae, showing two mandibular hooks; B – Muscid larvae, showing one mandibular hook

Photo 5: Caudal spiracles of *Parasarcophaga ruficornis* (Fabricius) in shallow depression.

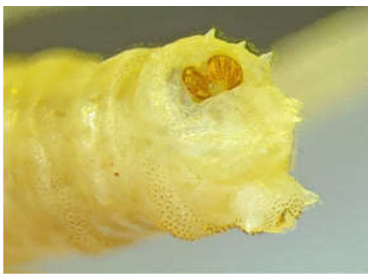


Photo 6: Caudal spiracles of *Chrysomya megacephala* (Fabricius) in deep cavity

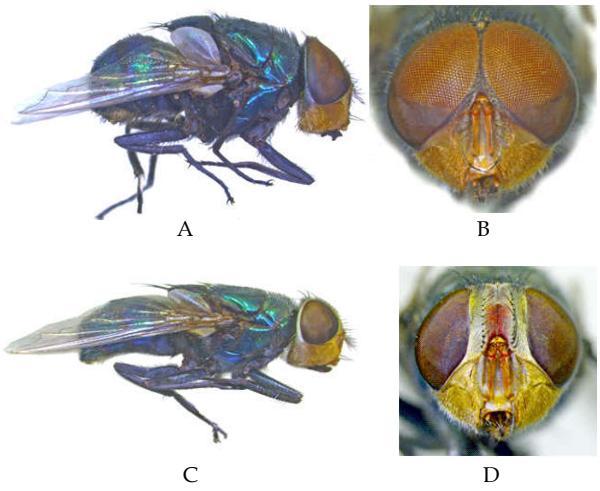


Photo 7: Adult flies of *Chrysomya megacephala* (Fabricius). A - Male, lateral view; B - Male face, front view showing eyes close together and large sized facets on upper region and smaller sized facets on lower region; C – Female, lateral view; D - Female face, front view showing widely separated eyes with uniformly sized facets.

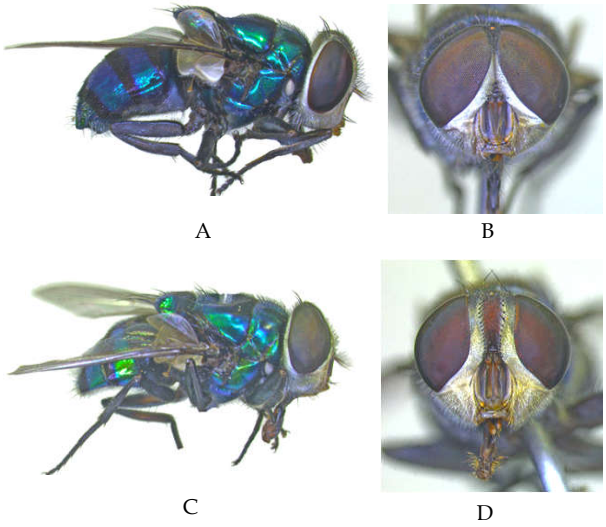


Photo 8: Adult flies of *Chrysomya rufifacies* (Macquart). A - Male, lateral view; B – Male face, front view showing eyes close together with similar sized facets; C – Female, lateral view; D – Female face, front view showing widely separated eyes.

Photo 9: Sarcophagid and Muscid flies. A – Sarcophagidae, *Parasarcophaga ruficornis* (Fabricius), dorsal view, showing three black stripes on mesothorax; B – Muscidae, dorsal view, showing four black stripes on mesothorax

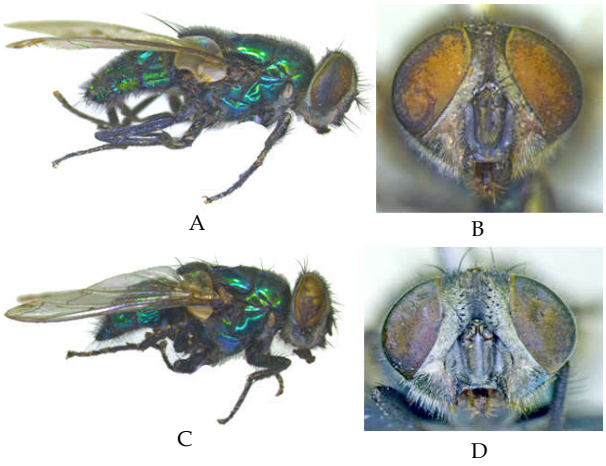


Photo 10: Adult flies of *Chrysomya nigripes* Aubertin.
A - Male, lateral view; B – Male face, front view showing eyes much closer to each other compared to female (see D); C – Female, lateral view; D – Female face, front view.

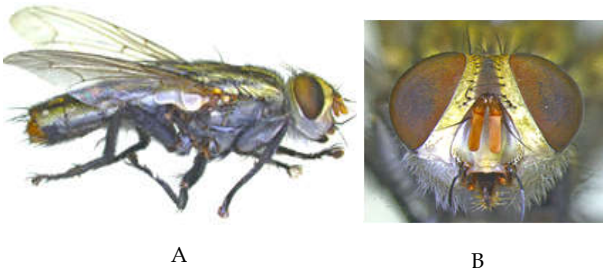


Photo 11: Adult fly and larval caudal spiracles of *Parasarcophaga ruficornis* (Fabricius).
A - Male, lateral view; B – Face, front view; C – Caudal spiracles of larva

Conclusion

Among the five species of flies recorded on human cadaver in this study, *Chrysomya megacephala* (Fabricus) and *Chrysomya rufifacies* (Macquart) are the most common and dominant species infesting throughout the year. *Chrysomya nigripes* Aubertin and species of Muscidae were the least common.

Parasarcophaga ruficornis (Fabricius) was prevalent mostly indoors.

Acknowledgement

1. Dr Viraktha math, Dr Yashwanth, all staff members, Dept of Entomology, GKVK, Bangalore
2. Staff members & Post graduates of department of Forensic Medicine & toxicology, BMC&RI, Bangalore

Conflict of Interest – NIL

Source of Funding – NIL

Ethical Clearence – Taken

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Original Article
Received on 22.08.2016, **Accepted on** 02.09.2016

Indian Journal of Forensic Medicine and Pathology
Volume 9 Number 4, October - December 2016
DOI: http://dx.doi.org/10.21088/ijfmp.0974.3383.9416.3

Profile of Medico Legal Cases in Hamdard Institute of Medical Institute and Associate Hospital New Delhi

P.C. Dikshit*, Amit Sharma, Shahina*****

Abstract

The causality department plays a vital role in treating in emergencies as well as in handling of legal implications of the cases. Common medico legal cases include alleged cases of assault, road traffic accidents, burns, poisoning, industrial accidents etc. In present study 925 medico-legal cases were reported to the causality department of Hamdard institute of medical science New Delhi during the period from 1st January 2014 to 31 December 2014. Study shows maximum number of cases were of physical assault (46.05%) followed by road traffic accidents (27.56%) and poisoning (8.97%) with over all male predominance (67.46%) and most vulnerable age group being 21-30yeas (39.89%) followed by 31-40 years (22.38%). These can be prevented by strict enforcement of law and order, proper education and awareness of road safety measures. Training of doctors who are involved in handling of medico legal cases is needed.

Keywords: Medico Legal Cases; Casualty; Incidence.

Introduction

Medico-legal case is a case of injury/illness where the attending doctor, after eliciting history and examine the patient, thinks that some investigation by law enforcement agencies is essential to establish and fix responsibility for the case in accordance with the law of the land [1,2]. Common medico-legal cases include alleged cases of assault, road traffic accidents, burns, poisoning, snake bite, insect bite, industrial accidents, alcohol intoxication etc. Medico-legal cases are an integral part of medical practice in emergency departments of major hospitals [3]. Profiling of medico-legal cases is an integral aspect for the prevention of preventable causalities in future and study the crime rate and pattern of crimes in that area.

Objectives

1. To study the Pattern of medico legal cases.
2. To study the epidemiology of such cases.

Authors Affiliation: *Professor & Head, **Associate Professor, ***Senior Resident, Department of Forensic Medicine, Hamdard Institute of Medical Sciences & Research, Jamia Hamdard, New Delhi-110062..

Reprints Requests: Amit Sharma, Associate Professor, Department of Forensic Medicine, Hamdard Institute of Medical Sciences & Research, Jamia Hamdard, New Delhi-110062.
E-mail: dr_amitsharma@hotmail.com

Method

The present study was retrospect analysis of all Medico-legal cases came to casualty department of Hamdard medical institute of medical science and research, associated hospital, New Delhi from 1st January 2014 to 31 December 2014. A total of 925 cases were included in the study and the cases with no medico-legal prospective were excluded from the study. Information regarding gender, age, demography and manner of causation was collected from the medico-legal register. The collected data was analysed, observations discussed and compared with other studies.

Observations and Results

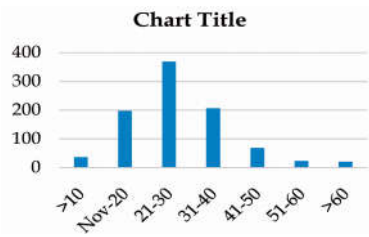

Chart 1: Age wise distribution of cases

Table 1: Age wise distribution of cases

S. No.	Age in years	No. of cases	Percentage (%)
1.	1-10	37	4
2.	11-20	198	21.41
3.	21-30	369	39.89
4.	31-40	207	22.38
5.	41-50	69	7.46
6.	51-60	24	2.59
7.	>60	21	2.27

Table 1 shows that maximum number of cases belongs to the age group of 21- 30 years followed by 31-40 yeas.

Table 2: Sex wise distribution of cases

S. No.	Sex	No. of cases	Percentage
1.	Males	624	67.46
2.	females	301	32.54

Table 2 shows the overall male predominance

Table 3: Pattern of cases

S. No.	Pattern of cases	No. of cases	Percentage
1.	Physical assault	426	46.05
2.	Rta	255	27.56
3.	poisoning	83	8.97
4.	unknown	31	3.35
5.	Hanging	30	3.24
6.	Fall from height	30	3.24
7	Self inflicted	17	1.84
8.	Fall of heavy object	16	1.73
9.	Electrocution	15	1.62
10.	Domestic violence	11	1.18
11.	Self fall	5	0.54
12.	Burn	3	0.32
13.	Sexual assault	2	0.22
14.	Drowning	1	0.11

Table 3 shows that physical assault cases account for maximum number of MLC cases, followed by road traffic accidents and poisoning

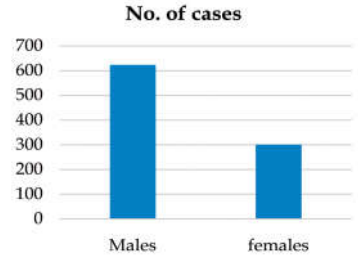

Chart 2: Sex wise distribution of cases

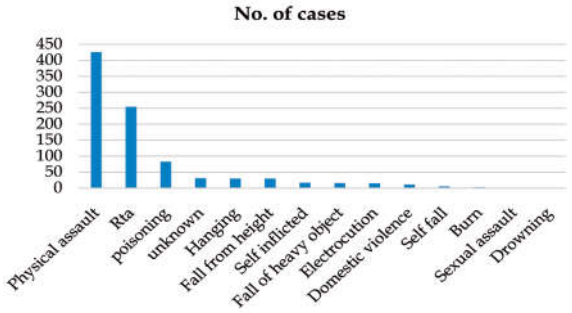

Chart 3: Pattern of cases

Discussion

In present study 925 cases were reported to the casualty department of Hamdard Institute of medical college and associated hospital New Delhi. During the period from 1st January 2014 to 31st December 2014. Study showed maximum number of cases were males (67.46%) as compared to females (32.54%). Similar finding were reported by Malik et al [4], Garge et al [5] and Dileep et al [3] this is to the fact that males are more involved in outdoor activities and ambulatory compared tofemales, so this makes them more vulnerable to accidents or injuries.

In this study majority of cases were from age group between 21-30years (39.89%) followed by 31-40 years (22.38%. similar finding were also reported by Garg et al [5], Sahadev et al [6], Harish et al [7], Sharma et al [8], Timsinha et al [9], Yadav et al [10] and Dileep et al [3]. As individuals of these age groups lead more active life, involve in outdoor activities, sports and recreation activities, there by exposing themselves to environmental factors.

In our study maximum number of medico-legal cases were due to were due to physical assault followed by road traffic accidents, poisoning. This is inconsistent with the studies conducted by Garg et al [5], Harish et al [7] and Dileep et al [3], in which road traffic accidents was most common cause followed by assault, mechanical injuries and poisoning.

In our study 3.35% cases were brought dead and or unconscious state and history given was insufficient to reveal the cause or manner of injury. In these cases even after the general examination, the cause and manner of death remained obscured.

Conclusion

The casualty department of any hospital not only caters to the needs of patients who reports in emergencies but also carry out legal responsibilities to examine, document and certify medico legal cases. This puts a lot of burden on causality department and on first contact doctor. The doctor those are involved in handling medico-legal cases need to be more trained.

Most of the time first contact doctor in causality is MBBS only. They are not experts in handling medico-legal cases so there is a needto increase time in practical training of the students during MBBS in the curriculum. The 15days posting under forensic medicine department during internship should be

mandatory for better exposure to medico-legal cases. Also, due to increase in violence and accidents, the need for round the clock availability of medico-legal experts is the need of the hour. This will provide great help to the law enforcing agencies, will safeguard the hospital from wrong or poor documentation and also will share the burden of work of causality doctors as documentation and certification of medico legal cases is a responsible and hectic job.

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Original Article
Received on 11.08.2016, **Accepted on** 22.08.2016

Indian Journal of Forensic Medicine and Pathology
Volume 9 Number 4, October - December 2016
DOI: http://dx.doi.org/10.21088/ijfmp.0974.3383.9416.4

Deaths in Police Custody: A Retrospective Analysis

Rambarapu Sudha*, B. Lakshmi Prasanna, Nishat Ahmed Sheikh*****

Abstract

Background: As per the Oxford dictionary custody means "protective care or guardianship of someone or something". In the legal parlance Custody is defined as any point in time when a person's freedom of movement has been denied by law enforcement agencies, such as during transport prior to booking, or during arrest, prosecution, sentencing, and correctional confinement. *Study design:* It's a Cross sectional retrospective analysis. *Material and Method:* Retrospective analysis of 90 cases of custodial deaths that have occurred in various Police custody of Andhra Pradesh. The cases collected for the study were over a period of more than two decade. Records pertaining to 90 cases could be gathered from various sources. Permissions and consents were procured before the study and clearance from the Institutional Ethical committee was obtained in advance. *Observation and Discussion:* Total 90 cases of custodial death were analyzed. Out of these, 89 were males and 1 was female. Age group of these cases were between 21 to 60 years, maximum cases (37 cases) being between 21 to 30 years and 34 cases in the age group of 31 to 40 years, whereas 11 cases in between 41-50 years age group. Out of 90 deaths, 19 were natural death of the unnatural deaths, 38 were suicides, 9 accidents, 30 were due to injuries. Among suicides the commonest mode of suicide is hanging. Out of 38 suicides, 28 cases were hanging, 5 cases of poisoning, 3 cases were of drowning. Death occurring in early age is of great concern and highlights the importance of effective implementation of screening and diagnostic program. Studies have concluded that natural and suicidal cases are more common in custody than accidental, homicidal or torture. *Conclusion:* Measures should be taken to provide a safe environment at the time of interrogation in police custody, following of code of conduct by the police. There is also a need for proper reform to avoid deaths due to suicide, violence and self-harm among the inmates. There is a need to have constant surveillance over them and install Cameras to supervise their activities to prevent violence and suicide.

Keywords: Police Custody Deaths; Torture; Victims; Suicide.

Introduction

Healthcare and happiness is a right of every person regardless of a profession and conditions of living. Slaves and prisoners have equal rights of health as other people in the community. It is the duty of state to provide good quality and cost effective health facility to everyone [1]. "Society can be measured by the way prisoners are treated." Winston Churchill. The motto of the National Human Rights Commission is "*Sarve Bhavantu Sukhinah*". Happiness and health for all is sought to be achieved through a rights-based regime where respect for human beings and their dignity is cardinal. President's assent to the Protection of Human Rights Act was a major breakthrough in this direction. Section 3 of the Act provides for the setting up of the National Human Rights Commission (NHRC) and Section 21 provides for the setting up of various States Commissions (SHRC) [2].

As per the Oxford dictionary custody means

Authors Affiliation: *Associate Professor, Dept. of Forensic Medicine, Osmania Medical College, Hyderabad Telangana State. **Professor, Dept. of Forensic Medicine, Kamineni Academy of Medical Sciences and Research Center, L. B. Nagar, Hyderabad. ***Professor, Dept. of Forensic Medicine, People's College of Medical Sciences and Research Center, Bhopal.

Reprints Requests: Nishat Ahmed Sheikh, Professor, Dept. of Forensic Medicine, People's college of Medical sciences & Research Centre, People's University. Bhanpur, Bhopal-462037, India.
E-mail: drnishatsheikh@gmail.com

"protective care or guardianship of someone or something". In the legal parlance Custody is defined as any point in time when a person's freedom of movement has been denied by law enforcement agencies, such as during transport prior to booking, or during arrest, prosecution, sentencing, and correctional confinement [3]. According to the report of the Asian Centre for Human Rights (ACHR), "Torture in India 2011", the National Human Rights Commission (NHRC) recorded a total of 14,231 deaths in custody in India between 2001 and 2010, which includes about 1,504 deaths in police custody and about 12,727 deaths in judicial custody. The ACHR report observes that these are only the cases reported to the NHRC, and do not include all cases of custodial deaths. The report attributes the deaths in custody to torture, denial of medical facilities and inhuman prison conditions [3].

As per the NHRC guidelines, all custodial deaths are to be reported within 24 hrs and post-mortem examination is to be conducted by a panel of doctors & videography has been made mandatory. NHRC Report from 2001 -02 to 2006-7 showed an increase in custodial deaths all over India [4]. Many of these deaths are premature deaths and can be prevented with proper care and treatment. In addition, having knowledge and data regarding such deaths is important to focus attention on medical services and can facilitate the implementation of preventive programs. Such studies would also guide the authorities in setting priorities for the allocation of their healthcare services [5].

Material and Method

This is a retrospective analysis of 90 cases of custodial deaths that have occurred in various Police custody of Andhra Pradesh. The cases collected for the study were over a period of more than two decade. Records pertaining to 90 cases gathered from various sources like Legal Cell of DGP's Office, from the reports of various Commissioners of Inquiries which were available in the library of AP Legislative Assembly as well relevant information was gathered from post-mortem reports and medical record files. An attempt was made to study and present 90 cases with regards to the circumstances of death, various causes of death, nature of death and lapses on the part of the doctors and various suggestions put forward in the judicial Commission to prevent recurrence of such deaths. The post-mortem examination of these cases was conducted in the mortuary of the institute as per the guidelines laid out by National Human Rights Commission. Causes of death were categorized under natural (disease process) and unnatural (suicides/accidents/homicides). Sufficient permissions and consents were procured before the study and clearance from the Institutional Ethical committee was obtained in advance.

Exclusion Criterion

Deaths in Judicial Custody, Mental Hospitals and encounter death were excluded from the study.

Observation and Discussion

In legal parlance custody is defined as any point in time when a person's freedom of movement has been denied by law enforcement agencies such as during transport prior to booking or during arrest, prosecution, sentencing and correctional confinement [6]. However the legal authorities are bound by the law to provide adequate necessary amenities to ensure the health and safety of persons in their custody, including timely medical assistance, and treating the inmates in a humane manner. The persons held in custody retain their basic constitutional right except for their right to liberty and a qualified right to privacy [7].

The person who is held in custody is totally dependent on his or her custodian for proper care and enough medical attention [8]. The custodians are bound by the law to provide adequate necessary amenities to ensure the health and safety of persons in their custody, including medical assistance and treating the inmates in a humane manner [6].

As per Figure 1, a total of 90 cases of custodial death were analyzed. Out of these, 89 were males and 1 was female. The findings are consistent with the other author studies.

As per Figure 2 Age group of these cases were between 21 to 60 years, maximum cases (37 cases) being between 21 to 30 years and 34 cases in the age group of 31 to 40 years, whereas 11 cases in between 41-50 years age group. No cases were found below the age of 20 or more than 60 years. Death occurring in early age is of great concern and highlights the importance of effective implementation of screening and diagnostic program. Routine enquiry about presence of any disease or taking treatment for any aliment will solve the problems in many cases. Similarly the elder prisoners should undergo routine evaluation and necessary treatment should be provided.

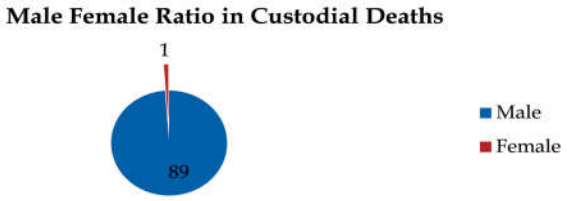

Fig. 1: Showing gender ratio in Custodial Deaths

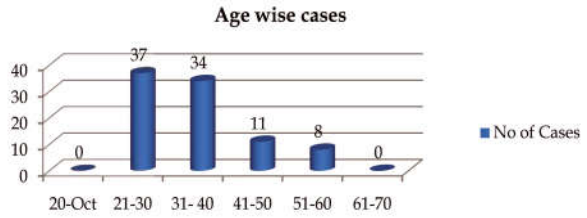

Fig. 2: Age wise no of custodial deaths cases

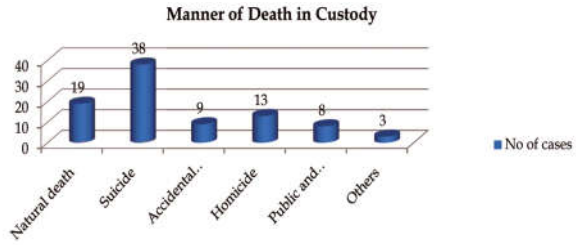

Fig. 3: Showing Manner of Death in Custody

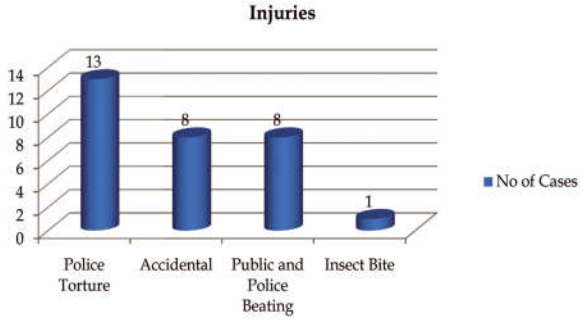

Fig. 4: Showing Deaths due to Injuries

Out of 90 deaths, 19 were natural death of the unnatural deaths, 38 were suicides, 9 accidents (including one insect bite), 30 were due to injuries. Out of 30 injuries 9 were due to accidental injuries, 13 were homicidal torture by the police, 8 cases were beaten by public and handed over to the police and again beaten by police. The causes of accidental deaths were as a result of deceased trying to escape from custody like jumping from a moving vehicle or while resisting arrest etc. Most of the deaths occur within a day or two of being apprehended. Most of the homicide cases were due to blunt trauma like beating, kicking, canning, pushing etc. According to the studies deaths in custody are not always un-natural, as opposed to general belief, but due to various cause ranging from natural diseases, intoxication, accidents and self-destructive behavior of the inmate to the tortures on the hand of authorities and/or fellow inmates [9,10]. Some of these studies have concluded that natural and suicidal cases are more common in custody than accidental, homicidal or torture [11], while other studies shown un-natural deaths to be more common [12].

Out of the 90 cases of custodial deaths 30 cases had injuries, 13 cases injuries were due to police torture, 8 cases reported to have injuries due to accident, whereas 8 cases injuries received due to public beating and again police torture, in one of the case the injuries sustained was due to insect bite.

Among suicides the commonest mode of suicide is hanging. Out of 38 suicides, 28 cases were hanging, 5 cases of poisoning, 3 cases were of drowning and other mode of suicides like self inflicted injuries (1) etc. Suicide in prison causes an enormous degree of distress to other prisoners, prison staff and of course, to the inmate's family and friends outside. Indeed, it is sometimes regarded as a testament to the failure of our penal institutions to fulfill their obligation to provide offenders with a humane and safe environment during the period of their incarceration [13]. In the custodial deaths, the deaths in prison outnumbered the death in police custody. The death in prison was natural in almost 85% cases and unnatural in 15% cases. Moreover all suicides in the custodial death occurred in the police cell [14]. Thus the suicide in prison was uncommon in India, which is in sharp contrast to that seen in developed countries. In Australia, almost 50% of all prison deaths were as a result of inmate suicide with hanging as the most common method [13]. Suicide is documented as the leading cause of death in prison in Canada [15], and in Britain [16] with hanging as the most common method. Moreover, Suicide in prison is much more common than suicide in community [15]. However, both England and US reports have noted the relative infrequency of suicide in special security hospitals [16,17]. The increase in custodial death is mainly due to increase in the number of suicide in police custody. The suicide in custody is worrisome and suggests lack of preventive effort by the authorities. As per the guidelines of NHRC, the government and the concerned authorities are taking all necessary precautions to prevent custodial death [18]. However, the prisoner who wants to commits suicide finds one or other new ways to end their life. So the concerned authorities have made various other stringent measures at police lockup and prison to prevent death in custody

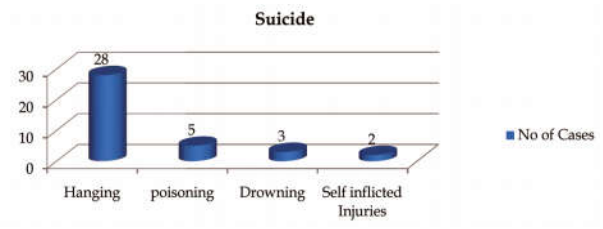

Fig. 5: Suicides in custody. Suicide: n=38

Suggestion and precautions for the medical experts in Custodial Death cases

- Case should be dealt with all equipped, well trained and well experienced experts.
- Model autopsy protocol should be followed to ensure a systematic and comprehensive examination to prevent the commission or lack of important details.
- Independent consultant must review the findings and opinion of the autopsy report.
- Autopsy should be thorough to be meaningful and conclusive.
- The medical investigator should leave access to the scene where the body is found.
- There should be coordination between the medical and non-medical investigator.
- The inquest findings should be consistent with the autopsy findings
- Adequate photographs are crucial for thorough documentation of autopsy findings and must demonstrate all injuries commented in the postmortem report.
- All skeletal injuries should be documented by X-rays.
- Keep records of all specimens saved, preserved all evidence and record the chain of custody.
- Perform appropriate toxicological, microscopic and bacterial tests and portions of tested samples retained to permit retesting.
- Circumstances of cases should be carefully evaluated before giving the opinion regarding the cause of death.
- A careful evaluation of the trauma should be done to determine whether the injuries sustained were before or after the arrest, and also to decide whether the injuries are accidental or self inflicted or due to police beating or homicidal.
- Opinion should be based on scientific facts and cause and manner of death should be determined with accuracy.
- Doctors should not refuse to conduct the postmortem examination.
- Proper preservation and early postmortem examination is necessary to minimize artefacts which could lead to error in interpretation of the findings.
- When death occurs in custody it should be autopsied in the presence of representatives of the family.
- Body should be preserved in cold storage in cases of delay in conducting the postmortem examination.
- The doctors conducting postmortem examination should come to a conclusion about the cause of death immediately and should avoid vague reporting like brain edema, lung edema, etc.
- Any pattern or practice that may have brought about the death should be noted to distinguish between natural, accidental or homicidal nature of deaths to aid in prosecution of the responsible.

Conclusion

Custodial deaths are among the most difficult and contentious deaths for investigation. The inmates in custody are marginalized populations that have poor access to healthcare in the community. The Magistrate inquest is conducted for all deaths in custody and is the only means of inquiry available to obtain information. Unfortunately, the inquiry reels around the cause of death and nothing substantial surfaces out regarding preventive aspect. The Article 21 of the Constitution of India enshrines the fact that no person shall be deprived of his life and personal liberty except according to the procedure established by the law.

Torture should be avoided under all circumstances. The government should take the responsibility to take legal, social, medical and psychological need of the victim of police violence and their families while the investigation is ongoing. Developing good practice standards on training; reviewing recommendations from NHRC, and monitoring progress in their implementation are some of the steps in a positive direction. Measures should be taken to provide a safe environment at the time of interrogation in police custody, following of code of conduct by the police.

There is also a need for proper reform to avoid deaths due to suicide, violence and self-harm among

the inmates. There is a need to have constant surveillance over them and install Cameras to supervise their activities to prevent violence and suicide.

Acknowledgement

Authors acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript. The authors are also grateful to authors /editors /publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

Conflict of Interest

The author declares no conflict of interest in the present study

Author Disclosures

Authors have no conflict of interest. This study was a part of departmental research activities of Forensic Medicine at Osmania Medical College, Hyderabad Telangana State.

Ethical Consideration

Clearance from the Institutional Ethical committee was obtained in advance.

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Original Article
Received on 16.09.2016, **Accepted on** 24.09.2016

Indian Journal of Forensic Medicine and Pathology
Volume 9 Number 4, October - December 2016
DOI: http://dx.doi.org/10.21088/ijfmp.0974.3383.9416.5

Clinical and Microbiological Profile of Chronic Non-Healing Ulcers in a Tertiary Care Teaching Hospital in North India

Shruti Barnwal*, Ravi Kant**

Abstract

Non-healing ulcers are an important cause of morbidity to the patients .Most of the patients present with chronic non healing ulcers over lower one third of the legs .However certain patients with peripheral arterial disease any have ulcer at any level below the arterial occlusion.Patients with diabetes are at greater risk for the non-healing ulcers due to peripheral neuropathy and and vascular compromise .The some times get secondarily infected which makes the condition more complicated for treatmen

Keywords: Non Healing Ulcers; Diabetes Mellitus; Vascular; Infection.

Introduction

Wound healing is a complex process that involves induction of an acute inflammatory process. The various steps responsible for healing are, regeneration of parenchymal cells, migration and proliferation of both parenchymal and connective tissue cells, synthesis of ECM proteins, remodeling of connective tissue and parenchymal components, also collagenization and acquisition of wound strength.

These phases are affected by various factors e.g.nutritional status, age, vascularity, endocrinal and neurological factors. Sometimes, the wound healing process does not proceed normally and chronic wound results.

Non-healing ulcers of foot are very common and represent a serious health problem. Foot ulcers in patients with diabetes, cause a major medical and economic problem. This is the leading cause of hospitalization for patients with diabetes mellitus[1]. As per the expectation the diabetic population is increasing so is the problem of diabetic foot ulcer. Presence of bacteria in the ulcers affects and delays the healing process. It also complicates the pathological picture of ulcers .Most of the times ulcers are generally treated empirically, but a directed therapy with a known causative organism can improve the outcome [2]. Diabetes mellitus is not the only cause of non-healing ulcers. There are some other conditions like peripheral vascular disease, peripheral neuropathy, coronary artery disease, paraplegia etc [3].

There are very few studies wherein the etiology of non-healing ulcers from non-diabetic and diabetic patients are studied.

Aim and Objective

Aim of the study was to asses the clinical and microbiological profile of the chronic non healing ulcer and the predisposing conditions in patients attending the out patient department of dermatology at govt doon medical college dehradun, India.

Material and Methods

It was a retrospective study. A total of 54 patients were enrolled from March 2015 to April 2016 who qualified for the inclusion critreria. Each patient was assessed clinically and was subjected to a structured proforma that included the history of wound ,its onset, duration, discharge any medication received by the patient etc. A detailed clinical examination of the wound was performed viz. type of wound its size, edge, floor, discharge, base and bleeding, relationship to the deeper structure, lymph nodes

Authors Affiliation: *Assistant Professor, Dermatology, Govt. Doon Medical College, Dehradun, Uttarakhand. **Associate Professor, Medicine, AIIMS Rishikesh, Dehradun, Uttarakhand.

Reprints Requests: Shruti Barnwal, Assistant Professor, Dermatology, Govt. Doon Medical College, Dehradun, Uttarakhand 248001
E-mail: drshruti2006@gmail.com

etc.All patients were assessed for pheripheral arterial and venous insufficiency.Wound swab culture was taken from the base of the ulcer and was sent for culture and sensitivity as per standard protocol.

Inclusion Criteria
Age >20 years and <60 years
Ulcers lasting more than 6 weeks
Wagner class 0,1,2

Exclusion Criteria
Trauma
Surgical interventions
Age <20 years and > 60 years
Wagner class more than 2

Results

In the present study the sex distribution was almost equal (Table 1).

So far as the duration of wound is concerned maximum patients (48%) were having duration between 7 to 11 months (Table 2). Diabetes remains the leading cause of non healing ulcers amongst males and females (Table 3). Mild anemia was noted in in more than 50% patients (Table 4) and total leucocyte count was normal in majority of patients (Table 5), seropurulent discharge was present in 57% patients (Table 6). Hypoalbuminemia was found in 48% cases with majority of the patients with normal total protein level (Table 7). All the patients were subjected to culture and sensitivity. Out of total 54 samples, growth was obtained in 46 samples (86%). Staphylococcus aureus dominated the isolated organisms, klebsiella was the least grown organism (Table 8).

Discussion

The intention of this study was find out the clinical and microbiological profile in chronic non healing ulcers. Most of the non healing ulcers were more than 6months old. Although there are studies that suggests the male preponderance [4], this was not our finding, majority of the patients were in the age range of 41-50 years. Diabetic foot infections are classified on the basis of severity by Wagner [5]. In our study total of 46 bacterial isolates were obtained. Majority of them were staphylococcus (38%) as found in other studies [6]. Among the gram negative Isolates proteous mirabilis outnumbered the other gram negative aerobic bacteria. There was no anaerobic bacteria isolated from the wound. This is because we have excluded the patients with deeper wounds. We have included only Wagner grades 0, I and II patients and not any grades above that.Study by Anandi et al [7] as well reports absence of anaerobic organisms from diabetic foot ulcers of Wagner grades 0 and I.Our results do agree with that of Anadi etal. Kavitha et al [8] have reported gram negative bacilli from 52.31% and Tiwari et al [9] have reported the same from 78% cases. In our study the incidence of gram negative infection is almost 62%.

Conclusion

From the present study we can conclude the clinico-etiology of non-healing ulcers in different patients groups. Diabetes is the most common predisposing disease and Staphylococcus aureus is the predominant causative organism.

Table 1: Showing age and sex distribution

Age & Sex Distribution				
Age	**Male**	**Female**	**Total**	**Percentage%**
20 – 30	4	3	7	12.96
31 – 40	9	7	16	29.62
41 – 50	11	9	20	37.03
51 - 60	6	5	11	20.37
Total	30	24	54	100

Table 2: Showing duration of wound

Duration of wound				
Duration(Months)	**Male**	**Female**	**Total**	**Percentage%**
2-6	3	2	5	9.25
7-11	13	13	26	48.14
12-16	7	5	12	22.22
17-21	7	4	11	20.37
Total	30	24	54	100

Table 3: Showing etiology of wound

	Etiology of Wound			
	Male	**Female**	**Total**	**Percentage%**
Diabetic	13	7	20	37.04
PVD	8	5	13	24.07
Vasculitic	5	8	13	24.07
Leprotic	4	4	8	14.81
Total	30	24	54	100

Table 4: Showing the hemoglobin levels

HB level (gm%)	**Male**	**Female**	**Total**	**Percentage%**
<9	7	5	12	22.22
09-11	15	13	28	51.85
12-15	8	6	14	25.92
Total	30	24	54	100

Table 5: Showing discharge from the wound

	Discharge of Ulcer			
	Male	**Female**	**Total**	**Percentage%**
Serous	7	12	19	35.18
seropurulent	20	11	31	57.4
serosangiuneous	3	1	4	7.4
Total	30	24	54	100

Table 6: Showing the total leucocyte count

TLC	**Male**	**Female**	**Total**	**Percentage%**
<4000	3	1	4	8
4000-11000	18	13	31	62
>11000	9	6	15	30
Total	30	20	50	100

Table 7: Showing total protien levels

	Total Protein Level			
Protein (gm %)	**Male**	**Female**	**Total**	**Percentage%**
<5.5	12	11	23	42.59
5.5-8	15	13	28	51.85
5.8	3	0	3	5.5
Albumin				
<3.5	17	9	26	48.14
3.5-5.5	11	11	22	40.74
>5.5	2	4	6	11.11
Globulin				
<2	11	9	20	37.03
2-3.5	18	9	27	50
>3.5	1	6	7	12.96

Table 8: Organisms isolated from the wound

Bacterial isolates	**Diabetic Group**	**Non-diabetic Group**
Staphylococcus aureus	**13(28%)**	**0 5 (10.8%)**
Proteus sp	7 (15%)	03(6.5%)
E.coli	5(10.8%)	02 (4.3%)
Pseudomonas aeruginosa	5(10.8%)	02(4.3%)
Klebsiella sp	03 (6.5%)	01 (2.17%)

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Review Article
Received on 31.10.2016, **Accepted on** 08.11.2016

Indian Journal of Forensic Medicine and Pathology
Volume 9 Number 4, October - December 2016
DOI: http://dx.doi.org/10.21088/ijfmp.0974.3383.9416.6

Between Eye Difference in Vitreous Electrolytes of Same individual for Identical Time Since Death

Tatiya Harish S.*, Taware Ajay A., Jadhav Vijay T.***

Abstract

Estimation of Time Since Death (TSD) is very important in routine forensic practice. Various techniques and parameters are utilised for determination of this TSD. One of the most recently and vastly studied method of determination of TSD is with the help of vitreous electrolyte concentration analysis. Recently workers have reported a significant between eye differences for same individual at identical TSD. This has doubted the use of vitreous electrolyte analysis in TSD determination. The review of literature highlights various causes behind this fact and need of more research in this topic as discussed in this article.

Keywords: Vitreous Electrolytes; Time Since Death; Between Eye Differences; Sampling Errors; Analytical Errors.

Introduction

In any postmortem examination determination of time since death i.e. the interval between death and time of examination of body is an important issue [1].

However no person knows exact time of his or her departure from this beautiful earth and no medical professional can tell the exact time since death of a person if he or she has not attended the patient at the time of his or her last breath [2].

The estimation of time since death is undoubtedly one of the most significant research in forensic medicine and yet it is still considered as to be most controversial and inaccurate one [3].

Repeated experience have taught the investigators that they should not rely on any single observation for estimating the time of death and also should wisely avoid to make any confident statements based on such single observations [4].

From the second half of 18th century the forensic experts across the globe started using various methods in combinations to estimate time since death. These methods included observations such as cooling of body, changes in eye, post mortem lividity, rigor mortis, signs of decomposition, contents of stomach and bowels, contents of urinary bladder and circumstantial evidences. Though these widely practiced methods give useful information regarding time since death, their range is too wide on most of the times. Hence attention of the researchers has now been drawn towards various biochemical parameters which can be used to narrow down the duration to be opined [5].

It is known that many of chemical changes start in the body immediately or shortly after death. It has also been observed that these changes progress in an orderly fashion till the disintegration of body. Changes in chemical constituents have its own time factor or rate of change. These changes occur especially in body fluids like blood, spinal fluid and vitreous humour of eye. Thus it was hypothesized and later confirmed that determination of the chemical quantity could help forensic pathologists to ascertain time since death more precisely [6].

These chemical changes have been largely studied in last few decades. Body fluids available for such chemical examination are whole blood, serum, CSF,

Authors Affiliation: *Assistant Professor, **Associate Professor, Department of Forensic Medicine and Toxicology, B J Govt Medical College and Sassoon General Hospitals, Pune, Maharashtra, India.

Reprints Requests: Tatiya Harish S., Assistant Professor, Department of Forensic Medicine and Toxicology, B J Govt Medical College and Sassoon General Hospitals, Pune-411001, Maharashtra, India.
E-mail: hstatiyakhalan7@gmail.com

aqueous humour and vitreous humour. Amongst all these body fluids the most widely studied and used method is estimation of vitreous humour potassium concentration [7].

Though no single measurement can give a complete and reliable estimate regarding the time since death, combinations of chemical determinations along with classical methods can be used as a helpful adjunct in cases of unwitnessed deaths[8].

As compared to other body fluids, vitreous humour of eye is stable and less susceptible to rapid chemical changes and contamination. It is also easily accessible and its composition matches a lot to that of aqueous fluid, cerebrospinal fluid and serum. Hence it is suitable for many analyses to estimate time since death [9].

For more than a quarter of a century, determination of time since death by evaluating vitreous biochemistry has been the subject of research in forensic pathology. Various studies performed across the globe to date have hypothesized a variety of linear or piecewise-linear relationships between vitreous humour potassium concentrations and time since death. There is a lack of agreement on different estimated intercepts and slopes of regression lines and also the different reliabilities of these estimates and this is due to the variable number of cases reported from study to study, difference in observed ranges of vitreous potassium and time since death and the un accommodated effects of factors including age of subject, amount of urea nitrogen, ambient temperature and presence of illness at the time of death on potassium concentration [10].

The accurate estimation of time since death carries great value in medico legal investigations of serious crimes. Hence several workers have studied and reported that the accurate prediction of time since death i.e. even within two hours, can be possibly made from vitreous humour potassium [11].

However few researchers have found a significant difference between the values of vitreous electrolyte concentration obtained from two eyes of same individual for identical postmortem interval. Current article is to highlight the work of those researchers and reasons for the same.

Vitreous Humor Between-Eye Differences or Differences between Vitreous Humour Biochemistry of Two Eyes of Same Individual for identical TSD.

The various factors may have a key role in the various disagreements regarding the utility of vitreous humor in TSD estimations. However, perhaps the most important concern in utilizing vitreous biochemistry for crucial forensic pathology determinations arises from the observed between eye differences in the same pair of eyes at identical TSD. Many researchers have assumed that the vitreous biochemical concentrations are identical and postmortem changes occur at the same rate in both the eyes. Recent observations have indicated that these presumptions may not be entirely true and between eyes differences at the same TSD have been documented. If these differences were to exist, it would grossly undermine the value of vitreous biochemistry in various forensic pathology applications.

Some early studies had reported that vitreous samples obtained from the same pair of eyes had near-identical biochemical values for the two eyes like Adelson et al [4], Sturner et al [12], Hughes[13], Lie [14] and Coe[15]. These investigators, however, did not provide the data or their statistical interpretation.

Key Messages

One should not blindly accept that the vitreous electrolytes will be same in two eyes of an individual. There is need to do more and more studies to verify the possibility and causes of between eye differences in vitreous electrolytes of same individual for identical TSD.

Balasooriya et al. [16] reported significant differences in various vitreous biochemical constituents from the same pair of eyes at identical TSD. The authors observed that each of the eyes exhibited independent values and nearly 19% of the results for vitreous potassium varied by more than 10% from the mean of the two values. Out of a total of 59 pairs of eyes, only six pairs had the same potassium concentration. Similar differences were also observed for vitreous sodium where 10% of the results varied by greater than 5% of the mean, vitreous urate where at least 19% of the cases had differences greater than 12% of the mean values of the two eyes.

Madea et al. [17] confirmed these findings and reported deviations up to 10% of the single values of both the eyes in the analysis of potassium, sodium, chloride and calcium. The authors, however, did not observe any such deviations for urea.

Pounder et al. [18] from a later study reported that between eye differences in potassium varied from 0 to 2.34 mmol/L or 0% to 21.8% of the mean. The authors suggested these differences to be significant and erratic, thereby questioning the practical usefulness of vitreous humor in evaluation of TSD. On the contrary, the authors reported that the differences observed for sodium and chloride were

tolerable using their methodology.

Tagliaro et al. [19] explored potassium concentration differences in the vitreous humor of two eyes using micro sampling technique with capillary electrophoresis. The authors reported that no significant differences existed in potassium concentration of the same pair of eyes at identical TSD, thus strengthening the application of vitreous humor as an important tool for TSD estimations.

Garg V. et al [3] studied 200 autopsy cases to find the changes in levels of vitreous humour potassium with time since death. The analyses showed highly significant increase in vitreous potassium with increasing time since death in linear fashion. They also found that when samples from both the eyes were taken at the same time and analyzed separately no significant difference was observed.

Mulla A. et al [20] conducted study to investigate the role of vitreous humour biochemistry in forensic pathology. This study hypothesized that the concentration of vitreous biochemical constituents in the same pair of eyes change at the same rate and this change that occurs in a time dependent fashion may be utilized in accurately estimating the TSD. The results of this study indicated that there were no significant between-eye differences for all of the vitreous biochemical constituents that were studied. The results of the present study suggest that the previously reported between eye differences for various vitreous biochemical constituents in the same pair of eyes are insignificant so far as forensic applications are concerned. Vitreous potassium is a useful biochemical marker for TSD estimations.

Discussion

A possible explanation for the ambiguous reports regarding between-eye differences at similar time since death was said to be either the variations in study methods or sample manipulations before analyses. A major variation was found in the aspiration techniques adopted by various investigators. Bito in 1977 reported that there is difference in concentrations of many solutes of the vitreous humor between anterior and posterior vitreous chambers [21].

According to Coe in 1989 the concentration of vitreous solutes next to the retina differed from the concentration in the central portion of the globe, and hence it is very important to aspirate vitreous humor as completely as possible. Such completely aspirated vitreous humour sample can reflect accurately the concentration levels of all solutes, and serves to eliminate any ambiguity that may occur due to selective vitreous humor aspiration [7].

The aspiration technique used by Balasooriya et al.[16] could highly give distorted values in each eye as they aspirated only the initial 1 mL volume of fluid. It is also interesting to note that, investigators like Coe [15] who removed all the available vitreous humor from both the eyes succeeded in demonstrating near identical concentrations for both eyes.

However in a study by Tagliaro et al. it was found that no statistically significant differences existed for potassium concentrations in the two eyes of the same individual when a micro sampling technique was used for sample collection [19]. The micro sampling technique i.e. aspiration of microliter amounts of fluid, used in their study was different from the technique of complete fluid aspiration employed in the previous studies. Hence it can be proposed that the difference in sampling technique may not be the sole reason that stands for the reported between-eye differences. This is authenticated by two previous studies of Madea et al [17] and Pounder et al[19] who assessed the effect of the sampling technique of the vitreous humor by aspirating the fluid in two installments and found no any significant influence of the sampling technique on the observed between-eye differences.

Hence it can be said that, even though the complete aspiration technique is ideal to give accurate vitreous solute concentration levels, certain other factors may also be responsible for the between-eye differences.

Such other factors may be like the different instrumentation methods used in different studies. It has been suggested that the concentration of vitreous humor constituents will vary with different instruments [22]. It is found that in studies that have proposed significant differences between the same pair of eyes, the method of sample analysis was direct or indirect potentiometry [16,17]. While studies in which near-identical concentration for various vitreous humor solutes were obtained, samples were analyzed by using flame photometry [12,15].

Also it is interesting to find that most of the analytical instruments used in various studies have been used for a clinical range of analysis where compensatory dilution has been an essential procedure in estimating a value for most of the postmortem vitreous humor constituents. Pounder et al. hypothesized that sample dilution prior to analysis can be reason for the between-eye differences in the same pair of eyes, and therefore they suggested measuring the samples undiluted [18]. However,

other studies that have reported no significant between-eye differences for vitreous constituents have performed the required dilutions [12,19].

According to few investigators the long time gap between vitreous-humor sample collection and analysis of the sample may be another responsible factor for the reported between-eye differences in the same pair of eyes. In some studies, the sample was kept frozen at -70°C before biochemical analysis. The improper storage conditions may have impacted the results little bit and it is argued that after indefinite storage at low temperatures, one cannot get accurate results regarding the biochemical concentrations of the vitreous humor [17]. Even though Pounder et al [18] used the technique of immediate analysis post-collection, they reported significant between-eye differences for potassium.

Hence during sampling of vitreous humour two precautions must be taken as

1. If vitreous aspirate is less than 0.5 ml, it may give unrepresentative results; owing to the uneven distribution of potassium within vitreous body [16]. Hence it is necessary to remove whole of the fluid from the eye that can be aspirated because the vitreous humour next to the retina has a highest concentration of solutes than the central portion of the globe until putrefaction sets in [5].
2. Secondly, the vitreous must be aspirated slowly to avoid tearing loose fragments of the tissue [12]. Such tissue fragments grossly distort the electrolytes in the vitreous, since it is from those cells from which most of the electrolytes are derived as mentioned by Coe [15] and Lie[23].

Conclusion

Finally it can be said that vitreous biochemistry, particularly vitreous potassium is useful in time since death estimation; and this utility of vitreous potassium cannot be doubted exclusively on the basis of these reported between eye differences. However more studies are required in future concentrating precisely on this topic.

Acknowledgement

Nil

Conflict of Interest

Nil

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Case Report
Received on 19.09.2016, **Accepted on** 28.10.2016

Indian Journal of Forensic Medicine and Pathology
Volume 9 Number 4, October - December 2016
DOI: http://dx.doi.org/10.21088/ijfmp.0974.3383.9416.7

Accessory Spleen at Autopsy: An Incidental Finding

Venkatesh Maled

Abstract

Spleen is a vascular organ situated in the left upper quadrant of the abdominal cavity. It appears approximately at sixth week of embryonic life. Like any other organ in the body it can display various developmental anomalies like agenesis, polyspleenia, splencunculi etc. Accessary spleen (AS) may be formed during embryonic life as a separated splenic tissue along the path of development. This may be mistaken for neoplasm/secondaries due to neoplasm etc. Apart from this AS has many clinical significance to the surgeon.

Keywords: Accessary Spleen; Autopsy; Congenital Anomalies.

Introduction

Spleen is a lymphatic vascular organ situated in the upper left quadrant of the abdominal cavity between the fundus of stomach and the diaphragm. The spleen appears approximately at the sixth week of embryologic life as a localized thickening of the coelomic epithelium of the dorsal mesogastrium near its cranial end. The process occurs in adjoining areas, which soon fuse to form a lobulated spleen. The spleen can display various developmental anomalies like agenesis, multiple spleens (polyspleenia), isolated small additional splencunculi etc [1].

Accessory spleens (AS) may be formed during embryonic development as ectopic or separated splenic tissue along the path from where the spleen forms at the midline to the spleen's final location on the left side of the abdomen [2]. Which are commonly mistaken for neoplasm or secondaries in case of malignancy investigations or surgeries. In cases of therapeutic splenectomy and ruptured spleen it is of clinical importance that the surgeon should have awareness of the presence of AS.

Authors Affiliation: Associate Professor, Dept. of Forensic Medicine, SDM College of Medical Sciences & Hospital, Dharwad- 580009, Karnataka, India.

Reprints Requests: Venkatesh Maled, Associate Professor, Dept of Forensic Medicine, SDM College of Medical Sciences & Hospital, Dharwad- 580009, Karnataka, India.
E-mail: drmaled_fm@yahoo.co.in

Case Report

A 40 years male body was brought for medicolegal autopsy with the history of road traffic accident. On external examination injuries were noted. On internal examination along with routine autopsy finding we found an AS attached to the peritoneum below the spleen measuring 1X1 cm (Figure 1 and 2). Gross examination of the tissue revealed the healthy splenic tissue/mass. Microscopic examination of the tissue revealed the typical structure of the spleen.

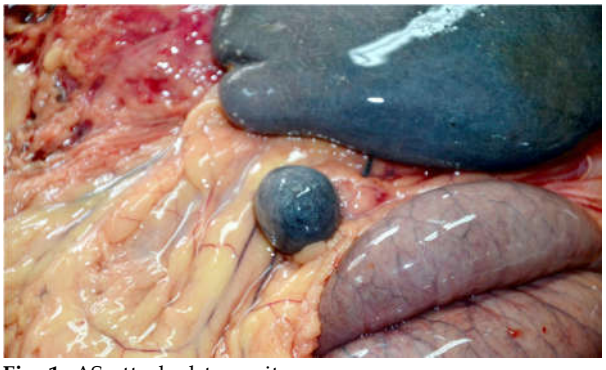

Fig. 1: AS attached to peritoneum

Fig. 2: Separated mass of AS measuring 1x1 cm

Table 1: Incidence of AS in different population

Author	Country	No of cases	Method	Incidence (%)
Yee et al	USA	25	Laparoscopic surgery	4.0
Winde et al	Germany	72	Open surgery	4.2
Park et al	USA	147	Laparoscopic surgery	15.0
Mortele et al	Belgium	1000	CT scan	15.6
Casaccia et al	Italy	309	Laparoscopic surgery	8.1
Ungor et al	Turkey	141	Foetal autopsy	13.5

Discussion

The AS is also known as splencunculi or splenules. The incidence of AS is 6-16% world wide as reported by various studies (Table 1) [3-8]. The largest CT scan based study conducted by Mortele et al reported the incidence of 15.6% in Belgium population [6]. AS are usually located near the spleens hilum, but they may be found at the tail of the pancreas, in the greater omentum, in the wall of the stomach, in the mesentry and even in the pelvis and the scrotum [1,2,9,10]. The size of the AS is usually about 1 cm in diameter but vary from microscopic deposits to 5 cm in diameter [11-14].

The present case revealed a round mass of 1 cm in diameter located at the greater omentum, which is the most common size and shape of the ASas per the previous studies [3-8]. location of the AS at the greater omentum is the second most common position as per the previous studies and hilum of the primary spleen remains the most common site [3-8].

An AS is an incidental finding of no clinical significance in majority of patients. AS are generally determined during radiological investigations or during open or laparoscopic surgeries and during medicolegal autopsy. AS are usually asymptomatic but some time reported as acute abdomen due the complications like torsion, spontaneous rupture, hemorrhage and formation of a cyst within. The torsion and ischemia of the AS can lead to gangrene, abscess, peritonitis etc [14,15].

The European Association of Endoscopic Surgery has recommended a routine search for AS intraoperatively along with preoperative CT scan to achieve the highest detection rates and to prevent disease recurrence, especially for autoimmune hematological disorders. However, the value of preoperative imaging to detect AS remains unclear [16]. Some researchers report that the sensitivity of detecting AS with preoperative CT is higher, but others report that laparoscopy has a higher sensitivity [16-18]. Quah et al. recently reported that the sensitivity of CT scan before laparoscopic splenectomy in detecting AS was 60%, whereas the sensitivity of laparoscopy in detecting AS was 93% [16]. It was also reported that Tc99m heat denatured red blood cell SPECT technique and reticuloendothelial system-specific contras tenhanced MRI may be used for detecting AS. [19,20]

It is concluded that, in addition to studies on CT scans and laparoscopic or open surgery series, autopsy series are useful for determining the incidences and the other features of AS in different populations. Surgeon should have awareness of the presence of AS and its clinical importance in day to day practice.

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