



JOURNAL OF

Punjab Academy of Forensic Medicine & Toxicology

ISSN: 0972-5687

Volume: 19, Number: 01

January to June

Publication: Half Yearly

2019



Editor-in-Chief
Dr. Parmod Kumar Goyal

A Peer Reviewed Journal on
Forensic Medicine, Toxicology, Analytical Toxicology, Forensic Science, Environmental Pollution,
Forensic Pathology, Clinical Forensic Medicine, Identification, Legal Medicine, State Medicine,
Medical Jurisprudence, Medical Ethics, Forensic Nursing, Forensic Odontology, Forensic Anthropology,
Forensic Psychiatry and other Allied branches of Medicine and Science
dedicated to administration of Justice.

OFFICIAL PUBLICATION OF
PUNJAB ACADEMY OF FORENSIC MEDICINE & TOXICOLOGY
Place of Publication: Bathinda (Punjab) India

- Indexed with Index Copernicus (Poland), Scopus (Elsevier Products), IndMed (ICMR New Delhi), Safetylit, Worldcat Library & WHO Hinari
- JPAFMAT is also having PubMed/NLM catalogue number (NLM Unique ID: 101232466).
- Available online at IndianJournals.com, pafmat.org and pafmat.com
- UGC Approved (Sr. No. 97, Journal No.19445)

PUNJAB ACADEMY OF FORENSIC MEDICINE AND TOXICOLOGY

(Registration No. 139 / 1998-99, Chandigarh)

HO: Department of Forensic Medicine, Govt. Medical College Patiala (Punjab) 147001

GOVERNING COUNCIL (2017 - 2019)

President

Dr. D. S. Bhullar

General Secretary

Dr. Akashdeep Aggarwal

Joint Secretary

Dr. Didar Singh Walia

Finance Secretary

Dr. Shilekh Mittal

Vice President

Dr. Rajiv Joshi

Web Master

Dr. Dildar Singh

Editor-in-Chief

Dr. Parmod Kumar Goyal

Joint Editor

Dr. Amandeep Singh

Assistant Editor

Dr. Satinder Pal Singh

Executive Members

Dr. Ashok Chanana

Dr. Preetinder S. Chahal

Dr. Amit Singla

Dr. Ishwar Tayal

Dr. Puneet Khurana

Dr. Ashwani Kumar

Dr. Dasari Harish

Dr. Ajay Kumar

Dr. Deep Rattan Mittal

Advisors

Dr. O.P. Aggarwal

Dr. Gurmanjit Singh

Dr. R.K. Gorea

Dr. S.S. Oberoi

Dr. K.K. Aggarwal

Dr. Vijaypal Khanagwal

Dr. Balbir Kaur

Dr. R.K. Sharma

Special Invitee

Dr. Adish Goyal

Dr. Mukul Chopra

FOUNDER GOVERNING COUNCIL OF PAFMAT

Patron

Dr Jagdish Gargi

President

Dr. R. K. Gorea

Executive Members

Dr A S Thind,

Vice President

Dr. D. S. Bhullar

Dr Jagjiv Sharma,

Advisors

Dr J. S. Dalal

Secretary

Dr. Sat Pal Garg

Dr Kuldeep Kumar,

Dr Harish Tuli

Treasurer

Dr. Nirmal Dass

Dr I. S. Bagga,

Dr Maj. Gen (Rtd.) Ajit Singh

Dr Baljit Singh

From the Desk of Editor-in-Chief

I am pleased to present the first issue of the year 2019 of Journal of Punjab Academy of Forensic Medicine & Toxicology. I am thankful to the authors and contributors for the scientific articles and research papers which are being published in this issue. I am also thankful to the editorial team for supporting me in its publication and the members of the Academy for giving me the opportunity to serve as Editor-in-Chief of the journal. My special thanks to Joint Editor Dr Amandeep Singh and Assistant Editor Dr Satinder Pal Singh for their support and sincere efforts for timely publication and release of this issue.

The Journal publishes original research papers, review articles, case reports and review of books on Forensic Medicine and Toxicology. The Journal highlights the achievements of the academy and its members. This journal is meant for achieving the aims and goals of the academy to expand the academic activities, spread the knowledge and latest research in the field of Forensic Medicine and Toxicology.

I am happy to announce that a new website of the journal has been created and can be accessed at pafmat.org. Authors can submit their papers by creating a user name and password on this site. The new website was officially launched on 17th November 2018 during the occasion of annual conference on Punjab Academy of Forensic Medicine and Toxicology. The Academy is very thankful to Dr Imran Sabri, Saudi Arabia for helping in designing and maintaining the new website, which is a mandatory requirement for PubMed indexing of the journal. Editorial team is trying their best for upgrading the indexing of the journal. However we need help from learned faculty for PubMed indexing of the journal.

Any suggestions and advice for further improving the standards and quality of the journal will be highly appreciated and may be sent to me through email or my whatsapp no. 9876005211.

ISSN Numbers:

ISSN-L: 0972-5687, p-ISSN: 0972-5687, e-ISSN: 0974-083X.

Indexed with:

IndexCopernicus <http://journals.indexcopernicus.com/karta.php?id=4715>

Scopus (SCI):

<http://www.scimagojr.com/journalsearch.php?q=19900194914&ip=sid&clean=0>

Volume of Distribution:

300 copies.

Funding Bodies: Punjab Academy of Forensic Medicine & Toxicology, Donations from Philanthropists and manuscript handling charges

Address for submission of articles Online (Soft Copy):

drparmodgoyal@gmail.com, editorinchief@pafmat.com

Copyright:

No part of this publication may be reprinted or republished without the prior permission of Editor-in-Chief of Journal of Punjab Academy of Forensic Medicine & Toxicology. Submission of all papers to the journal is understood to imply

that it is not being considered for publication elsewhere.

Submission of multi-authored paper implies that the consent of each author has been taken. Researchers/Authors should adhere to publication requirements that submitted work is original, not plagiarized, ethical and has not been published elsewhere.

Every effort has been made not to publish any inaccurate or misleading information. However, the Editor-in-Chief, the Joint Editor or any member of the editorial committee accept no liability in consequences of any such publications. For any further information/query please contact with Editor-in-Chief.

Dr Parmod Goyal

Professor & Head (Forensic Medicine)
Coordinator, Medical Education Unit
Member Secretary, Ethics Committee, Adesh University
Member, Curriculum Committee
Convener, BOS(PG) Adesh University, Bathinda
Coordinator, Body Donation Programme
FAIMER 2013, ACME 2015
Editor in Chief, Journal of Punjab Academy of Forensic
Medicine and Toxicology (JPAFMAT)
Adesh Institute of Medical Sciences & Research, Bathinda

JOURNAL OF

Punjab Academy of Forensic Medicine & Toxicology

ISSN: 0972-5687

Volume: 19, Number: 01

January to June

Publication: Half Yearly

Contents

*From the Desk of Editor-in-Chief	1
*Contents	2-4
* Editorial : Social, Medical And Human Rights Issues Related To Transgenders In India Manoj Kumar Pathak, Rohini, Srishti Rai, Madhu Upadhyay	5-15
*Original Research Papers	
1. Profile of Poisoning In Autopsy Cases In Bangalore North : A Ten Year Retrospective Study Akshith Raj S Shetty, S Praveen, Girish Chandra YP, Basappa S Hugar, Jayanth SH, S Harish	16-20
2. Pattern of Injuries in Fall from Height Cases : An Autopsy Based Study Mukesh Kumar Bansal, Charak Sangwan, Priyanka Garg, Anil kumar, Mukesh Yadav	21-24
3. Orbital and Eye Ball Dimensions on Computed Tomography for Gender and Age Differences in Iranian Population Azadeh Memarian, Fatemeh Noorian Zavareh, Nahid Dadashzadeh Asl, Roya Kordrostami, Elham Zarei, Shahrokh Mehrpisheh	25-28
4. Age Estimation Using Non Destructive Approach : A Comparative Forensic Analysis Kusum Singal, Neelkamal Sharma, S.C. Narula, Vikas Kumar, Permila Singh	29-34
5. Control Measures for Hospitalitermes Monoceros, a Major Pest of Sugarcane by Using Medicinal Plants Lovleen, Perna	35-58
6. Determination of Sex from the Length of Mesosternum (Body of Sternum) in Indore Region, Madhya Pradesh Pankaj Nema, Priyanka K. Nema, S.K. Dadu, B.K. Singh, A.K. Rastogi, Rahul Agrawal, Devesh Pataria, Mohit Shrivastava	39-42
7. Determination of Sex from Upper end of Right Radius in Western Rajasthan Sample Sanjana Devi	43-46
8. Traditional Versus Analytical Examination of Addition as Tampered Documents Shipra Rohatgi, Shruti Gupta, Madhulika Sharma	47-50
9. Amel Gene Profiling from Toothbrush for Sex Determination Among Tanzanians in Surabaya, East Java. Simon Martin Manyanza Nzilibili, Ahmad Yudianto	51-54
10. Adequacy of Dental Education and Environment Health Sonu Gupta, Ravinder Nath Bansal, Surender Pal Singh Sodhi, Samriti Bansal	55-62
11. Estimation of Range of Natural Variations Contemplating Time Interval in Handwritten Scripts Shruti Gupta, Rakesh K. Garg, S K Shukla, Tamanna Jaitly	63-67
12. A Study of Age Estimation through Epiphyseal Union of Medial End of Clavicle in an urban population of Haryana Varun Garg, Ashish Singla, Kunal Khanna, S K Dhatarwal, Vijaypal Khanagwal	68-75
13. Medicolegal Study of Deaths Due To Hanging In Varanasi Region Satish Kumar Khalkho, Manoj Kumar Pathak	76-83
14. Knowledge of Fresh Interns About Legal Aspects of Medicine Smitha Rani, Chinmayi Y, Arun M	84-87

JOURNAL OF

Punjab Academy of Forensic Medicine & Toxicology

ISSN: 0972-5687

Volume: 19, Number: 01

January to June

Publication: Half Yearly

Contents

15. **Firing Range Estimation of handguns (0.32 Revolver & 7.65 mm Pistol) by Studying Inorganic Constituents of Firearm Discharge Residue Particles Using Scanning Electron Microscope coupled with Energy dispersive X-rays and Atomic Absorption Spectrometer flame** 88-93
Puneet Puri, Preeti Grewal, R.K. Sarin, S.K. Shukla
16. **Variant alleles observed in short tandem repeat (STR) analysis of random Indian population** 94-95
Monika Chakravarty, Nidhi Singh, Damini Varshney, Dhruw Sharma, Naresh Kumar, Prateek Pandya, Anupuma Raina,
17. **Gender and Age Differences in Eruption Age of Third Molar Teeth in Iranian Children and Young Adults** 96-99
Seyed Javad Mirhadi, Saeed Mohammadi, Azadeh Memarian, Samad Moini, Katayoun Mottaghi, HoseinJavadi Vasigh, Khatere Asadi,
18. **Effect of Room Temperature and Length of Exposure to recover quality DNA from Earphone Swab-Derived 126bp and 143bp Mitochondrial DNA at D-loop region** 100-107
Ahmad Yudianto, Simon Martin Manyanza Nzilibili
19. **A Study on Snake Bite Poisoning in a Tertiary Care Hospital in Rural Odisha, India** 108-112
Ashim Mishra, S.N. Mohanty, Prateek Rastogi
20. **Estimation of Stature and Gender from Percutaneous Tibial Measurements in population from North-West Uttar Pradesh.** 113-116
Somshekhar Sharma, Rahul G Peddawad, Gurmanjit Maan
21. **Clinico-Epidemiological Profile of Snake Bite Patients in a Tertiary Healthcare Centre in South India** 117-124
Vrinda Lath, Shankar M Bakkannavar, Ranjith Immanuel James, Pradeep Kumar G
22. **Age Estimation of Individuals Beyond 45 Years of Age By CT Scan of Skull** 125-128
Kanika Kohli, O.P. Aggarwal, Amit Mittal
23. **A study of deaths due to burn injuries brought for medico legal autopsies at a tertiary care hospital in northern region of Himachal Pradesh.** 129-132
Susheel Sharma, Vijay Arora, Vipul Parmar, Saurbh Sharma, Mukul Sharma

***Case Reports**

1. **Role of Vaginal Swab Examination to Apprehend the Culprit in Sexual Assault & Murder Case** 133-135
Naresh Kumar, Amit Chauhan, Ritika Gupta, Deepa Verma, S.K. Shukla
2. **Acrylamide Versus Agarose Efficiency to recover Trace DNA (without PCR) on 70% Ethylated Swab Versus Distilled Water Swab from Spectacles** 136-141
Simon Martin Manyanza Nzilibili, Muh. Abduh Dwi Putra, Ahmad Yudianto
3. **Writing On Unconventional Surface** 142-144
Sarita Sharma, Jyoti Singh, Anurag Sharma, Deepa Verma, Madhulika Sharma, S.K Shukla
4. **Scanning Electron Microscopic Finding of Heart in a Case of Celphos Poisoning** 145-147
Manoj Kumar Pathak, Rohini

JOURNAL OF

Punjab Academy of Forensic Medicine & Toxicology

ISSN: 0972-5687

Volume: 19, Number: 01

January to June

Publication: Half Yearly

Contents

- 5. Human Genetic Identification in Paternity testing by multiplex STR analysis using ABI 3500 xl Genetic Analyzer** **148-150**
Monika Chakravarty, Nidhi Singh, Damini Varshney, Dhruw Sharma, Naresh Kumar, Prateek Pandya, Anupuma Raina

***Review Article**

- 1. Application of Saliva in Forensic Odontology : An Update** **151-154**
Abraham Johnson
- 2. A Survey of Techniques used for Ink Examination** **155-161**
Mandeep Kaur Purba, Shruti Gupta, Reeta Rani Gupta, S.K. Shukla
- 3. Frequently Asked Questions Regarding Court Evidence through Video-conferencing in Medicolegal Cases** **162-168**
Vivekanshu Verma, Santosh Kumar Verma
- 4. Systematic Review of Causes of Head Trauma in Age Groups 0-14 Years Versus 0-20 Years in Iran** **169-172**
Siamak Soltani, Kamran Aghakhani, Saeed Mohammadi, Shahrokh Mehrpisheh
- 5. The role of Insects in Forensic investigation: A review on modern techniques** **173-175**
Hussein R. Mahmood, Taif Mothhar Al-Khalidi, Abbas H. Burhan, Mohammed M. Alkhuzai

***Commentary (Scientific Correspondence)**

- 1. Significance of Accreditation of Forensic Science Laboratories in India** **176-184**
Madhavi Jog, Aastha Pandey
- 2. The Protection of Children from Sexual Offences Act, 2012** **185-190**
Kunal Khanna, Vijay Pal

***Instructions to Authors** **191**

***Life Membership Form** **192**

***Life Members PAFMAT** **193-194**

ERRATA

Volume 2, Number 1 at Page No 3, Sr No:15, under contents for Title “ Bisexual variation in Age Determination from fusion of Xiphoid process with the body of sternum in Indore Region, MadhyaPradesh” Authors are Pankaj Nema, Priyanka Kolhe Nema, S.K.Dadu, B.K Singh. Inadvertently other names were written. The Editorial Team sincerely apologises for the same.

Editorial

Social, Medical And Human Rights Issues Related To Transgenders In India

1. **Manoj Kumar Pathak**, Professor and Head, Department of Forensic Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi, U.P., India
2. **Rohini**, Research Scholar, Department of Forensic Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi, U.P., India
3. **Srishti Rai**, Research Scholar, Department of Forensic Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi, U.P., India
4. **Madhu Upadhyay**, Associate Professor, Department of Community Medicine, University College of Medical Sciences & GTB hospital, Dilshad Garden, New Delhi, India

ABSTRACT :

The main problems that are being faced by the transgender community are of discrimination, unemployment, lack of educational facilities, homelessness, lack of medical facilities like HIV care and hygiene, depression, hormone pill abuse, tobacco and alcohol abuse, problems related to marriage and adoption. There are approximately 4.8 million transgender people in India. 2/5th of homosexuals in country have faced blackmail after the 2013 ruling. LGBT people are not allowed to do sex reassignment surgery, to make same sex marriages and acquire civil rights (as a married couple). They are not allowed to adopt a child, access to IVF, commercial surrogacy, entry into the military services as well as not authorised for blood donation. Among men who have sex with men, rates of human papilloma virus-associated anal cancers are 17 times higher than those of heterosexual men. International Statistical Classification of Diseases (ICD) or the American Diagnostic Manual of Mental Disorder (DSM5) refers it as Gender Dysphoria.

Corresponding Author

Dr. Manoj Kumar Pathak,

Professor Department of Forensic Medicine,
Institute of Medical Sciences, Banaras Hindu University,
Varanasi, Uttar Pradesh,
Contact : +91 94501-79177
E-mail : drmanojpathak@gmail.com

Article History:

Received: 31 October 2018
Received in revised form: 31 December 2018
Accepted on: 31 December 2018
Available online: 1 June 2019

KEYWORDS : Transgender, LGBT, Queer, Transsexuals, Transvestism, SRS(Sex Reassignment Surgery)

INTRODUCTION :

"Sex is what you are born with, gender is what you recognise and sexuality is what you discover"-A.Chettiar^[1]

Concepts of gender identity differ from that of sexual orientation. Sexual orientation describes an individual's enduring physical, romantic, emotional or spiritual to another person, while gender identity is one's personal sense of being a man or a woman.^[2]

It is the movement across a socially imposed boundary away from an unchosen starting place. Some people move away from their birth assigned gender, because they feel strongly that they properly belong to another gender, in which it would be better for them to live.^[3]

Sex assigned at birth is based on anatomy and biology, Gender identity expands beyond the binary male and female and includes persons who think of their gender as containing elements of both or neither. Sexual orientation refers to how one thinks of their physical or emotional attraction to others.^[2]

According to a study on LGBT rights in India published on Wikipedia Psychiatrist John F. Oliven of Columbia University coined the term transgender in his 1965 reference work Sexual Hygiene and Pathology, writing that the term which had previously been used, transsexualism, "is misleading; actually, 'transgenderism' is meant, because sexuality is not a major factor in primary transvestism." The term transgender was then popularized with varying definitions by various transgender, transsexual, and transvestite people, including Virginia Prince, who used it in the December 1969 issue of Transvestia, a national magazine for cross dressers she founded. By the mid-1970s both trans-gender and Trans people were in use as umbrella terms, and 'transgenderist' was used to describe people who wanted to live cross-gender without sex reassignment surgery (SRS). By 1976, transgenderist was abbreviated as TG in educational materials^[4].

A lot of acronyms are used by members of the T section of the LGBTIQQA (Lesbian, Gay, Bisexual, Transgender, Queer, Questioning and Allies).

Despite the distinction between sexual orientation and gender, throughout history the gay, lesbian and bisexual subculture was often the only place where gender –variant people were socially accepted in the gender role they felt they belonged to; especially during the time when legal or medical transitioning was impossible.^[3]

In April 2014, the Supreme Court of India declared transgender to be a "third gender" in Indian law. The term 'transgender people' is generally used to describe those who transgress social gender norms. Transgender is often used as an umbrella term to signify individuals who defy rigid, binary gender constructions and who express or present a breaking and/or blurring of culturally prevalent stereotypical gender roles. Transgender people may live full- or part-time in the gender role 'opposite' to their biological sex. In contemporary usage, "transgender" has become an umbrella term that is used to describe a wide range of identities and experiences, including but not limited to: pre-operative, post-operative and non-operative transsexual people (who strongly identify with the gender opposite to their biological sex); male and female 'cross-dressers' (sometimes referred to as "transvestites", "drag queens", or "drag kings"); and men and women, regardless of sexual orientation, whose appearance or characteristics are perceived to be gender atypical. A male-to-female transgender person is referred to as 'transgender woman' and a female-to-male transgender person, as 'transgender man'. The terms 'transgender' or 'transgender populations/people', used in this brief, while more encompassing.

According to a study on LGBT rights in India published on Wikipedia South Asia (modern-day India, Pakistan, Bangladesh and Nepal) has traditionally recognized a third gender population, considered by society as neither male nor female. Such individuals are known as *hijras* or alternatively *hijadas* (Hindi: हिजड़ा; Bengali: হিজড়া; Nepali and Marathi: हिजड़ा). In Telugu, they are referred to as *napunsakudu* (నాపుంసకుడు), in Urdu as *khawajasara* (خیوجا سارا), in Gujarati as *pavaiyaa* (પાવૈયા), in Tamil as *thirunangayar/thirunangai* (திருநங்கை), in Punjabi as *khusra* (ਖੁਸਰਾ), in Odia as *napunsak* (ନାପୁଂସକ), in Sindhi as *khadro* (ڪڏرو), in Malayalam as *sandan* (ഷണ്ടന്), in Kannada as *chhakka* (ಚಕ್ಕ), in Konkani as *izddo*, in Manipuri as *nupimanbi*, in Kashmiri as *napumsakh* (নপুংসখ), in Assamese as *npunnsk* (নপুংসক), in Santali as *cakra*, in Sanskrit as *klība* (क्लीब), and in Mizo as *mil tilreh*. In English language publications, these terms are given to eunuchs, intersex people or transgender people.^[4]

Not all transgender people seek medical interventions, and

some may seek none. The current standard of care is to allow each transgender person to seek only those interventions they desire to affirm their own gender identity.^[5]

Transgender community in India (made up of Hijras and others) has a long history in Indian history and in Hindu mythology.^[6]

MATERIAL AND METHOD:

A thorough search related to LGBTQ was done from various sources including online resources (e.g. Wikipedia on LGBT rights in India), various journals, books available on this topic and various acts passed by parliament of India on article 377 and LGBTQ issues (Supreme Court judgment 2014, protection of transgender right bill 2016). As well as recent judgment by Hon'ble Supreme Court on September 6, 2018 pertinent to LGBTQ community and their discrimination issues were systematically reviewed and been incorporated in this study. Map study was done on different organizations working for status improvement of LGBTQ community in different states of India.

RESULTS:

History

The Khajuraho temples, famous for their erotic sculptures, contain several depictions of homosexual activity. Historians have long argued that pre-colonial Indian society did not criminalize same-sex relationships, nor did it view such relations as immoral or sinful. Transgender individuals held high positions in courts of Mughal rulers in the 16th and 17th centuries. Hinduism, India's largest religion, has traditionally portrayed homosexuality as natural and joyful, though some Hindu texts do contain injunctions against homosexuality. Hinduism also acknowledges a third gender known as *hijra*. There are multiple characters in the Mahabharata who change genders such as Shikhandi who is born female but identifies as male and eventually marries a woman. Bahuchara Mata is the goddess of fertility, worshipped by *hijras* as their patroness. Modern societal homophobia was introduced to India by the European colonizers and the subsequent enactment of Section 377 by the British, which stood for more than 70 years after Indian independence.

The Goa Inquisition once prosecuted the capital crime of sodomy in Portuguese India, but not lesbian activity.

During the Mughal Empire, a number of the preexisting Delhi Sultanate laws were combined into the Fatawa-e-Alamgiri, mandating a common set of punishments for Zina (unlawful intercourse). Babur was known to have a crush on a boy, and recorded it in his memoirs. Other prominent Mughal men who engaged in homosexuality include Ali Quli Khan, and poet Sarmad Kashani who had such a crush on a Hindu boy that he

went to his home naked. In contrast, homosexual acts were regarded as taboo among the common people.

The British Raj criminalized anal sex and oral sex (for both heterosexuals and homosexuals) under Section 377 of the Indian Penal Code, which entered into force in 1861. This made it an offence for a person to voluntarily have "carnal intercourse against the order of nature."

In 1884, a court in north India, ruling on the prosecution of a *hijra*, commented that a physical examination of the accused revealed she "had the marks of a habitual catamite" and commended the police's desire to "check these disgusting practices".

CHALLENGES :

Problems are greatest where people cannot be open about their sexual orientation and gender identity. Encouraging greater LGBT acceptance and access to healthcare will be critical to improving outcomes and experiences for LGBT communities. Understanding gender identity and sexual orientation, addressing health disparities and creating positive healthcare experiences requires an understanding of the diversity of cultural expression and lives of LGBT persons. Providers must be able to distinguish gender identity from sexual orientation.

Justice K S Radhakrishnan noted in his decision that, "Seldom, our society realises or cares to realize the trauma, agony and pain which the members of Transgender Community undergo, nor appreciates the innate feelings of the members of Transgender community, especially of those whose mind and body disown their biological sex", adding:

Non-recognition of the identity of Hijras/transgender persons denies them equal protection of law, thereby leaving them extremely vulnerable to harassment, violence and sexual assault in public spaces, at home and in jail, also by the police. Sexual assault, including molestation, rape, forced anal and oral sex, gang rape and stripping is being committed with impunity and there are reliable statistics and material to support such activities. Further, non-recognition of identity of Hijras/transgender person's results in them facing extreme discrimination in all spheres of society, especially in the field of employment, education, healthcare etc.

People have a gender identity that differs from the sex they were assigned at birth. Gender identity describes a person's internal sense of gender, over the past decade; LGBT people have gained more and more tolerance in India, especially in large cities. Nonetheless, most LGBT people in India remain closeted, fearing discrimination from their families, who might see homosexuality as shameful. Reports of honour killings, attacks, torture, and beatings of members of the

LGBT community are not uncommon in India. Discrimination and ignorance are particularly present in rural areas, where LGBT people often face rejection from their families and forced opposite-sex marriages.

ISSUES RELATED TO TRANSGENDERS

A. HEALTH RISKS

Numerous studies highlight health disparities involving the care of lesbian, gay, bisexual and transgender people. Lesbian and bisexual women are less likely to receive recommended preventive screenings such as breast, cervical and colorectal cancer screenings. These disparities are compounded by structural barriers to healthcare, including decreased access to medical care, lack of awareness to the unique health needs of LGBT individuals and stigma and discrimination towards the LGBT community. Chronic exposure to high levels of stress from real or anticipated discrimination referred to as "minority stress" may be an important factor contributing to the poor health outcomes experienced by LGBT populations. It is evident that many LGBT individuals experience health disparities across their life-cycle. While LGBT youth are at greater risk of suicide and homelessness, on the other hand elderly LGBT individuals face barriers to health because of isolation and fewer family supports.

Sexual health

Hijras/TG communities face several sexual health issues including HIV. Both personal and contextual level factors influence sexual health condition and access to and use of sexual health services. Among men who have sex with men, rates of human papilloma virus-associated anal cancers are 17 times higher than those of heterosexual men. In 2014, men who have sex with men accounted for 83% of primary and secondary syphilis infections, where the sex of the sexual partner was known.

Mental health

Mental health needs of Hijras/TG communities are barely addressed in the current HIV programs. Some of the mental health issues reported in different community forums include depression and suicidal tendencies, possibly secondary to societal stigma, lack of social support, HIV status, and violence-related stress. Most transgender people, especially youth, face great challenges in coming to terms with one's own gender identity and/or gender expression which are opposite to that of the gender identity and gender role imposed on them on the basis of their biological sex. They face several issues such as: shame, fear, and internalized transphobia; disclosure and coming out; adjusting, adapting, or not adapting to social pressure to conform; fear of relationships or loss of relationships; and self-imposed limitations on expression or

aspirations.

Alcohol and substance abuse

Available evidence suggests the need to address alcohol and substance use among Hijras/TG communities. An unknown but significant proportion of Hijras/TG communities consume alcohol possibly to forget stress and depression that they face in their daily life. Hijras provide several reasons justifying their alcohol consumption that range from the need to 'forget worries' (because there is no family support or no one cares about them) to managing rough clients in their sex work life. However, alcohol use is associated with inability to use condoms or insist their clients to use condoms and thus increase risk for HIV transmission and acquisition.

Social Exclusion of Hijras/Transgender Women

Our culture today tries to reduce the wide range of liveable body types into two and only two genders, one of which is subject to greater social control than the other, with both genders being based on genital sex.

LIVES THAT DO NOT CONFORM TO THIS DOMINANT PATTERN ARE GENERALLY TREATED AS HUMAN GARBAGE.

Social Exclusion Framework is seen as having particular salience in addressing the barriers to meeting the Millennium Development Goals, particularly where these relate to exclusionary social relations and institutions. TG communities have been excluded from effectively participating in social and cultural life; economy; and politics and decision-making processes.

Exclusion from family and society

Despite Indian society's general climate of acceptance and tolerance, there appears to be limited public knowledge and understanding of same sex sexual orientation and people whose gender identity and expression are incongruent with their biological sex. Most families do not accept if their male child starts behaving in ways that are considered feminine or inappropriate to the expected gender role. Parents may provide several reasons for doing so: bringing disgrace and shame to the family; diminished chances of their child getting married to a woman in the future and thus end of their generation (if they have only one male child); and perceived inability on the part of their child to take care of the family. Thus, later transgender women may find it difficult even to claim their share of the property or inherit what would be lawfully theirs. Sometimes, the child or teenager may decide to run away from the family not able to tolerate the discrimination or not wanting to bring shame to one's family. Some of them may eventually find their way to Hijra communities.

Discrimination in healthcare settings

Hijras face discrimination even in the healthcare settings. Often, healthcare providers rarely had the opportunity to understand the sexual diversities and they do not have adequate knowledge about the health issues of sexual minorities. Thus, TG people face unique barriers when accessing public or private health services. Barriers in accessing HIV testing, antiretroviral treatment and sexual health services have been well documented.

B. EXCLUSION FROM ECONOMIC PARTICIPATION AND LACK OF SECURITY

Hijras/TG communities face a variety of social security issues. Since most Hijras run away or evicted from home, they do not expect support from their biological family in the long run. Subsequently, they face a lot of challenges especially when they are not in a position to earn (or has decreased earning capacity) due to health concerns, lack of employment opportunities, or old age. Some of the important issues and concerns faced by Hijras/TG communities in relation to social security measures are given below.

Lack of livelihood options

Most employers deny employment for even qualified and skilled transgender people. Sporadic success stories of self-employed Hijras who run food shops, or organise cultural programs are reported in some states. Lack of livelihood options is a key reason for a significant proportion of transgender people to choose or continue to be in sex work - with its associated HIV and health-related risks.

Lack of specific social welfare schemes and barriers to use existing schemes

Although Social welfare departments provide a variety of social welfare schemes for socially and economically disadvantaged groups, so far, no specific schemes are available for Hijras except some rare cases of providing land for Aravanis in Tamil Nadu. Stringent and cumbersome procedures need for address proof, identity proof and income certificate, hinder even deserving people from making use of available schemes.

Most of the social welfare schemes are not much known to these people.

Lack of access to Life and Health insurance schemes

Most Hijras are not under any life or health insurance schemes because of lack of knowledge; inability to pay premiums; or not able to get enrolled in the schemes. Thus, most rely on the government hospitals in spite of the reality of the pervasive discrimination.

EXCLUSION FROM POLITICAL PARTICIPATION

Legal, civil, and political rights

In 1871, the British enacted the Criminal Tribes Act, 1871, under which certain tribes and communities were considered to be 'addicted to the systematic commission of non-bailable offences'. These communities and tribes were perceived to be criminals by birth, with criminality being passed on from generation to generation.

In 1897, the Criminal Tribes Act of 1871 was amended and under the provisions of this statute, "a eunuch [was] deemed to include all members of the male sex who admit themselves or on medical inspection clearly appear, to be impotent". The local government was required to keep a register of the names and residences of all the eunuchs who are "reasonably suspected of kidnapping or castrating children or of committing offences under Section 377 of the Indian Penal Code. And "any eunuch so registered who appear dressed or ornamented like a woman in a public street.....or who dances or plays music or takes part in any public exhibition, in a public street.....[could] be arrested without warrant and Hijras were also reportedly harassed by police by threatening to file a criminal case under Sec-377 IPC.

In July 2009, the Delhi High Court ruled that consensual same-sex relations between adults in private cannot be criminalized. Especially, getting legal recognition of gender identity as a woman or transgender woman is a complicated process.

Transgender people now have the option to vote as a woman or 'other'. However, the legal validity of the voter's identity card in relation to confirming one's gender identity is not clear. Hijras had contested elections in the past. It has been documented that the victory of a transgender person who contested in an election was overturned since that person contested as a 'female', which was thus considered a fraud and illegal.

Challenges in collectivization and strengthening community organisations

A recent mapping study showed that only 103 organizations and networks (that include agencies providing services for MSM) were found to be working with transgender people in India. Even among these, only half (46/103) of these organisations are community-owned organisations. Many have faced a lot of challenges in community mobilisation and legally registering their organisations.

Stringent registration and legal procedures

Some of the legal provisions (e.g., Indian Trust Act, Societies registration Act) that enable a group of individuals to form a legal association pose challenges for Hijras/TG communities. For example, the need of address proof and identity proof of all

members of the group is the basic requirement to register an association. However, most Hijras/TG do not have identity and/or address proof or documents only with their male identity.

Lack of sensitivity among public department officials

In spite of the above challenges, a few CBOs of Hijras across India were able to meet the legal requirements for registration. However, Hijras reportedly had issues with the government officials who are in-charge of processing the registration formalities they were asked unnecessary and irrelevant queries and there was unnecessary delay.

Buying or hiring office space

Hiring an office space for the legal association is very difficult. Even if they get one, the landlords quote unfair rent prices.

Lack of funding support

Hijras/TG associations rarely get external financial support. Even those funders who might want to support primarily want to fund for HIV prevention activities. Through the National AIDS Control Programme, only a few CBOs of TG/Hijras have been granted TI projects.

Need for community systems strengthening

Many of the existing Hijra/TG organizations lack basic systems that are essential for effectively running an organization. It is crucial that the capacity of these organisations be enhanced for effective community mobilisation and providing quality services.

Creating a congenial atmosphere

Hospitals and clinics can take a number of steps to create a welcoming and safe space for LGBT patients. This goes on by establishing and communicating a no discriminating policy that clearly includes gender identity, gender expression and sexual orientation protections. Additionally, hospitals and clinics can develop and implement an equal visitation policy to ensure equal visitation for LGBT patients from same sex partners, parents and other family and friends. State training in LGBT patient-centred care is also a key component of creating inclusive health environments. This includes covering LGBT cultural competency, caring for LGBT patients, creating an inclusive environment for LGBT patients and staff and other topics important for LGBT health. As hospitals and clinics continue to adopt electronic health records, collecting sexual orientation and gender identity information becomes increasingly important to delivering personalised care to LGBT individuals.

BIOLOGICAL BASIS:

Gender identity, like other aspects of temperament awaits the third trimester, when the brain as a whole is growing. The time

around birth may be when the brain's gender identity is being organised. The gender identity being able to be envisioned as a cognitive lens. A lens in the brain controls who to focus on as a "TUTOR". Transgender identity is then the acceptance of a tutor from the opposite sex. Degrees of transgender identity and of gender variance generally, reflect different degrees of single-mindedness in the selection of the tutor's gender. The development of gender identity thus depends on both brain state and early postnatal experience, because brain state indicates what the lens is, and environmental experience supplies the image to be photographed through the lens and ultimately developed immutably into brain circuitry. Once gender identity is set, like other basic aspects of temperament, life proceeds from there. Sexual orientation is one of the many biological phenomena which is natural and inherent in an individual and is controlled by neurological and biological factors. The science of sexuality has theorized that an individual exerts little or no control over who he/she gets attracted to. Any discrimination on the basis of one's sexual orientation would entail a violation of the fundamental right of freedom of expression. Despite this statement from the IPS, conversion therapies are still performed in India. These practices usually involve electroconvulsive therapy (which may lead to memory loss), hypnosis, the administration of nausea-inducing drugs, or more commonly talk therapy where the individual is told that homosexuality is caused by "insufficient male affirmation in childhood" or "an uncaring father and an overbearing mother". Conversion therapy can lead to depression, anxiety, seizures, drug use and suicidal tendencies for the individuals involved. Although it seems to be same, transgender and Intersex activist communities are quite different. Intersex activism tends to focus on ending the practice of performing "normalization" surgery on infants born with noticeably ambiguous genitals; transgender people are rightly considered to face different kinds of problems with the medical establishment. Deborah Rudacille in his paper "Endocrine Disrupting Chemicals and Trans-sexualism (2001) linked transgender to falling sperm counts among human males with endocrine disrupting chemicals found in pesticides and food additives in the environment. Intersex conditions are far more common than most people realise; reliable estimates put the number at about 1 in 2000 births. Current theories favour sex-linked neurological differences in the brain.

Human rights

The Hon'ble Supreme Court of India declared that:

1. Hijras, eunuchs, apart from binary gender, be treated as "third gender" for the purpose of safeguarding their rights under Part – III of our Constitution and the laws made by the Parliament and the State Legislature.

2. Transgender persons' right to decide their self-identified gender is also upheld and the Centre and State governments are directed to grant legal recognition of their gender identity such as male, female or as third gender.

3. The Court direct the Centre and the State governments to take steps to treat them as socially and educationally backward classes of citizens and extend all kinds of reservation in cases of admission in educational institutions and for public appointments.

4. The Centre and State Governments are directed to operate separate HIV sero-surveillance Centre since Hijras / Transgenders face several sexual health issues.

5. The Centre and State Governments should seriously address the problems being faced by hijras/transgenders such as fear, shame, gender dysphoria, social pressure, depression, suicidal tendencies, social stigma, etc. and any insistence for *Sex Reassignment Surgery (SRS)* for declaring one's gender is immoral and illegal.

6. The Centre and State Governments should take proper measures to provide medical care to the transgenders in the hospitals and also provide them with separate public toilets and other facilities.

7. The Centre and State Governments should also take steps for framing various social welfare schemes for their betterment.

8. The Centre and State Governments should take steps to create public awareness so that the transgenders will feel that they are also part and parcel of the social life and be not treated as untouchables.

9. The Centre and State Governments should also take measures to regain their respect and place in the society which once they enjoyed in our cultural and social life.

Contemporary times

There have been isolated initiatives for offering mainstream jobs to qualified transgender women for LIC of India.

The state government of Andhra Pradesh has ordered the Minority Welfare Department to consider hijras as a minority and to develop welfare schemes for them.

The Department of Social Welfare in the states of Tamil Nadu has recently established 'Aravanigal/Transgender Women Welfare Board' for addressing the social welfare issues of Aravanis/Hijras.

In 2003, the Indian Government said that legalizing homosexuality would "open the floodgates of delinquent behavior".

In 2009, the Delhi High Court decision in *Naz Foundation v. Govt. of NCT of Delhi* found Section 377 and other legal prohibitions against private, adult, consensual, and non-

commercial same-sex conduct to be in direct violation of fundamental rights provided by the Indian Constitution. Section 377 stated that: "Whoever voluntarily has carnal intercourse against the order of nature with any man, woman or animal, shall be punished with [imprisonment for life], or with imprisonment of either description for a term which may extend to ten years, and shall also be liable to fine," with the added explanation that: "Penetration is sufficient to constitute the carnal intercourse necessary to the offence described in this section."

According to a previous ruling by the Indian Supreme Court, decisions of a high court on the constitutionality of a law apply throughout India, and not just to the state over which the high court in question has jurisdiction.

There have been incidents of harassment of LGBT groups by authorities under the law.

On 23 February 2012, the Ministry of Home Affairs expressed its opposition to the decriminalization of homosexual activity, stating that in India, homosexuality is seen as being immoral. The Central Government reversed its stance on 28 February 2012, asserting that there was no legal error in decriminalizing homosexual activity. The shift in stance resulted in two judges of the Supreme Court reprimanding the Central Government for frequently changing its approach to the issue.

On 11 December 2013, the Supreme Court set aside the 2009 Delhi High Court order decriminalizing consensual homosexual activity within its jurisdiction.

Human Rights Watch expressed concerns that the Supreme Court ruling would render same-sex couples vulnerable to police harassment, stating that "the Supreme Court's ruling is a disappointing setback to human dignity, and the basic rights to privacy and non-discrimination" The Naz Foundation stated that it would file a petition for review of the court's decision. Activist group Kavi's Humsafar Trust has reported that two-fifths of homosexuals in the country had faced blackmail after the 2013 ruling.

On 28 January 2014, the Supreme Court of India dismissed the review petition filed by the Central Government, the Naz Foundation and several others against its 11 December verdict on Section 377. The bench explained the ruling by claiming that: "While reading down Section 377, the High Court overlooked that a minuscule fraction of the country's population constitutes lesbians, gays, bisexuals or transgender people, and in the more than 150 years past, less than 200 persons have been prosecuted for committing offence under Section 377, and this cannot be made a sound basis for declaring that Section ultra vires Articles 14, 15 and 21.

On 18 December 2015, Shashi Tharoor, a member of the Indian National Congress party, introduced a bill for the repeal of Section 377, but it was rejected in the House by a vote of 71-24. Shashi Tharoor is planning to re-introduce the bill.

On 2 February 2016, the Supreme Court decided to review the criminalization of homosexual activity. In August 2017, the Supreme Court unanimously ruled that the right to individual privacy is an intrinsic and fundamental right under the Indian Constitution. The Court also ruled that a person's sexual orientation is a privacy issue, giving hopes to LGBT activists that the Court would soon strike down Section 377.

In January 2018, the Supreme Court agreed to refer the question of Section 377's validity to a large bench, and heard several petitions on 1 May 2018. In response to the court's request for its position on the petitions, the Government announced that it would not oppose the petitions, and would leave the case "to the wisdom of the court". A hearing began on 10 July 2018, with a verdict expected before October 2018. Activists view the case as the most significant and "greatest breakthrough for gay rights since the country's independence", and it could have far-reaching implications for other Commonwealth countries that still outlaw homosexuality.

On 6 September 2018, the Supreme Court issued its verdict. The Court unanimously ruled that Section 377 is unconstitutional as it infringed on the fundamental rights of autonomy, intimacy and identity, thus legalizing homosexuality in India. The Court explicitly overturned its 2013 judgement.

Criminalizing carnal intercourse is irrational, arbitrary and manifestly unconstitutional.

— *Chief Justice Dipak Misra*

History owes an apology to these people and their families. Homosexuality is part of human sexuality. They have the right of dignity and free of discrimination. Consensual sexual acts of adults are allowed for [the] LGBT community.

— *Justice Indu Malhotra*

It is difficult to right a wrong by history. But we can set the course for the future. This case involves much more than decriminalizing homosexuality. It is about people wanting to live with dignity.

— *Justice Dhananjaya Y. Chandrachud*

Furthermore, it ruled that any discrimination on the basis of sexual orientation is a violation of the Indian Constitution:

Sexual orientation is one of the many biological phenomena which is natural and inherent in an individual and is controlled by neurological and biological factors. The science of sexuality has theorized that an individual exerts little or no

control over who he/she gets attracted to. Any discrimination on the basis of one's sexual orientation would entail a violation of the fundamental right of freedom of expression.

The Supreme Court also directed the Government to take all measures to properly broadcast the fact that homosexuality is not a criminal offence, to create public awareness and eliminate the stigma members of the LGBT community face, and to give the police force periodic training to sensitize them about the issue.

Legal experts have urged the Government to pass legislation reflecting the decision, and frame laws to allow same-sex marriage, adoption by same-sex couples and inheritance rights.

Sex with minors, non-consensual sex (rape) and bestiality remain criminal offences. The Supreme Court ruling may not extend to the state of Jammu and Kashmir, which is governed by its own criminal law, the Ranbir Penal Code (RPC). Legal opinion is divided on whether the Supreme Court judgment applies to the state or not. Per a 1995 judgment of the state High Court, when an IPC (Indian Penal Code) provision is struck down on grounds of violating the Constitution, its corresponding provision in the Ranbir Penal Code too would be struck down. However, it has yet to be determined if Section 377 of the RPC is identical to its IPC version. LGBT activists in Jammu and Kashmir have already announced their intention to challenge the RPC.

Transgender rights: evolution with time

Hijras were legally granted voting rights as a third sex in 1994. Due to alleged legal ambiguity of the procedure, Indian transgender individuals have difficulties accessing safe medical facilities for surgery.

On 15 April 2014, the Supreme Court of India declared transgender people a socially and economically backward class entitled to reservations in education and jobs, and also directed union and state governments to frame welfare schemes for them. The Court ruled that transgender people have a fundamental constitutional right to change their gender without any sort of surgery, and called on the Government to ensure equal treatment for transgender people. The Court also ruled that the Indian Constitution mandates the recognition of a third gender on official documents. In light of the ruling, government documents, such as voter ID cards, passports and bank forms, have started providing a third gender option along side male (M) and female (F), usually designated "other" (O), "third gender" (TG) or "transgender" (T).

In 2013, transgender and gender activists S. Swapna and Gopi Shankar Madurai from Srishti Madurai staged a protest in the Madurai collectorate on 7 October 2013 demanding

reservation and to permit alternate genders to appear for examinations conducted by TNPSC, UPSC, SSC and Bank exams. Swapna, incidentally, had successfully moved the Madras High Court in 2013 seeking permission to write the TNPSC Group II exam as a female candidate. Swapna is the first transgender person to clear TNPSC Group IV exams.

On 24 April 2015, the Rajya Sabha unanimously passed the *Rights of Transgender Persons Bill, 2014* guaranteeing rights and entitlements, reservations in education and jobs (2% reservation in government jobs), legal aid, pensions, unemployment allowances and skill development for transgender people. It also contains provisions to prohibit discrimination in employment as well as prevent abuse, violence and exploitation of transgender people. The bill also provides for the establishment of welfare boards at the center and state level as well as for transgender rights courts.

Social Justice and Empowerment Minister Thaawar Chand Gehlot stated on 11 June 2015 that the Government would introduce a new comprehensive bill for transgender rights in the Monsoon session of Parliament. The bill would be based on the study on transgender issues conducted by a committee appointed on 27 January 2014. According to Gehlot, the Government intends to provide transgender people with all rights and entitlements currently enjoyed by scheduled castes and scheduled tribes.

The *Transgender Persons (Protection of Rights) Bill, 2016*, which was initially introduced to Parliament in August 2016, was re-introduced to Parliament in late 2017. Some transgender activists have opposed the bill because it does not address issues such as marriage, adoption and divorce for transgender people. Akkai Padmashali criticised the bill's definition of transgenderism, which states that transgender people are "based on the underlying assumption of biological determinism".

Third-gender literature and studies

Vaadamalli by novelist Su. Samuthiram is the first Tamil novel about the local aravani community in Tamil Nadu, published in 1994. Later, transgender activist A. Revathi became the first hijra to write about hijra issues and gender politics in Tamil. Her book is part of a research project for more than 100 universities. She is the author of *Unarvum Uruvum* (Feelings of the Entire Body), the first of its kind in English from a member of the hijra community. She also acted and directed several stage plays on gender and sexuality issues in Tamil and Kannada. *The Truth about Me: A Hijra Life Story* by A. Revathi is part of the syllabus for final year students of The American College in Madurai. The American College is the first college in India to introduce third gender literature and studies with research-oriented seminars. Later, Naan

Saravanan'sAlla (2007) and Vidya'sI Am Vidya (2008) were among early transwoman autobiographies.

Recognition of Same Sex Relationship

The states of Tamil Nadu and Kerala were the first Indian states to introduce a transgender welfare policy. According to the transgender welfare policy, transgender people can access free sex reassignment surgery (SRS) in government hospitals (only for male-to-female); free housing program; various citizenship documents; admission in government colleges with full scholarship for higher studies; alternative sources of livelihood through formation of self-help groups (for savings) and initiating income-generation programs (IGP). Tamil Nadu was also the first state to form a transgender welfare board with representatives from the transgender community. In 2016, Kerala started implementing free surgery in government hospitals.

In July 2016, the state of Odisha enacted welfare benefits for transgender people, giving them the same benefits as those living below the poverty line. This was aimed at improving their overall social and economic status, according to the Odisha Department of Social Security.

In April 2017, the Ministry of Drinking Water and Sanitation instructed states to allow transgender people to use the public toilet of their choice.

In October 2017, the Karnataka Government issued the "State Policy for Transgender, 2017", with the aim of raising awareness of transgender people within all educational institutions in the state. Educational institutions will address issues of violence, abuse and discrimination against transgender people. It also established a monitoring committee designed with investigating reports of discrimination.

On 28 November 2017, N. Chandrababu Naidu, the Chief Minister of Andhra Pradesh, announced the enactment of pension plans for transgender people. On 16 December 2017, the Andhra Cabinet passed the policy. According to the policy, the State Government will provide an amount of ₹1,500 per month to each transgender person above the age of 18 for social security pensions. In addition, the Government will construct special toilets in public places, like malls and cinema halls, for transgender people.¹

In January 2018, the Kashmiri Finance Minister introduced a proposal to the Jammu and Kashmir Legislative Assembly that would grant transgender people free life and medical insurance, and a monthly sustenance pension for those aged 60+ and registered with the Social Welfare Department. Transgender activists have criticized aspects of the bill, including its requirement to establish medical boards to issue "transgender certificates".

DISCUSSION :

The Supreme Court has held that the right of self-identification of gender is part of the right to dignity and autonomy under article 21 of the constitution. It is understandable that no aspect of an individual is more private than sexual relations and hence criminalization of sexual preference is a violation of right to privacy. However, objective criteria may be required to determine one's gender in order to be eligible for entitlements. The transgender would have the right 'self-perceived' gender identity. A district screening committee would issue a certificate of identity to recognize transgender persons. Certain criminal and personal laws that are currently in force only recognize the gender of 'man' and 'women'. It is unclear how such laws would apply to transgender persons who may not identify with either of the two genders.

The Yogyakarta Principle animates Article 14. Homosexuals have a fundamental right to live with dignity, are entitled to be treated as human beings and imbibe the spirit of fraternity. Tragedy and anguish inflicted by section 377 should be remedied.

Macaulay's legacy exists 68 years after the coming of a liberal Constitution. Human instincts to love have been constrained. Sexual orientation has been the reason for blackmail on the internet. Decriminalization of homosexuality is only the first step.

In a community-based study, nearly one-third of non-heterosexual individuals met diagnostic criteria for a mental disorder and/or reported suicide attempt. LGBT students regularly experience bullying at school; end up homeless; are more likely to contemplate or attempt suicide; and the LGBT community has elevated rates of poverty, food insecurity, and joblessness compared to the general population.

The World Health Organization (WHO) recognizes this deficiency, stating that the limitation in available data "points to the fact that LGBT people often experience poorer health outcomes than the general population and face barriers to health care that profoundly affect their overall health and well-being." Changing the culture of medicine to welcome lesbian and bisexual women in one's medical practice and applying one's knowledge about the specific health issues of this underserved population is essential to their achieving health equity. Part of identifying and serving lesbians and bisexual women is to create a welcoming clinical space where each person is cared for and respected for all of who they are-including their sexual orientation. Many transgender women have lesbian or bisexual sexual orientations. It is important to understand that sexuality (including identity, attraction, and behavior), like gender identity and gender expression, is a spectrum and may vary over time with age, cultural context, and region.

Transgender people may avoid seeking care to prior discrimination or disrespect in a clinical setting. One in three transgender people report delay in seeking care due to prior discrimination, and one in five reports being refused medical care due to their transgender identity.

The estimated size of MSM population was 7.4% as against the overall adult HIV prevalence of 0.36%. The estimated size of MSM and male sex worker population in India (latter presumably includes Hijras/TG communities) is 2,352,133 and 235,213 respectively. No reliable estimates are available for Hijras/ TG women. Until recently, hijras/transgender people are included under the category of MSM in HIV sentinel sero-surveillance. Recent studies among hijras/transgender women have indicated a very high HIV PREVALENCE (17.5% to 41%) among them. A study conducted in a Mumbai STI clinic reported very high HIV seroprevalence of 68% and high syphilis prevalence of 57% among Hijras. In Southern India, a study documented a high HIV seroprevalence (18.1%) and syphilis prevalence (13.6%) among Hijras. A study conducted in Chennai documented high HIV and STI prevalence among Aravanis: 17.5% diagnosed positive for HIV and 72% had at least one STI (48% tested seropositive for HSV-1; 29% for HSV-2; and 7.8% for HBV)

Published data on sexual risk behaviors of hijras/ TG women are limited but available data indicate high risk sexual behaviors. The available information from the Integrated Biological and Behavioral assessment (IBBA) survey conducted in 2007 in selected districts of Tamil Nadu, reported that, among Hijras/TG, the condom use during last anal sex with commercial male partners and 81% with non-commercial male partners in 85% and 81% respectively. Also, the survey documented low level of consistent condom use among hijras/TG women: 6% with commercial male partners and 20% with non-commercial male partners.

As for now the government is working towards the betterment of transgender community in April 2017, the Ministry of Drinking Water and Sanitation instructed states to allow transgender people to use the public toilet of their choice.

Most transgender people, especially youth, face great challenges in coming to term with one's own gender role imposed on them on the basis of their biological sex, they face several issues such as: shame, fear, and internalized transphobia; disclosure and coming out; adjusting, adapting, or not adapting to social pressure to conform; fear or relationships or loss of relationships; and self-imposed limitations on expression or aspirations.

CONCLUSION :

Society owes an apology to the LGBTQ community for the

years of stigma imposed on them. Certain people are not cut out to be heterosexual and we don't need to castigate them, we don't need to punish them, to ostracize them.

Our society needs to understand the fact that: "Transgender is an adjective not a pronoun."

While social and cultural acceptance of the LGBT community has improved its certain parts of the world, many LGBT individuals continue to experience discrimination, stigmatization and violence. Inequitable healthcare and practices, lack of awareness to LGBT health issues, and limited understanding of the unique health needs of LGBT individuals contribute to decreased access to care and disparities in health outcomes for LGBT individuals.

Addressing these barriers will require improved data collection on the LGBT population; understanding of the intersectionality of gender identity, sexual orientation, race/ethnicity, and other determinants of health; and outcomes-focused research across the life course. In striving to deliver high-quality care experiences for all patients, hospitals, clinics, and providers will have to focus on meeting the needs of LGBT community.

The reality is that, the transgender community in India lives in a precarious environment dominated by oppression, discrimination and systemic exclusion. It cannot be forgotten that discrimination is antithesis of equality and that it is the recognition of equality which will foster the dignity of every individual. "Seldom, our society realizes or cares to realize the trauma; agony and pain which the members of Transgender community neither undergo, nor appreciate the innate feelings of the members of the Transgender community, especially of those whose mind and body disown their biological sex".

The physical environment of a hospital or clinic is important, but the majority of clinical spaces do not signal that they are safe place LGBT patients, most healthcare posters, pamphlets, and materials feature heterosexual individuals or couples; adding LGBT –friendly images and text can help signal that the hospital or the clinic is a safe space for sexual and gender minorities.

Last year, a nine-judge bench of the honourable Supreme Court headed by the Chief Justice, in its judgment regarding privacy as a fundamental right, declared that sexual orientation is an essential attribute of privacy and discrimination against an individual on the basis of sexual orientation which is deeply offensive to the dignity and self-worth of an individual. Miniscule fraction of the country's population that constitutes the LGBT community is not a sustainable basis to deny them the right to privacy.

"Every citizen of India should get equal opportunity in

employment as well as each and every sphere of life, according to their qualification, experience and aspirations, without any discrimination on the basis of sex, gender or sexual orientation or any other criteria."

REFERENCES :

1. Agarwal S. Civil and political rights of transgenders in Indian constitutional perspective. International Journal of law and legal jurisprudence studies. 2017;4 (4):144-160
2. Jameson, Fauci, Kasper, Hauser, Longo, Loscalzo, Harrison's Principle of Internal Medicine, 20th edition. 2018, Mac Graw Hills education, ch 393; 1-8.
3. Stryker S, Transgender History, 2008, Seal Press.
4. LGBT Rights in India; <https://www.wikipedia.org>
5. Maxine A. Papadakis, Stephen J. McPhee; Current Medical Diagnosis and Treatment, 2019, Mc Graw Hill Education; chapter 42
6. Hijras/transgender women in India: HIV, Human Rights and Social Exclusion; UNDP, issue brief, December 2010.

Original Research Paper

Profile of Poisoning in Autopsy Cases in Bangalore North : A Ten Year Retrospective Study

1. Akshith Raj S Shetty, Assistant Professor*
2. S Praveen, Professor & Head*
3. Girish Chandra YP, Professor*
4. Basappa S Hugar, Associate Professor*
5. Jayanth SH, Assistant Professor*
6. S Harish, Professor*

*Department of Forensic Medicine, MS Ramaiah Medical College, Bangalore

ABSTRACT :

Introduction : Poisoning pattern of a region is very crucial in determining the health need of the society and planning the infrastructure and manpower requirement for dealing these cases effectively and efficiently.

Aim : The aim of present study is to study the profile of deaths due to poisoning in autopsy cases and to analyse the socio etiological factors involved.

Materials and Methods : The study is a ten year retrospective study conducted at Department of Forensic Medicine, M S Ramaiah Medical College during the period from 1st January 2006 to 31st December, 2015. All cases of deaths due to poisoning brought for medico legal autopsy were studied. The data obtained was computed and descriptive analysis of baseline characteristics were analysed and summarized.

Results : In our study, 7% of the total cases autopsied in the study period belonged to death due to poisoning of which maximum numbers of 420 (70%) cases were seen in male and 180 (30%) cases were female and male to female ratio being 2.33:1. Most of the poisoning cases were reported in the 21-30 years age group. Majority of cases (76%) were suicidal in manner. Majority of cases were due to OP poisoning.

Conclusions : Thus, the study concluded that males are higher in number, and poisoning is seen most commonly among people from the age group of 21-30 years. During the span of the study, poisoning due to insecticide were predominant.

Corresponding Author :

Dr. Akshith Raj S Shetty

MD, Assistant Professor,

Department of Forensic Medicine, MS Ramaiah Medical College, MSRIT PO, MSR Nagar, Bangalore 560054

Contact : +91 99454-46766

E-mail : aksrash@gmail.com

Article History:

Received: 4 October 2018

Received in revised form: 4 November 2018

Accepted on: 4 November 2018

Available online: 1 June 2019

KEYWORDS : Retrospective Study, Profile of Poisoning, Manner of Death, Organophosphorus.

INTRODUCTION :

Poison is any substance that either destroys life or impairs seriously the functions of one or more organs of the body. Poison can be defined as, A substance (solid, liquid or gas) which if introduced in a living body or brought in contact with any part thereof will produce ill health or death by its constitutional or local effects or both^[1]. Poisoning is a common form of purposeful self-harm in the developing world. The mortality and disabilities due to poisoning are increasing because people use highly poisonous chemicals for suicides, which are easily available. Poisoning both accidental and intentional is a significant contributor to mortality and

morbidity throughout the world. Modern development in all fields leads to newer poisonous substances and more number of poisoning cases. Advanced medical treatment and awareness cannot wholly control this issue^[2].

World Health Organization (WHO) conservatively estimates that about 3 million cases of poisoning occur every year in the world and about 2,20,000 (2.2 Lac) deaths occur due to poisoning. Of these 99% of fatal poisoning occurs in developing countries particularly among agriculture workers.^[3] The commonest cause of poisoning in India and other developing countries is pesticides, the reasons being agriculture-based economics, poverty unsafe practices,

illiteracy, ignorance and lack of protective clothing and easy availability of highly toxic pesticides and among pesticides. The exact incidence of poisoning in India is uncertain due to lack of data at central level as most cases are not reported, and as mortality data are a poor indicator of incidence of poisoning. Poisoning pattern of a region is very crucial in determining the health need of the society and planning the infrastructure and manpower requirement for dealing these cases effectively and efficiently. This pattern depends on the availability of poisons in that region, occupation, culture, beliefs, demography, socio-economic status, education and customs of the region. Studies of these factors will be useful not only in management of poisoning cases but also identify administrative requirement and help in planning appropriate preventive measures. In this context, the present study was carried out with the objective to investigate the poisoning trends in the last ten years in Bangalore North.

MATERIAL AND METHODS :

The study is a ten year retrospective study conducted at Department of Forensic Medicine, M S Ramaiah Medical College during the period from 1st January 2006 to 31st December, 2015. All cases of deaths due to poisoning brought for medico legal autopsy were studied. A standard proforma specially designed was used to collect information regarding age, socio-economic background, manner, type of poison and regarding hospitalization and survival period. Standard autopsy protocol was followed and relevant samples / viscera subjected to chemical analysis. Unidentified bodies where chemical analysis was negative and Bodies in advanced state of decomposition were excluded. Descriptive analysis of baseline characteristics were analysed and summarized.

RESULTS:

Of the total 8609 autopsy cases during the study period, 601 were attributed to poisoning accounting for 7% (Table 1)

Table 1 : Year Wise Distribution of Poisoning Cases

Year	Total Autopsies	Total Poisoning Cases	%age
2006	791	62	7.8
2007	811	54	6.7
2008	896	60	6.7
2009	819	60	7.3
2010	894	63	7.0
2011	911	72	7.9
2012	963	70	7.3
2013	907	73	8.0
2014	815	41	5.0
2015	802	46	5.7
Total	8609	601	7

Sex Distribution of Poisoning cases:

420 cases were males and 180 cases were females, one case was a transgender. The males accounting for 70% of the cases and male to female ratio being 2.33:1.

Age Distribution of Poisoning cases:

Most of the poisoning cases were reported in the 21-30 years age group as shown in Table 2 followed by 3rd decade and above 40 years.

Table 2 : Age Wise Distribution of Poisoning Cases

Age Group	0 to 10 years	11 to 20 years	21 to 30 years	31 to 40 years	> 40 years
2006	2	11	23	15	11
2007	1	11	18	14	10
2008	1	3	21	21	14
2009	0	10	25	11	14
2010	0	8	22	15	18
2011	2	13	23	19	15
2012	3	11	21	15	20
2013	5	13	27	16	12
2014	0	5	15	9	12
2015	0	5	13	12	16
Total	14	90	208	147	142

Alleged manner of death:

Majority of cases (76%) were suicidal in manner followed by (22.5%) accidental in manner. (Table 3)

Table 3 : Alleged Manner of Death from History

Manner of Death	Suicidal	Accidental	Homicidal	Dyadic/ Pedicide suicide
2006	47	15	0	0
2007	41	13	1	0
2008	46	13	0	0
2009	51	9	0	0
2010	48	15	0	0
2011	52	20	0	0
2012	47	16	3	4
2013	57	14	0	2
2014	32	9	0	0
2015	35	11	0	0
Total	456 (76%)	135 (22.5%)	04 (0.5%)	06 (1%)

Majority of cases (76%) were suicidal in manner followed by (22.5%) accidental in manner.

Type of poison:

Majority of cases were due to OP poisoning followed by phosphide poisoning. (Table 4)

DISCUSSION:

The study showed a preponderance of death due to poisoning among males accounting to 70% of the total cases. These findings are similar to findings of other studies

Table 4 : Type of Poison

Types of Poison	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	Total
Organophosphorus (OP)	36	31	32	29	31	35	33	34	13	21	295
Alcohol + OP	1	5	1	5	1	4	4	4	1	1	27
Alcohol	9	6	4	5	7	8	8	1	4	6	58
methyl alcohol	0	0	2	0	0	0	0	0	0	0	2
Phosphide	4	5	6	10	14	8	14	17	11	8	97
Organochlorine	0	0	1	1	0	0	0	0	0	0	2
Pyrethroid	2	2	0	0	0	2	1	3	4	1	15
Barbiturate/BZD	1	1	2	1	0	2	0	0	0	0	7
Corrosive	0	0	0	4	3	1	2	4	2	1	17
Supervasmol	0	0	0	0	0	1	1	0	1	1	4
drugs of abuse	0	1	2	0	0	1	0	2	3	2	11
snake bite	2	0	0	1	2	4	1	2	0	2	14
Ant bite	0	0	0	0	1	0	0	0	0	0	1
CO	3	0	0	0	1	1	0	0	1	0	6
Iron	0	0	0	0	0	1	1	1	0	0	3
scorpion sting	0	0	1	0	0	0	0	1	0	0	2
kerosene	0	1	0	0	0	1	1	0	0	0	3
Carbamate	1	0	2	1	0	0	0	0	0	0	4
Prescription drugs	1	1	1	2	2	2	0	0	0	2	11
Miscellaneous	2	0	2	1	1	2	0	5	3	1	17
cyanide	0	1	3	0	0	0	0	0	0	0	4

conducted by KSN Reddy, Gannar OG at Gulbarga which shows that 65.65% of cases were male^[4]. According to study of Vinay Shetty 51.5% of cases were males^[5]. In a study by Prajapati et al 65.4% were males, Male: Female ratio being 1.8:1. Sharma in his study conducted at Jammu has reported the Male: Female ratio being 2.5:1. This high incidence in males is attributed to point that they are more exposed to stress and are the earning members of a large family. A study by Ramesha et al noted the incidence due to poisoning was more common among males (75.4%) than females (24.3%)^[6]. However, another study showed that it was more among females (52.3%) than males (47.7%)^[7]. Pokhrel et al found that females were more susceptible to the intentional poisoning than male^[8].

The present study showed a predominance in 21-30 years age group, this age range is more prone for domestic, educational and employment related stress. Poisoning is most common among people from the age group of 21-30 years and then shows a progressive fall as the age progresses according to a study by Celine et al^[9]. Gargi et al observed that, majority of the victims were in the age group of 21-30 years (45.59%)^[10]. Dhanya et al stated that maximum victims were from the age group 15-30 (58.58%)^[11]. Ali et al also found that majority of

the cases was young people from the age group 16-40 years (about 80%)^[12]. Another study has come up with very similar findings i.e. maximum victims were from the age group of 15-30 years^[13].

Majority of cases (76%) were suicidal in manner followed by (22.5%) accidental in manner. Suicide was the commonest manner of poisoning and many authors made this observation based on history provided by the investigating officers or relatives prior to autopsy. In a study it was observed that Maximum poisoning is attributed to suicidal reasons (89%) with accidental (8%) being the second. Accidental poisoning is prevalent among children as they are vulnerable to consume unknown substances. Homicidal poisoning is still rare and account for only 3% of total^[13]. Ali et al. in a study on clinical pattern and outcome of OP poisoning showed that suicide is the most common mode of poisoning and reported in 65% cases, followed by accidental (27%) and homicidal (8%)^[12]. Therefore, suicide is still the leading cause of poisoning followed by accidental and homicidal. Moreover, most of the suicides were attributed to family problems and depression.

Roberts et al mentioned that use of a poison for a purpose is

determined by several factors including its easy availability in the market, price and popularity among the masses and appropriate laws concerning the poisonous agent^[14]. Many studies have shown that deliberate self-poisoning has a far higher mortality than accidental poisoning^[15]. The act of self-harm has been done to express anger, rebellion or revenge by causing distress to another person in some cultures. Many studies emphasize that not all people who die following acts of self-harm, wished to die^[16]. The role of intent in an attempted suicide is controversial. Determinants for a fatal event include poison's toxicity, time taken in receiving clinical attention and the efficacy of medical treatment. WHO class I hazardous pesticides are easily available in developing countries and the virtually non-existent medical services in the developing world ensure that the mortality rate for deliberate self-poisoning is at least 20 times higher in these countries^[17].

Majority of cases were due to OP poisoning followed by phosphide poisoning. Majority of poisoning cases were due to organo phosphorus compound (OPC) (36.0%)^[6]. Report of a study showed that 67.8% of poisoning cases was due to aluminium phosphide^[18]. OP pesticides were responsible for most deaths in most series of self-poisoning cases^[19]. Dhanya et al observed that Organophosphorus (OP) poisoning constitute maximum number of cases (37.25%) followed by unspecified drugs in Calicut^[11]. Gupta et al confirmed through chemical analysis report that insecticide was the commonest poison (72.44%) followed by aluminium phosphide (14.28%) and acid (0.63%)^[18]. However, the present study agreed with Dhanya et al and Gupta et al and observed that Organophosphorus poisoning constitute major chunk of total cases (49%) in Bangalore North. It appears that OP poisoning constitutes majority of cases because of easy availability, lowcost, unregulated sale and presence in majority of households in this region. Pesticide poisoning is a major public health problem in developing world^[20]. Intentional self-poisoning was the major cause of death due to poisoning^[21,22].

CONCLUSION:

The present study was done to study the pattern of poisoning in the Bangalore north, The Male: Female ratio for poisoning in this region was 2.3:1 with maximum cases in the 21-30 years age group. OP poisoning (49%) is still the leading cause of poisoning in this region followed by phosphide poisoning (16%). Suicidal poisoning was responsible for 76% of poisoning followed by accidental manner. By addressing the issues related to these victims and by implementing the preventive measures like using dedicated suicide help lines for the depressed & the suicidal, and counselling to deal with love failure, exam stress and inter-familial disputes which are reason for most cases, deaths due to suicidal poisoning can

be reduced thereby helping in betterment of the society.

Funding : Nil

Conflicts of Interest : None

REFERENCES:

1. Reddy KSN, Murty OP. The Essentials of Forensic Medicine and Toxicology : 32nd Ed. Hyderabad: K Suguna Devi; 2013:473
2. Gupta BD, Vaghela PC. Profile of fatal poisoning in & around Jamnagar. JIAFM. 2005; 27(3):145-8.
3. World Health Organisation. Guidelines for poison control. Bulletin 1999; Geneva, World Health Org.
4. Gannur DG, Maka P, Reddy KSN. Organophosphorus compound poisoning in Gulbarga –A Five year study. Indian Journal of Forensic Medicine and Toxicology. 2014; 2(1):3-11
5. Shetty V, Pawar GS. Profile of poisoning cases in district and Medical College Hospital of North Karnataka. Indian Journal of Forensic Medicine and Toxicology. 2008; 2(2):26-8
6. Ramesha KN, Rao KB, Kumar GS. Pattern and outcome of acute poisoning cases in a tertiary care hospital in Karnataka, India. Indian J Crit Care Med. 2009; 13:152-5.
7. Khadka SB, Khadka SB. A study of poisoning cases in emergency Kathmandu Medical College Teaching Hospital. Kathmandu Univ Med J 2005; 3:388-91.
8. Pokhrel D, Pant S, Pradhan A, Mansoor S. A comparative retrospective study of poisoning cases in central, zonal and district hospitals. Kathmandu University Journal of Science Engineering and Technology. 2008; 1(5): 40-8.
9. Celine TM, Antony J. A profile of acute poisonings: A retrospective study. Journal of the Scientific Society. 2015; 42(3): 156-60
10. Gargi J, Rai H, Chanana A, Raj G, Sharma G, Bagga IJS. Current Trends of Poisoning. A Hospital Profile, J Indian Med Assoc. 2006 Feb; 104(2):72-3.
11. Dhanya SP, Dhanya T H, Bindu Latha R Nair, Herna C G. A Retrospective Analysis of the Pattern of Poisoning in Patients Admitted to the Medical College Hospital. Calicut Medical Journal 2009; 7(2):1-8.
12. Ali P, Anwer A, Bashir B, Jabeen R, Haroon H, Makki K. Clinical pattern and outcome of Organophosphate poisoning. JLUMHS, 2012; Jan-Apr 11 (1):15-8.
13. Saxena V, Atal DK, Das S. Retrospective Analysis of Pattern of Poisoning in Uttarakhand – J Indian Acad Forensic Med. July-September 2014; 36(3):230-3
14. Roberts DM, Karunarathna A, Buckley NA, Manuweera

- G, Sheriff MH, Eddleston M. Influence of pesticide regulation on acute poisoning deaths in Sri Lanka. Bulletin of the World Health Organization, 2003; 81(11):789-98.
15. De silva P .The logic of attempted suicide and its linkage with human emotion – suicide in srilanka kandy institute of fundamental studies.1989;1:25-40
 16. Aggarwal NK, Aggarwal BBL.:Trends of poisoning in Delhi : JIAFM.1998;20(2):32-6.
 17. Rao V : Attempted suicide. Ind. J. Psy.1965;7(4):253-64.
 18. Gupta BD, Vaghela PC.Profile of fatal poisoning in & around Jamnagar, JIAFM, 2005; 27(3):145-8.
 19. Eddleston M. Patterns and problems of deliberate self-poisoning in the developing world. QJM 2000;93:715-31.
 20. Jeyaratnam J. Acute pesticide poisoning:A major global health problem.World Health Stat Q 1990;43:139-44.
 21. Van der Hoek W, Konradsen F, Athukorala K, Wanigadewa T. Pesticide poisoning: A major health problem in Sri Lanka. Soc Sci Med.1998;46:495-504.
 22. Eddleston M, Sheriff MH, Hawton K. Deliberate self harm in Sri Lanka: An overlooked tragedy in the developing world. BMJ 1998;317:133-5.

Original Research Paper

Pattern of Injuries in Fall from Height Cases : An Autopsy Based Study

1. **Mukesh Kumar Bansal**, Assistant Professor, Department of Forensic Medicine & Toxicology, Government Allopathic Medical College, Banda (U.P.)
2. **Charak Sangwan**, Assistant Professor, Department of Forensic Medicine & Toxicology, Rajkiya Medical College, Jalaun (U.P.)
3. **Priyanka Garg**, Lecturer, Department of Physiology, Government Allopathic Medical College, Banda (U.P.)
4. **Anil kumar**, Director Professor, Department of Forensic Medicine & Toxicology, Maulana Azad Medical College, New Delhi.
5. **Mukesh Yadav**, Principal, Government Allopathic Medical College, Banda (U.P.).

ABSTRACT :

Introduction : Falls from height are the one of the major trauma cases presenting to emergency departments and often cause mortality and morbidity.

Materials and Methods : Forty cases of fall from height which occurred in National Capital Region (NCR) of Delhi were analysed in department of Forensic Medicine & Toxicology, Safdarjang Hospital, New Delhi with aim to correlate the pattern of injuries with site of primary impact and manner of death.

Results and Conclusions : Out of 40 cases, 85% were male victims. Majority of victims belonged to age group 21-50 years. Most of the cases had history of fall from buildings. In 37 cases (92.5%), manner of death was accidental. In most of the cases, height of fall was less than 10 meters. Primary head impact was most common type of impact followed by primary side impact. Cranio-cerebral damage was most common cause of death. Most commonly injured abdominal organ was liver followed by spleen. Death occurred within 24 hours of incidence in majority cases. Most of cases happened due to lack of appropriate fencing or barriers along balconies & terrace of buildings. Uniform policy should be made by developmental authorities in order to reduce such type of cases. It is advised to adopt various safety measures to prevent fall from height cases.

Corresponding Author

Charak Sangwan, Assistant Professor,
Department of Forensic Medicine & Toxicology, Rajkiya
Medical College, Jalaun (U.P.)
Contact : +91 84330-28732
E-mail : sangwancharak@gmail.com

Article History:

Received: 9 October 2018
Received in revised form: 9 November 2018
Accepted on: 9 November 2018
Available online: 1 June 2019

KEYWORDS : Height of fall, primary impact, subarachnoid haemorrhage, cranio-cerebral damage

INTRODUCTION:

Fall from height is a major cause of trauma, next to traffic accidents in both developed and developing countries. In India, fall from height is one of the common causes of severe blunt trauma. According to International classification of diseases (ICD 9), a fall from height is an event where a person falls to a ground from upper level. In current scenario, fall from height's deaths are rising due to increased construction activities in urban areas. Fall from height generally occurs in accidents and suicides & rarely in homicides.

Death due to fall from buildings are common in the national capital region (NCR) of Delhi due to multi-story buildings culture. In majority of these cases, reliable eye witnesses are not present at scene of incidence. This creates hurdles not only for investigating officer but also for forensic expert to differentiate blunt trauma cases due to fall from height from

other causes of blunt trauma (Road traffic accidents, blow to body etc). Absence of reliable & accurate information also poses problems to reach on a conclusion regarding manner of death.

In fall from height cases, damage to body is due to the energy absorption at impact site. This energy relates to the kinetic energy and causes injuries to body parts. The velocity is closely related to the height of the fall. Pattern of injuries on body depends upon multiple factors like height of fall, velocity, site of primary impact, elasticity & viscosity of contact tissue, ground surface etc^[1]. There is paucity of adequate literature regarding pattern of injuries in fall from height cases. The aim of present study is to correlate the pattern of injuries with the primary site of impact. This study also focuses to determine the manner of death by examining pattern of injuries.

MATERIALS AND METHODS:

The present study was conducted in the Department of Forensic Medicine & Toxicology, Vardhman Mahavir Medical College (VMMC) & Safdarjang Hospital, New Delhi, from April 2016 to September 2016 on forty cases of fall from heights which occurred in National Capital Region (NCR) of Delhi and were brought to Mortuary for medicolegal autopsy.

Information regarding case details like age, sex, height of fall, place of fall, manner of fall, site of primary impact etc. was collected from inquest papers, interviewing investigating officer and relatives of the deceased. A proforma was filled for above mentioned parameters. Body was examined thoroughly externally and internally to find out the site of primary impact.

RESULTS & DISCUSSION:

In the present study, out of 40 cases with history of fall from height, 34 (85.0%) were male victims and 6 (15.0%) were female victims. Our results have been supported by previous studies done by Lalwani S et al.² and Kumar JVK et al.³

Maximum cases belonged to the age group of 21-30 years (25%), followed by age group 31-40 & 41-50 years (17.5%). Majority of victims belonged to age group 21-50 years (60%). Similar observations have also been reported by Prathapan V et al.⁴ and Kohli A et al.⁵ Half of the female victims belonged to age group 1-10 years. (Table 1)

Table 1 : Age & Sex of Victim

Age Group (Yrs)	Male		Female		Total	
	No.	%	No.	%	No.	%
1-10	3	7.5	3	7.5	6	15.0
11-20	4	10.0	1	2.5	5	12.5
21-30	10	25.0	0	0.0	10	25.0
31-40	7	17.5	0	0.0	7	17.5
41-50	6	15.0	1	2.5	7	17.5
51-60	4	10.0	0	0.0	4	10.0
61-70	0	0.0	1	2.5	1	2.5
Total	34	85.0	6	15.0	40	100

Majority of victims had history of fall from buildings (70%) followed by history of fall from tree (10%) and stairs/ladder (10%). A study done by Kumar JVK et al.³ found two third of fall occurred from buildings. In contrast to present study, Prathapan V et al. showed that 63% cases were of fall from tree and 26% were of fall from buildings. Reason for this may be that present study was done in NCR region having multi-story building culture. (Table 2).

In the present study, it was very difficult to find out the site of primary impact. It was decided on the basis of data obtained from the investigating officers, deceased's relatives and interpretation and evaluation of injuries on the body of victims⁶. In 24 cases, the primary site of impact was head (60%) followed by sides of body in 11 cases (27.5%). Primary

Table 2 : Place of Fall & Sex of Victim

Place of fall	Male		Female		Total	
	No.	%	No.	%	No.	%
Building	23	57.5	5	12.5	28	70.0
Trees	4	10.0	0	0.0	4	10.0
Stairs/ladder	4	10.0	1	2.5	5	12.5
Miscellaneous	3	7.5	0	0.0	3	7.5
Total	34	85.0	6	15.0	40	100

feet impacts were noted in 4 cases (10.0%). Site of primary impact could not be determined in one case. A study by Prathapan V et al.⁴ on fall from heights- pattern of injuries found 53% cases have head as primary site of impact. Kumar JVK et al. found 46.6% cases of primary head impact & 36.6% cases of primary side impact.

Maximum victims (30%) belonged to injuries sustained from height group of 5-10 meters followed by 0-5 meter (25%). In the 30-35 meters range, there was only one case having history of head impact. There was no case where height of fall exceeds 35 meters. In majority of cases with primary head impact (79.1%), height of fall was less than 10 meters while majority cases (63.6%) with primary side impact had more than 10 meter height of fall. A study done by Kumar JVK et al.³ on height of fall showed almost similar results. (Table 3)

Table 3 : Pattern of Impact in Relation to Height of Fall

Height of fall (meters)	Site of Primary Impact				Total	
	Head	Side of Body	Feet	Un-known	No.	%
0-5	8	2	0	0	10	25.0
5-10	11	1	0	0	12	30.0
10-15	4	3	1	0	8	20.0
15-20	1	3	2	1	7	17.5
20-25	0	1	1	0	2	5.0
25-30	0	1	0	0	1	2.5
Total	24	11	4	1	40	100

In majority of cases (92.5%), manner of death was accidental while only 3 cases (7.5%) have history of suicide. There was no case where history of homicide presents. Other authors like Jagannatha SR et al.⁷, Prathapan V et al.⁴, Richter D et al.⁸ also reported similar findings regarding manner of death. (Table 4)

Table 4 : Manner of Death in Relation to Primary Impact

Height of fall (meters)	Site of Primary Impact				Total	
	Head	Side of Body	Feet	Un-known	No.	%
Suicide	1	0	2	0	3	7.5
Homicide	0	0	0	0	0	0.0
Accident	23	11	2	1	37	92.5
Total	24	11	4	1	40	100

In majority of cases, place of incidence was urban region while in 13 cases, it was rural region. (Table 5)

In maximum cases (82.5%), ground surface was made of hard material (concrete) followed by soft materials as grass & sand

Table 5 : Place of Incidence Wise Distribution of Cases

Place of Incidence	Male	Female	Total	
			No.	%
Urban	29	3	27	67.5
Rural	10	3	13	32.5
Total	34	6	40	100

(17.5%). There is huge difference (65%) between them. These findings have been supported by a study done by Mustafa İcer MD et al.⁹ (Table 6)

Table 6 : Ground Surface-Wise Distribution of Cases

Ground Surface	Male	Female	Total	
			No.	%
Concrete	29	4	33	82.5
Grass	4	1	5	12.5
Sand	1	1	2	5.0
Total	34	6	40	100

In about two third cases, cause of death was cranio-cerebral damage followed by haemorrhagic shock (15%). In 26 victims having cranio-cerebral damage as cause of death, primary site of impact was head (50%) followed by body sides (12.5%). Spinal cord damage was cause of death in four victims; all have head as primary site of impact. In four cases, cause of death was combination of cranio-cerebral damage & haemorrhagic shock. Studies on head injuries as cause of death done by Kohli A et al.⁵ & Kumar J.V.K. et al.³ observed almost similar results. (Table 7)

Table 7 : Cause of Death in Relation to Primary Impact

Cause of Death	Head	Body Side	Feet	Un-known	Total	
					No.	%
Cranio-cerebral damage (a)	20	5	0	1	26	65.0
Spinal cord damage (b)	4	0	0	0	4	10.0
Haemorrhagic shock ©	0	3	3	0	6	15.0
Combination of (a + c)	0	3	1	0	4	10.0
Total	24	11	4	1	40	100

Table 8 shows that abrasions were the most common external

injury. They alone comprised 16 cases (40%) and along with other injuries comprised 27 cases (67.5%). Contusion was least common external injury having 3 cases (7.5%) alone. On the other hand, laceration alone was present in 8 cases (20%) and along with other injuries comprised 16 cases (40%).

Table 8 : Pattern of External Injuries

Type of External Injuries	Total	
	No.	%
Abrasion only	16	40
Contusion only	3	7.5
Laceration only	8	20.0
Abrasion+ contusion	2	5.0
Abrasion + laceration	4	10.0
Contusion+ laceration	1	2.5
Abrasion+ Contusion+ Laceration	3	7.5

The pattern of external injuries in study done by Hartshorne et al.¹⁰ differs from the present study. Reason for this is that in present study almost two thirds cases have history of fall from buildings where scalp contusions are uncommon while in ground level falls, scalp contusions are quite common.

Cranio-cerebral damage was the predominant feature in primary head impact cases. It included skull fractures and various types of intracranial haemorrhages. In 24 cases of primary head impacts, 16 cases (66.6%) showed fractures of skull. In 11 cases of primary side impacts, 6 cases (54.5%) showed fracture of skull bones. In four cases of primary feet impacts, there was not even a single case of skull fracture. This result coincides with the findings in the study conducted by Prathapan V et al.⁴ In all types of primary impact, different types of intracranial haemorrhage were observed. Of the 40 cases studied, intracranial bleeding was present in 32 victims (80%). Maximum cases (21) were having history of primary head impact followed by primary side impact (9 cases). Subarachnoid haemorrhage was commonest (93.7%) followed by subdural haemorrhage (68.7%). Other authors like Goonetilleke et al.¹, Hartshorne et al.¹⁰ also reported similar findings. In the present study, 19 victims had injury to brain substance, out of which in 11 cases (57.89%), site of primary impact was head. These injuries were contusions and lacerations. Study done by Prathapan V et al.⁴ noted almost similar results. (Table 9)

Table 10 showed that visceral organs like liver, spleen and lungs were injured in present study. Liver was most commonly involved organ (5 out of 11 cases) in primary side impact. In one case of primary feet impact, liver was involved. Spleen showed injury in four cases, out of which in 3 cases, site of impact was primary side impact. The heart and kidneys damage were not present in any case. Heart was not damaged

Table 9 : Injuries to Head in Relation to the Site of Primary Impact

Type of Injury	Head Impact	Body Side Impact	Feet Impact	Un-known Impact	Total	
					No.	%
Skull Fracture	16	6	0	0	22	55.0
Intracranial haemorrhage	21	9	1	1	32	80.0
Injury to brain	11	6	1	1	19	47.5

due to protective mechanism provided by rib cage. Abdominal organs like liver, spleen don't have such type of protective mechanism so involved in 10 cases (25%). Due to location and small size, kidneys escaped damage. A study done by Hartshorne et al.¹⁰ showed different results because in ground level falls, injury to chest & abdomen is very rare. It can be inferred that as the height of fall increases, there are higher chances of injuries to chest & abdomen.¹¹

Table 10 : Injuries to Visceral Organs in Relation to the Site of Primary Impact

Type of Injury	Head Impact	Body Side Impact	Feet Impact	Un-known Impact	Total	
					No.	%
Lungs	0	2	0	0	2	5.0
Liver	0	5	1	0	6	15.0
Spleen	0	3	1	0	4	10.0
Heart	0	0	0	0	0	0.0
Kidney	0	0	0	0	0	0.0

Table 11 shows that majority of victims (60%) died within 24 hours after incidence of fall from height. The results coincides with the study done by Kohli A et al.⁵ Reason for this may be that in majority of cases, cause of death was cranio-cerebral damages which were serious in nature and left less time for any medical intervention.

Table 11 : Time Interval Between Injury and Death

Time Interval	Male	Female	Total	
			No.	%
0-24 hours	19	5	24	60
1-2 days	6	1	7	17.5
2-7 days	9	0	9	22.5
>7 days	0	0	0	0.0
Total	34	6	40	100

Summary & Conclusions:

- Out of 40 cases, majority (85%) were male victims.
- Majority of cases (60%) belonged to age group 21-50 years.

- Majority of cases (70%) had history of fall from buildings. In majority cases (92.5%), manner of death was accidental. Reason behind this may be lack of appropriate fencing/ barrier on terrace/balcony.
- In majority cases (55%), height of fall was less than 10 meters. In majority cases (60%), place of incidence was urban region. Ground surface was made of hard material (Concrete) in most cases. Present study was done in Delhi & adjoining areas having urbanisation. This may be reason behind this.
- In majority cases, primary site impact was head followed by side impact. In majority cases, cause of death was cranio-cerebral damage. In primary feet impact cases, most common cause of death was haemorrhagic shock.
- Abrasion was most common external injury followed by laceration.
- Liver was most common abdominal organ damaged followed by spleen.
- In majority case, death occurred within 24 hours of incidence.

REFERENCES :

1. Goonetilleke UKDA. Injuries caused by falls from heights. Med. Sci. Law.1980;20 (3):262-75.
2. Lalwani S, Agnihotri AK et al. Patterns of injuries in fatal falls from heights- a retrospective review. J. Forensic Med. Toxicology.1999;16(2):38-46.
3. Kumar JVK, Srivastava AK. Pattern of injuries in fall from height. J.Indian Acad Forensic Med.2013; 35:47-50.
4. Prathapan V, Umadethan B. Fall from heights-pattern of injuries. International Journal of Biomedical Research.2015; 6(01):8-13.
5. Kohli A, Banerjee KK. Pattern of injuries in fatal falls from buildings. Med.Sci.Law.2006; 46(4):335-41.
6. Mac Laughlin H L.Trauma. W. B Saunders Company, Philadelphia.1960; 146-571.
7. Jagannatha SR, Pradeep Kumar MV et al. Injuries due to fall from height-a retrospective study. Journal of Forensic Medicine & Toxicology .2010; 27(1):47-50.
8. Hahn MP, Richter D et al. Injury pattern after a fall from a great height-an analysis of 101 cases.Unfallchirurg.1995; 98(12): 609-13.
9. Icer M, Giloglu C et al. Factors affecting mortality caused by falls from height. Ulus Travma Acil Cerr Derg.2013;19(6):529-35.
10. Hartshorne NJ, Harruff RC et al. Fatal head injuries in ground-level falls. Am.J. Forensic Med. Pathol.1997;18(3): 258-64.
11. Atanasijevic TC, Savic SN et al. Frequency and severity of injuries in correlation with the height of fall. J. Forensic Sci. 2005; 50(3): 608-12.

Original Research Paper

Orbital and Eye Ball Dimensions on Computed Tomography for Gender and Age Differences in Iranian Population

1. **Azadeh Memarian**, Assistant Professor of Forensic Medicine, Iran University of Medical Sciences, Tehran, Iran.
2. **Fatemeh Noorian Zavareh**, Forensic Medicine Specialist, Iran University of Medical Sciences, Tehran, Iran.
3. **Nahid Dadashzadeh Asl**, Forensic Medicine Specialist, Iran University of Medical Sciences, Tehran, Iran.
4. **Roya Kordrostami**, Forensic Medicine Specialist, Iran University of Medical Sciences, Tehran, Iran.
5. **Elham Zarei**, Assistant Professor of Radiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.
6. **Shahrokh Mehrpisheh**, Assistant professor of Neonatology, Mazandaran University of Medical Sciences, Sari, Iran

ABSTRACT :

Introduction : Nowadays, examination of skeletal components is frequently used for gender and age estimation. The present study aimed to assess the value of the orbital and eye ball dimensions on computed tomography for gender and age differences in Iranian population.

Materials and Methods : Present study was performed on 100 individuals (60 males and 40 females) with the age range of 18-87 years old who referred to Rasoul-e-Akram hospital in Tehran between 2008-2015 and subjected to orbital CT scanning because of different clinical reasons. Height of orbital margin, width of orbital margin, depth of orbit, and inter-orbital distances including sagittal, transverse, and axial dimensions of the orbit on both sides were measured in both genders using the orbital CT scan and reviewed on the Picture Archiving and Communication system (PACS).

Results : Comparing different orbital dimensions between men and women showed no difference except for only left orbital depth that was significantly higher in men as compared to women. Based on the values of area under the curve, none of the orbital dimensions have potential to discriminate between males and females.

Conclusions : The orbital dimensions may not be reliable parameters for gender identification in Iranian population.

Corresponding Author :

Dr. Fatemeh Noorian Zavareh,

MD,

Department of Forensic Legal Medicine, Rasoul-e-Akram Hospital, Tehran, Iran.

Contact : 009802166551201

E-mail : fatemenooriandr@gmail.com

Article History:

Received: 16 October, 2018

Received in revised form: 16 December, 2018

Accepted on: 16 December 2018

Available online: 1 June 2019

KEYWORDS : Gender Identification; Orbital Dimensions; Iranian

INTRODUCTION:

Skeletal system is an excellent and applied option for anthropological and forensic investigations. Skull and facial components vary significantly from male to female and also from one to another ethnicity that the male features are frequently more pronounced than female features^[1]. Nowadays, examination of skeletal components is frequently used to gender identification based upon the appearances of skull, pelvic, sternum, long bones, and scapula^[2]. However the value of the skeletal components is different for gender differentiation as 95% for the pelvic, 90% for the skull, 80% for the long bones, and 98% for combining skull and pelvic^[3]. In total, the differences between men and women depend to skeleton along with other reproductive, hormonal, physical, and behavioral characteristics that totally form sexual dimorphism^[4]. The assessment of sexual dimorphism in skeletal system is naturally based on the two factors including the differences in skeletal sizes and also in functionality related

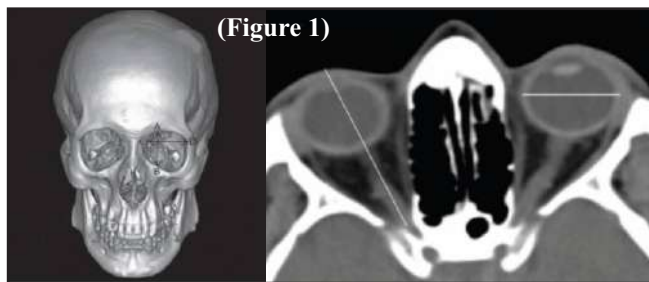
to size differences^[5]. In other words, bone-based gender determination is performed on the two approaches including morphological approach (based on the anatomical differences in the bones) and osteometric approach (based on bone dimensions). In skull, the difference in some features are highlighted in the two genders including general appearance, supraorbital ridges, orbits, glabella, zygomatic arches, mastoid processes, mandible and palate^[6-8].

The orbit is a complex, conical structure formed by different skeletal elements with the base opening towards the front and the apex towards the back of the head. The orbit contains, above all, the eyeball, the optic nerve and the eyeball muscles^[9]. All these structures are tightly bound by ligaments, supporting elements made of connective tissue, and by adipose tissue. In total, two main skeletal components of orbit include orbit size (such as the lengths between the lateral, medial, and inferior margins and the optic canal) and orbital angles (such as the angle between the medial and lateral wall, the angle between

superior and inferior walls, the angle in the inferior wall, and also the angle between the Frankfurt plane and the inferior margin)^[10]. Each of these sizes and angles may be useful to male from female sex differentiating and thus can be helpful in legal medicine to predict gender of cadavers particularly in mutilation, accidents, burnings, and also in damages caused by the wars and natural disasters. However the role of these orbital sizes and angles to discriminate male from female genders remained unclear. The present study aimed to assess the value of these orbital parameters to gender identification in Iranian population.

MATERIALS AND METHODS :

After taking approval from Ethical Committee of Iran University of Medical Sciences and informed consent from the participants, the present retrospective study was done on 100 individuals (60 men and 40 women) with age range of 18-87 years (who underwent orbital CT scan for various clinical reasons) at Rasoul-e-Akram hospital, Tehran between 2008-2015. Persons without identification documents or with pathological orbital situations including orbital or pre-orbital tumors or trauma were excluded. Eligible persons were enrolled after authentication by the national ID card or birth certificate or health insurance. The different orbital dimensions such as height of orbital margin (It is the height of the orbital aperture between incisura supra-orbital and the zygomatico-maxillary suture), width of orbital margin (It is the width of the orbital aperture between the dacryon and the frontozygomatic suture), depth of orbit, and inter-orbital distances including sagittal, transverse, and axial diameters of the orbit (**Figure 1**) on both sides were measured on CT scan and reviewed on the Picture Archiving and Communication system (PACS). Sagittal, transverse, and axial (anterior-to-posterior) diameters of both eyeballs were measured by the PACS SOFTWARE computer program.



(Figure 1)

All measurements were made using the same window, contrast, and brightness. The transverse and the sagittal diameters were measured twice from outer edge of fibrous coat (sclera to sclera), inner edge of fibrous coat (retina to retina) and from cornea to the inlet of the optic channel in the axial CT scan view.

All measurements were taken by two experts and the agreement between them was measured to minimize technical measurement error. In final, the mean of diameters measured by the two experts were stated as the final diameters. For statistical analysis, outcomes were shown as mean $\pm$ standard deviation (SD) for quantitative variables and by absolute frequencies and percentages for categorical variables. Normality of data was analyzed by the Kolmogorov-Smirnoff test. Categorical variables were compared using chi-square test or Fisher's exact test. Quantitative variables were compared with t-test or Mann-Whitney U test. The association between the quantitative variables was measured using the Pearson's or Spearman's correlation tests. The statistical software SPSS version 16.0 for windows (SPSS Inc., Chicago, IL) was used, for the statistical analysis. P values of 0.05 or less were considered statistically significant.

RESULTS:

Out of 100 individuals (60 men and 40 women) 30 aged less than 30 years, 20 ranged 30 to 39 years, 20 ranged 40 to 49 years, 11 ranged 50 to 59 years, 14 ranged 60 to 69 years, and 5 aged equal to or higher than 70 years. Comparing different orbital diameters between men and women showed no difference except for only left orbital depth that was significantly higher in men as compared to women. Our study showed no significant correlation between age and all orbital dimensions (**Table 1**). In total, comparing each dimension between the right-sided and the left-sided showed no significant difference (**Table 2**). Also, the comparison of each diameter across the age subgroups showed no meaningful difference. Using measurement of the area under the Receiver Operating Characteristic (ROC) curve (**Table 3**), none of the orbital dimensions could discriminate male from female gender in our population.

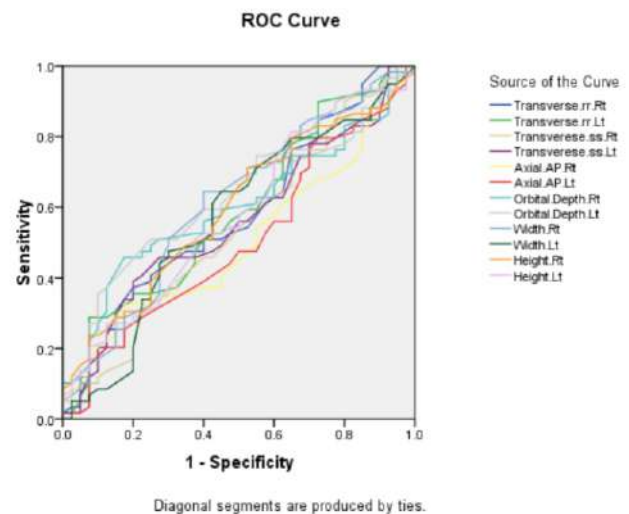


Table 1: Orbital Diameters Assessed by CT Scan in Men and Women and Correlation of Age with Orbital Diameters Assessed by CT Scan

Dimension (in mm)	Age			Sex	
	Men	Women	P-value	r correlation coefficient	P-value
Right r-r transverse diameter	22.79 ± 1.11	22.36 ± 1.31	0.091	-0.188	0.061
Left r-r transverse diameter	22.71 ± 1.29	11.32 ± 1.28	0.143	-0.173	0.087
Right s-s transverse diameter	29.06 ± 1.19	25.11 ± 1.18	0.304	0.186	0.064
Left s-s transverse diameter	25.22 ± 1.25	24.96 ± 1.21	0.322	0.107	0.293
Right axial AP diameter	24.37 ± 1.17	24.36 ± 1.08	0.962	0.106	0.874
Left axial AP diameter	24.39 ± 1.15	24.39 ± 1.20	0.987	0.050	0.624
Right orbital depth	49.26 ± 6.61	48.79 ± 2.79	0.626	-0.069	0.492
Left orbital depth	50.12 ± 3.46	48.74 ± 2.87	0.033	-0.019	0.851
Right orbital width	34.21 ± 2.09	33.48 ± 1.87	0.069	-0.058	0.569
Left orbital width	33.92 ± 2.07	40.41 ± 44.33	0.321	-0.112	0.269
Right orbital height	37.78 ± 2.28	37.12 ± 1.68	0.096	0.026	0.796
Left orbital height	37.78 ± 2.35	37.11 ± 2.03	0.138	0.017	0.866

Table 2: Comparing Orbital Diameters Between Right and Left Sides

Dimension (mm)	Right	Left	P-value
r-r transverse diameter	22.61 ± 1.21	22.55 ± 1.29	0.425
s-s transverse diameter	27.50 ± 1.27	25.11 ± 1.23	0.302
Axial AP diameter	24.38 ± 1.13	24.39 ± 1.16	0.821
Orbital depth	49.06 ± 5.43	49.56 ± 3.29	0.296
Orbital width	33.94 ± 2.03	36.54 ± 2.20	0.365
Orbital height	37.53 ± 2.08	37.51 ± 2.24	0.707

Table 3: The area under the ROC curve for discriminating male from female gender using orbital dimensions

Area Under the Curve					
Test Result Variable(s) (mm)	Area	SE	P-value	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Right r-r transverse diameter	0.582	0.058	0.167	0.468	0.697
Left r-r transverse diameter	0.589	0.058	0.135	0.474	0.703
Right s-s transverse diameter	0.540	0.059	0.496	0.424	0.657
Left s-s transverse diameter	0.560	0.058	0.310	0.446	0.675
Right axial AP diameter	0.501	0.058	0.986	0.386	0.616
Left axial AP diameter	0.504	0.059	0.946	0.388	0.620
Right orbital depth	0.600	0.057	0.092	0.489	0.711
Left orbital depth	0.625	0.056	0.035	0.515	0.735
Right orbital width	0.614	0.058	0.056	0.500	0.727
Left orbital width	0.572	0.060	0.228	0.454	0.689
Right orbital height	0.597	0.057	0.101	0.485	0.710
Left orbital height	0.583	0.058	0.164	0.468	0.697

DISCUSSION:

Despite accurate measurement of different orbital components, the measures of these features in different races remains unclear. Necessity for determining differences in orbital diameters as one of the main tools to gender identification, we attempted to determine and compare different orbital dimensions aided by CT scanning in Iranian population. One of the major and important limitations in our investigation was the multiracial characteristics of the Iranians and thus for precise determination of orbital diameter in our population, it needs a great population-based study using

cluster sampling method and using a sample from all identified ethnicities of the country. However, in the present study, we pointed a great referral general hospital in the capital of the country with references from whole of the country that might cover the pointed limitation. Despite to attempt to minimize study limitations, our study showed no difference in all orbital dimensions between the two genders. In other words, none of the orbital diameters measured by CT scan could identify male from female genders among Iranian population. Also, the right orbits were determined to be similar to the left ones in orbital dimensions. It was in contrary to similar study in the same population by Khademi et al^[11] that showed a significant difference in height, depth, and width between the right and left orbits. However, some authors could find similar results on the lack of gender difference in orbital dimensions. In a study by Ezeuko et al^[12], there was no statistically significant difference in the percentage frequency of any range of all the parameters studied between males and females. Contrarily, it has been shown by some authors that different orbital dimensions are different between men and women and thus can be used for gender identification^[13-18]. In total, our study could not demonstrate gender and age difference in orbital diameters. This is at variance with the study by Igbigbi and Ebite^[8] with adult Malawians, which found higher orbital index among the females than the males. In contrast, Munguti et al.^[16] agreed that there was no statistical significant difference between male and female orbital indices to warrant its use in sexing the crania from a black Kenyan population. In fact, paradoxical results have been obtained on the value of orbital measurements on gender identification in various ethnical populations. These findings support the need for population-based studies on craniometry. The present study, however, showed no difference in orbital dimensions according to the age subgroups with evidently hinting weakness in the use of orbital anthropometry in sex and age determination. In other words, according to study results, the use of other skeletal dimensions such as in the skull or limbs may be more reliable and applicable than orbital dimensions to identify gender in our population.

Limitations : It is better to repeat this work in a larger sample size to be sure about the general use of the result of the study.

AUTHORS' CONTRIBUTIONS : AM and FNZ: conception of the research idea, study design, data collection, analysis and interpretation, and manuscript write-up. NDA, SM, RK and EZ: data collection, analysis and interpretation, and manuscript write-up.

REFERENCES :

1. Gupta A, Kumar K, Shetty DC, Wadhwan V, Jain A, Khanna KS. Stature and gender determination and their correlation using

- odontometry and skull anthropometry. *J Forensic Dent Sci.* 2014;6:101-6.
2. Nieves JW, Formica C, Ruffing J, Zion M, Garrett P, Lindsay R, et al. Males have larger skeletal size and bone mass than females, despite comparable body size. *J Bone Miner Res.* 2005;20:529-35.
3. Günay Y, Altinkök M, Cagdir S, Kirangil B. Gender determination with skull measurements. *J Forensic Med.* 1997;13:4-8.
4. Rose AJ, Rudolph KD. A review of sex differences in peer relationship processes: potential trade-offs for the emotional and behavioral development of girls and boys. *Psychol Bull.* 2006;132:98-131.
5. Seeman E. Clinical review 137: Sexual dimorphism in skeletal size, density, and strength. *J Clin Endocrinol Metab.* 2001;86:4576-84.
6. Mall G, Hubig M, Buttner A, Kuznik J, Penning R, Graw M. Sex determination and estimation of stature from the long bones of the arm. *Forensic Sci Int.* 2001;117:23-30.
7. Meyerrose GE, Wong CY, Yung BC, Sostre S. Men, women, and bone scintigraphy: gender identification on bone scans. *Clin Nucl Med.* 1994;19:636-9.
8. Luo L, Wang M, Tian Y, Duan F, Wu Z, Zhou M, et al. Automatic sex determination of skulls based on a statistical shape model. *Comput Math Methods Med.* 2013;2013:251628.
9. Takahashi Y, Miyazaki H, Ichinose A, Nakano T, Asamoto K, Kakizaki H. Anatomy of deep lateral and medial orbital walls: implications in orbital decompression surgery. *Orbit.* 2013;32:409-12.
10. Turvey TA, Golden BA. Orbital anatomy for the surgeon. *Oral Maxillofac Surg Clin North Am.* 2012;24:525-36.
11. Khademi Z, Bayat P. Computed tomographic measurements of orbital entrance dimensions in relation to age and gender in a sample of healthy Iranian population. *J Curr Ophthalmol.* 2016;28:81-4.
12. Ezeuko VC, Udemezue OO. Sex cannot be determined through orbital margin anthropometry: A radiologic study of the aditus orbitae of adult Igbo of Nigeria. *Annals of Bioanthropology.* 2015;3:50.
13. Adebisi S. Sex identification from the skull of the Hausa/Fulani in Northern Nigeria. *Annals of African medicine.* 2003;2:22-6.
14. Ezeuko C, Aligwekwe A, Udemezue O, Ejimofor O. Orbit dimensions and bony interorbital distance in south-east Nigerians: A radiologic study. *J Exp Clin Anat.* 2007;6:47-50.
15. Ebeye O, Otikpo O. Orbital index in Urhobos of Nigeria. *IOSR Journal of Dental and Medical Sciences.* 2013;8:51-3.
16. Munguti J, Pamela M, Fawzia B. Sex differences in the cranial and orbital indices for a black Kenyan population. *International Journal of Medicine and Medical Sciences.* 2013;5:81-4.
17. Ezeuko VC, Iniabohs O, Ferdinand A. Radiologic evaluation of the orbital index among the Igbo Ethnic Group of Nigeria. *Eur j anat.* 2015;19:9-14.
18. Memarian A, Aghakhani K, Mehrpisheh S, Fares F. Gender determination from diagnostic factors on anteroposterior pelvic radiographs. *J Chin Med Assoc.* 2017;80:161-8.

Original Research Paper

Age Estimation Using Non Destructive Approach : A Comparative Forensic Analysis

1. **Kusum Singal**, B.D.S, M.Sc (Forensic Science) Senior Research Fellow, Department of Genetics (Forensic Science), Maharshi Dayanand University, Rohtak
2. **Neelkamal Sharma**, Assistant Professor, Department of Genetics, Maharshi Dayanand University, Rohtak
3. **S.C. Narula**, Professor, Department of Periodontics, PGIDS, Rohtak
4. **Vikas Kumar**, Senior Research Fellow, Department of Genetics, Maharshi Dayanand University, Rohtak
5. **Permila Singh**, Assistant Professor, Department of Statistics, Government College for Women, Rohtak

ABSTRACT :

Introduction: In the field of forensic science, age assessment through teeth is believed to be the most appropriate and reliable source of age estimation in contrast to other skeletal remains.

Aims and Objectives: The present examination was directed with the aim (i) To assess the area of pulp (AP) and area of tooth (AT) of various teeth using digital dental radiographs (ii) To correlate chronological age of the subjects with AP/AT.

Materials and Methods: The sample consisted of Radiovisiographs (RVGs) of 320 subjects (160 males and 160 females) having age ranged from 15 to 54 years. Two radiographic measurements (AP and AT) were performed on RVGs of four monoradicular teeth (maxillary canine, mandibular canine, maxillary second premolar and mandibular first premolar) using computer-aided imaging software ADOBE Photoshop version CS6. AP/AT was calculated for each sample. Data was subjected to correlation and regression analysis using SPSS version 21.

Results: A significant negative correlation was reported between chronological age and AP/AT of four teeth. Gender-wise and teeth-wise linear regression equations were derived. For analysis, Pearson correlation and descriptive statistics was done. AP/AT of maxillary second premolar was better correlated with chronological age followed by maxillary canine in both the genders.

Conclusion: The outcome of study demonstrated that the AP/AT of monoradicular teeth is a reliable statistical tool for age estimation. Maxillary second premolar and Maxillary canine was observed to be more appropriate for age estimation when contrasted with mandibular canine and mandibular first premolar.

Corresponding Author

Dr. Neelkamal Sharma

Assistant Professor,

Department of Genetics, MDU Rohtak

Contact : +91 97285-34170

E-mail : neelforensics@gmail.com

Article History:

Received: 20 October, 2018

Received in revised form: 20 December, 2018

Accepted on: 20 December, 2018

Available online: 1 June 2019

KEYWORDS : Age Estimation, Radiovisiographs, Statistical, Reliable, Non-Destructive, Forensic Anthropology, Statistical Tools, Regression etc.

INTRODUCTION :

Age estimation is required in both living as well as dead. But in present, worldwide socio-political scenario, there is an growing demand of age estimation in living persons like instances associated with refugees, asylum seekers, immigrants etc wherein other skeletal remains cannot be used for age estimation.¹ That's why in living individuals specifically, radiographic age estimation approach sounds to be most favored one technique due to their simple, feasible and nondestructive nature.² Although radiographic age estimation can be performed using radiographic images of bones as well as teeth however when compared individually teeth being much less influenced by taphonomic factors was determined to

better age indicator. Precise and accurate age estimate from teeth makes them one of the most appropriate evidence.^{3,4} Moreover, methodologies like digitization of all radiographs and computer-assisted imaging software will lessen the subjective errors and therefore upgrade the unwavering quality, exactness, and accuracy of results.^{6,7}

Firstly, Cameriere (2004) utilized computerized periapical radiographs to check pulp tooth area ratio and evaluate secondary dentine deposition in a canine tooth only without considering the sexual orientation factor.⁸ Numerous examinations have been done till date to discover the connection of tooth area ratio with age and this method was observed to be exceptionally feasible in age estimation system

in distinctive populations.⁹⁻¹⁴ Because of the fluctuation of results, there is a strong need to derive teeth specific and gender particular age estimation models.¹⁵

The present study was conducted in the Department of Genetics (Forensic Science), Maharshi Dayanand University (MDU) Rohtak, Haryana with plan to measure AP/AT on RVGs of various teeth (maxillary canine, maxillary second premolar, mandibular canine, and mandibular first premolar) utilizing digital imaging software and to derive teeth-specific and gender-specific regression equations and models for age estimation among Haryana population.

MATERIALS AND METHODS :

Study protocol was reviewed and permitted by the Institutional Human Ethical Committee of Maharshi Dayanand University Rohtak (Ethical clearance dated 4th July 2017). The sample consisted of 1280 RVGs belonging to 320 subjects (160 males and 160 females) with age range of 15 to 54 years. RVGs were gathered from subjects visiting to the Department of Oral Diagnosis Medicine and Radiology, Post Graduate Institute of Dental Sciences (PGIDS), Rohtak and PDM Dental College and Research Institute, Bahadurgarh, Haryana for their routine dental treatment. The subjects were separated into 4 age gatherings; each of them consisted of an interim of 10 years as follows: group A (15-24 years), group B (25-34 years), group C (35-44 years), group D (45-54 years) (Table 1).

Gnatus radiographic imaging system with standard specifications (7-10 mA, 0.05 sec exposure time, 70 kV voltage), Kodak sensor and XCP-CP sensor holder were used for taking RVGs of the study subjects. Radiographic images were taken only after performing the oral clinical examination. To normalize the angulations and magnification errors amongst x-rays, grid was used and all measurements were taken in the form of ratios only.

Inclusion criteria :

- Presence of 4 selected teeth (Maxillary canine, mandibular canine, maxillary second premolar and mandibular first premolar).
- RVGs chosen were of the subjects matured in the vicinity of 15 and 54 years.
- The chosen teeth were completely emitted into the oral cavity.
- Only good quality radiographs having good contrast were selected.

Exclusion criteria :

- Teeth with any pathology, for example, caries or periodontitis were excluded.
- Malaligned or pivoted teeth and teeth with any prosthetic fittings were not included.

- Radiographs showing poor visibility, distorted image were also excluded from the study.

Radiographic Measurements : Radiographic pictures of all teeth were dealt with utilizing computer-assisted imaging program ADOBE Photoshop version CS6. All the radiographs were subjected to the two radiographic measurements (i) Area of the tooth (AT) (ii) Area of pulp (AT) as proposed by Cameriere et al.¹²⁻¹⁵ For AT, twenty focuses were set on the tooth outline and tooth area were displayed by the program and for AP, ten focuses were marked on the outline of the tooth pulp chamber on all RVGs. All traced ten points were joined to assess the pulp area. The proportion of pulp area and tooth area was calculated as AP/TA.

Statistical Analysis : All the measurements and chronological age of the subjects was entered into the Microsoft Excel sheet. Same researcher performed all the measurements. To test the intra-observer reproducibility and reliability, a random sample of 20 RVGs was reconsidered following an interim of 15 days. Intraobserver errors were quantified using concordance coefficient ($p < 0.5$). Statistical analysis was performed utilizing SPSS (Statistical Package for the Social Sciences) version 21. Different statistical tests were used to determine the age of the subjects. Pearson's correlation coefficient was figured to decide the relationship between AP/TA and chronological age. Descriptive statistics was performed gender-wise and for each tooth separately. P value was set at < 0.05 to check the significance level of the variables. Correlation coefficients were assessed for every tooth as well as for males and females independently. The assessed age was then contrasted with the chronological age of the subject.

RESULTS :

1280 RVGs of 320 subjects were analyzed in the present examination after satisfying the selection criteria. Sample divided into four study groups (Table 1).

Table 1: Age and Gender Wise Distribution of Subjects.

Age Group	No. of Males	No. of Females	Total no. of Subject
Group A (15-24)	40	40	80
Group B (25-34)	40	40	80
Group C (35-44)	40	40	80
Group D (45-54)	40	40	80
Total	160	160	320

A very good agreement was found between intraobserver measurements. $P < 0.05$ was considered significant. Descriptive Statistics was done that demonstrated the mean chronological age, mean of AP/AT and standard deviations of AP/AT (Table 2 for females and Table 3 for males). Pearson correlation coefficients between chronological age and

predictive variables were also ascertained (Table 4,5). The significance threshold was set at 5%. Linear regression equations were developed for different teeth in males and females study samples independently (Figure 1 and 2).

Table 2 : Descriptive Statistics of the Female Study Subjects

Teeth	N	Mean of AP/AT	Std. Deviation of AP/AT
Maxillary Canine	160	.1264186	.03367179
Mandibular Canine	160	.1399574	.04831820
Maxillary Second Premolar	160	.1382047	.03984417
Mandible First Premolar	160	.1342175	.04101365

Table 3 : Descriptive Statistics of the Male Study Subjects

Teeth	N	Mean of AP/AT	Std. Deviation of AP/AT
Maxillary Canine	160	.1435432	.03494493
Mandibular Canine	160	.1254999	.04295860
Maxillary Second Premolar	160	.1404678	.04000472
Mandible First Premolar	160	.1449072	.04288036

Table 4 : Pearson Correlation Between AP/AT and Chronological Age and Standard Error of Estimate For Different Teeth in Female Study Sample

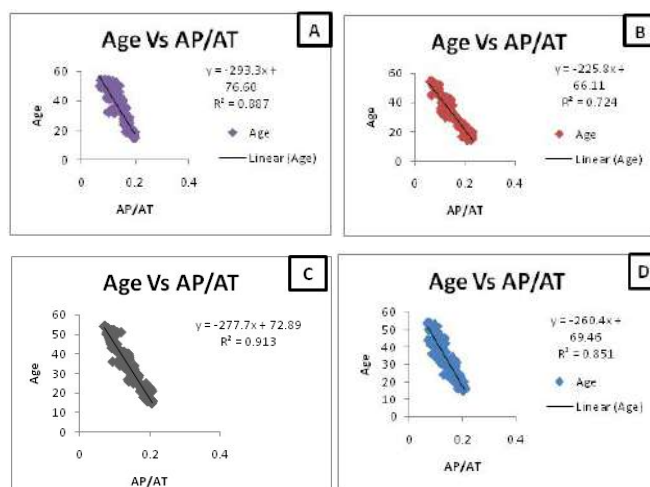
Tooth	Pearson Correlation (r)	Std. Error of Estimate	P
Maxillary Canine	-.942	3.8831	<0.001
Mandibular Canine	-.923	4.4804	<0.001
Maxillary Second Premolar	-.956	3.4135	<0.001
Mandible First Premolar	-.851	6.2439	<0.001

A strong negative correlation was found between chronological age and AP/AT. Mean chronological age for the study subjects was 34.500 with standard deviation 11.579.

Table 5 : Pearson Correlation Between AP/AT and Chronological Age and Standard Error of Estimate For Different Teeth in Male Study Sample

Tooth	Pearson Correlation (r)	Std. Error of Estimate	P
Maxillary Canine	-.963	3.1406	<0.001
Mandibular Canine	-.885	5.4039	<0.001
Maxillary Second Premolar	-.968	2.8957	<0.001
Mandible First Premolar	-.846	6.1999	<0.001

Figure 1: Showing scatter diagram and regression equations for all the selected teeth for the female study samples. A) Maxillary Canine B) Mandibular Canine C) Maxillary Second Premolar D) Mandibular First Premolar. (y represents estimated age, x is AP/AT, R^2 indicates regression coefficient)

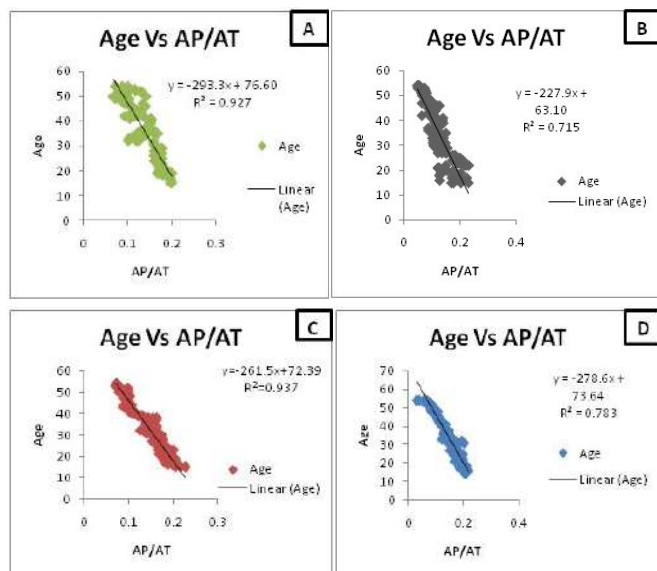


DISCUSSION :

Age estimation is one of the most critical assignments to be finished by specialists in the field of Forensic Anthropology and Forensic Medicine. Several techniques for age estimation using skeletal remains including dental evidence have been developed.¹⁰ But while utilizing bones as an apparatus for age estimation, their strong reliance on the operator and their poor precision in ages displays their genuine reliability confinement as contrasted with dental remains.¹¹

In recent years, different dental age estimation strategies in view of morphological, biochemical, radiological and histological qualities of dentition have been used by specialists for medicolegal purposes.^{16,17} Radiographic dental age

Figure 2 : Showing scatter diagram and regression equations for all the selected teeth for the male study samples. A) Maxillary Canine B) Mandibular Canine C) Maxillary Second Premolar D) Mandibular First Premolar (y represents estimated age, x is AP/AT, R² indicates regression coefficient)



estimation procedures are considered to be one of the most reliable and standard means for age estimation. These methods are based on evaluation of various parameters. Assessment of secondary dentin deposition is one that can be observed radiographically by the reduction in pulp area.^{10,11}

Literature provided a no. of studies based on quantification of the decrease in dental pulp space for age estimation using dental radiographs. Previous examinations demonstrated that the median error rate between the estimated age and the chronological age was approximately four years. Most of the studies were performed on maxillary and mandibular canine teeth using peri-apical and panoramic radiographs only.^{18,19} That why there is a strong need for effective, nondestructive and reliable statistical tool for age estimation with less error rate.

Present study was performed with the aim to determine whether the correlation between dental pulp dimensions and age are significant enough for use as an independent age determination strategy or not. The data for the present study consisted of the tooth and pulp measurements done on 1280 RVGs of the maxillary canine, maxillary second premolar, mandibular canine and mandibular first premolar teeth collected from 320 individuals (160 males, 160 females) having age range 14-54 years (divided into four groups A, B, C,

and D)(Table 1). Only monoradicular teeth were chosen for the study. Purpose behind choosing canines and premolars was that these are less inclined to attrition and durable in nature that means can be assessed in the older age groups. Either from the left or right side teeth was decided for taking RVGs, in light of the fact that it has been clarified that there is no critical difference between permanent teeth from the left or right side of the jaw.¹⁷

Utilization of RVGs rather than periapical radiographs conquers the necessity for scanning and digitalization of radiographs. Paralleling technique was used for better and accurate RVGs. This technique causes less image distortion. Roots and crowns of all the selected teeth were indicated extremely well empowering the study of pulp chamber and tooth. Dental age was evaluated using pulp tooth area ratio method.

Three sets of data were considered; tooth and pulp measurement, ratio values for tooth/pulp measurement, and mean values of AP/AT for different teeth in male and female study group (Table 2, 3). Descriptive Statistics was performed for male and female study subjects separately. In female study subjects mean value AP/AT varies from 0.12-0.13 with standard deviation .03+0.1, while in male study subjects it was 0.12-0.14 with standard deviation .03+0.1 (Table 2,3).

Stepwise regression analysis was done to derive tooth-specific and gender-specific formula for age estimation. Scatter plot curve showed a significant negative correlation between chronological age and AP/AT indicating that all the selected variables contributed significantly to the age estimation yielding the unique linear regression formula for various teeth. All the morphological variables explain approximately 71.5% to 93.7% of variations in estimated chronological age (figure 1 and figure 2).

The results demonstrated that tooth pulp area ratio changes with age. The obtained regression plots showed that data point equations did not plot outside the expected boundaries and explaining the variations ($R^2=71.5\%-93.7\%$) (figure 1 and 2). Regression equations obtained for maxillary canine and mandibular canine were similar to the previous studies conducted.^{14,15} The variations found may be due to the difference in sample size and geographic variations of the study subjects as the previous studies were performed on Portuguese population by Cameriere et al, in south Indian population by Jeevan et al. and in Iranian population by Dehghani et al.¹⁴⁻²⁰

Pearson correlation coefficient showed a negative and significant correlation between radiographical variable i.e. AP/TA and chronological age ($r = -.942$ for maxillary canine, $r = -.923$ for mandibular canine and $r = -.956$ for maxillary

second premolar and -0.851 for mandibular first premolar) in females (Table 4). In males study samples, Pearson correlation coefficient varies from -0.846 in respect to mandibular first premolar to -0.968 in respect to maxillary second premolar (Table 5). Better value of Pearson coefficient indicated that all morphological variables were significantly correlated with age and were equivalently justified by results of previous studies.²¹⁻

24

The standard error of estimate fluctuates from 3.4-6.2 years i.e minimum in maxillary second premolar and maximum in the mandibular first premolar indicating that maxillary second premolar is a superior indicator for age as compared to other studied teeth (Table 3,4). The mean difference between actual and estimated age was lower for maxillary second premolar. When maxillary and mandibular canines were compared, upper ones (3.00 ± 0.9) turned to be a better age predictor as compared to lower ones (3.20 ± 1.12). This is in accordance to the study directed by Cameriere's et al.¹⁴⁻¹⁵ Maxillary teeth showed better correlation reason might be because of secondary dentine deposition found to be more regular, distinct and consistent in maxillary teeth as compared to those of mandibular teeth.²⁵ It is believed that the standard error of estimate ought to be equivalent or less than 10 years to be considered as adequate for age estimation.

In 2013, Joseph et al. played out an examination on mandibular premolars using tooth pulp area method.²⁶ No statistically significant difference was observed between evaluated age and chronological age in the that study. Singaraju et al. likewise concluded that there is no significant difference between estimated age and chronological age in a study conducted by them on single-rooted teeth, using orthopantomographs which are in agreement to results of the present study.²⁷

Among the four age groups studied, the best outcome for age estimation was seen in initial 3 age groups i.e Group A (15-24), B (25-34), C (35-44) with the mean difference between chronological and estimated age of 2.18 ± 0.84 , 3.11 ± 0.6 and 3.21 ± 0.44 years respectively. Group D showed the least significant results (4.11 ± 2.84). Age group specific results are in accordance with outcomes of past examination led by Jeevan et al. recommending the application of these age formulas in younger age group as compared to older age group.¹⁸ Better results in the younger age groups can be explained by the factors that quality of RVGs may get diminished with age. Another factor for this can be regular deposition of secondary dentin in younger than the older ones. The outcomes showed that this non-destructive and quick strategy provides a very less error of age estimation (> 5 years) as compared to the most skeletal age estimation tools.

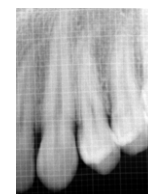
CONCLUSION :

Present study concluded that non-destructive approach i.e radiographic approach is a extraordinary method for dental age estimation as compared to other strategies. The pulp tooth area ratio method was observed to be a reliable, statistically accurate age indicator in living individuals. The highest correlation was shown by maxillary second premolar irrespective of gender. Maxillary canine proved to be the other suitable teeth followed by mandibular canine and mandibular first premolar amongst all studied teeth indicating the significance of development of teeth-wise linear equations for age estimation. Future research can be directed utilizing numerous monoradicular as well as multi radicular teeth simultaneously to enhance accuracy and to diminish standard error of age estimation.

Abbreviations

RVGs- Radiovisiograph

AP/AT- Area of Pulp/Area of tooth



FUNDING :

The financial support provided by UGC, New Delhi in the form of 'UGC-JRF Fellowship' (Letter No F.15-9 (JUNE 2014)/2014(NET), helped the author in preparation of this research article.

ACKNOWLEDGEMENTS :

The authors are extremely thankful to Dr. Mandeep Singh Virdi, Principal, PDM Dental College and Research Institute, Bahadurgarh for allowing us for data collection. Our sincere thanks to Dr. Cheena Singh, Assistant Professor, Department of Oral medicine and Radiology, PDIGS, Rohtak for her assistance. We are deeply indebted to all the people who have participated in this study by providing their RVGs.

REFERENCES :

1. Sharma R, Srivastava A. Radiographic evaluation of dental age of adults using Kvaal's method. J Forensic Dent Sci. 2010; 2(1): 22-26.
2. Cunha E, Baccino E, Martrille L, Ramsthaler F, Prieto J, Schuliar Y. The problem of aging human remains and living individuals: a review. Forensic Sci Int. 2009;193(3):1-13.
3. D'Ortenzio L, Prowse T, Inskip M, Kahlon B, Brickley M. Age estimation in older adults: Use of pulp/ tooth ratios calculated from tooth sections. Am J Phys Anthropol. 2017; 8(2):1-10.
4. Jain S, Nagi R, Daga M, Shandilya A. Shukla A. Tooth coronal index and pulp/tooth ratio in dental age estimation on digital panoramic radiographs-A comparative study. Forensic Sci Int. 2017; 277:115-21.

5. Zafer JE. Age estimation from pulp/tooth area ratio in maxillary incisors among Egyptians using dental radiographic images. *J Forensic Legal Medicine*. 2011; 33:62-65.
6. Shah P, Venkatesh R. Pulp/tooth ratio of mandibular first and second molars on panoramic radiographs: An aid for forensic age estimation. *J Forensic Dent Sci*. 2016; 8: 112-18.
7. Seema B, Vinod VC, Priyanka N, Ashwin D. Estimation of Age by Pulp-Tooth Area Ratio Using Three Computer Aided Software's. *Quality in Primary Care* 2016; 24(4): 161-166.
8. Cameriere R, Ferrante L, Cingolani M. Variations in pulp/tooth area ratio as an indicator of age: a preliminary study. *J Forensic Sci*. 2004;49:317-9.
9. Cameriere R, Brogi G, Ferrante L, Mirtella D, Vultaggio C, Cingolani M. Reliability in age determination by pulp/tooth ratio in upper canines in skeletal remains. *J Forensic Sci*. 2006;51:861-4.
10. Marroquin T, Karkhanis S, Kvaal S, Vasudavan E, Kruger M. Age estimation in adults by dental imaging assessment systematic review, *Forensic Sci Int*. 2017; 275:203-11.
11. Singal K, Sharma N. Dental radiology: An adjunctive aid in age estimation. *Annals and Essences of Dentistry*. 2017;9(3):8c-11c.
12. Cameriere R, Ferrante L, Belcastro MG, Bonfiglioli B, Rastelli E, Cingolani M. Age estimation by pulp/tooth ratio in canines by periapical X-rays. *J Forensic Sci*. 2007;52:166-70.
13. Cameriere R, De Luca S, Aleman I, Ferrante L, Cingolani M. Age estimation by pulp/tooth ratio in lower premolars by orthopantomography. *Forensic Sci Int*. 2012; 214: 105-112.
14. Cameriere R, Ferrante L, Belcastro MG, Bonfiglioli B, Rastelli E, Cingolani M. Age estimation by pulp/tooth ratio in canines by mesial and vestibular X-rays. *J. Forensic Sci*. 2007; 52:1151-55.
15. Cameriere R, Cunha E, Sassaroli E, Nuzzolese E, Ferrante L. Age estimation by pulp/tooth area ratio in canines: Study of a Portuguese sample to test Cameriere's method. *Forensic Sci Int*. 2009; 193: 128e1-128e6.
16. Babshet M, Acharya AB, Naikmasur VG. Age estimation in Indians from pulp/tooth area ratio of mandibular canines. *Forensic Sci Int*. 2010; 197:125-29.
17. Feisthamel A, Kay M. Forensic Age Determination By Digital Radiographic Measurement of the Dental Pulp. MSU Graduate Thesis; 2012.
18. Jeevan MB, Kale AD, Angadi PV, Hallikerimath S. Age estimation by pulp/tooth area ratio in canines: Cameriere's method assessed in an Indian sample using radiovisiography. *Forensic Sci Int*. 2011; 204: 209e1-209e5.
19. Dehghani M, Shadkam E, Ahrari F, Dehghani M. Age estimation by canines' pulp/tooth ratio in an Iranian population using digital panoramic radiography, *Forensic Sci Int*. 2018;220: 120-124.
20. Singla Y, Sharma R, Mishra A, Sharma R. "Age estimation by forensic Odontology" *International Journal of Current Research in Life Sciences*. 2018; 7 (05): 2097-2100.
21. Shrestha M. Comparative Evaluation of Two Established Age Estimation Techniques (Two Histological and Radiological) by Image Analysis Software using Single Tooth. *J Forensic Res*. 2014; 5:237-9.
22. Nallamala S, Guttikonda VR, Manchikatl PK, Taneeru S. Age estimation using exfoliative cytology and radiovisiography: A comparative study. *J Forensic Dent Sci*. 2017;9:144-8.
23. Juneja M, Devi YB, Rakesh N, Juneja S. Age estimation using pulp/ tooth area ratio in maxillary canines-A digital image analysis. *J Forensic Dent Sci*. 2014;6:160-5.
24. Azevedo Ade C, Alves NZ, Michel-Crosato E, Rocha M, Cameriere R, Biazevic MG. Dental age estimation in a Brazilian adult population using Cameriere's method. *Braz Oral Res*. 2015;29 (4):114-8.
25. Afify MM, Zayet MK, Mahmoud NF, Ragab AR. Age estimation from pulp/tooth area ratio in three mandibular teeth by panoramic radiographs: Study of an Egyptian sample. *J Forensic Res*. 2014;5:1-5.
26. Joseph CC, Reddy BH, Cherian NM, Kannan SK, George G, Jose S. Intraoral digital radiography for adult age estimation: A reliable technique. *J Indian Acad Oral Med Radiol*. 2013;25:287-90.
27. Singaraju S, Sharada P. Age estimation using pulp/tooth area ratio: a digital image analysis. *J Forensic Dent Sci*. 2009; 1: 37-41.

Original Research Paper

Control measures for *Hospitalitermes Monoceros*, a major pest of sugarcane by using medicinal plants

1. Lovleen, Assistant Professor*

2. Prerna, M.Sc. Student*

*Department of Zoology, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara

ABSTRACT :

The present research execution deals with control of *Hospitalitermes monoceros* (termite) common pest of sugarcane, using water and ethanolic extract of medicinal plants including *Aegle marmelos*, *Calotropis procera* and *Datura stramonium*. For execution of present research exploration, specific insects were captured from sugarcane field and acclimatized to laboratory conditions. Thereafter, insects were exposed to water and ethanolic extract of selected medicinal plants for 24 hours. Mortality was reported after 24 hours, in each experimental stock along with respective control. The procured data was analysed by applying Z- test and it was concluded that selected botanical extracts induced statistically significant mortality in treated pest as compared to untreated pests ($p < 0.05$). Furthermore, it was observed that ethanolic extract of selected medicinal plants induced significant mortality in specific pest than control stocks.

Corresponding Author :

Dr. Lovleen

Assistant Professor,

Department of Zoology, School of Bioengineering and Bioscience, Lovely Professional University, Phagwara, Punjab

Contact : +91 94178-52950

E-mail : lovleenmarwaha@gmail.com

Article History:

Received: 16 July, 2018

Received in revised form: 23 December, 2018

Accepted on: 23 December, 2018

Available online: 1 June 2019

KEYWORDS : *Hospitalitermes monoceros*, *Aegle marmelos*, *Calotropis procera*, *Datura stramonium*, ethanol extract and water extract

INTRODUCTION : *Saccharum officinarum* (Sugarcane) is a species of tall perennial grass that belong to Tribe-Andropogoneae. The specific crop requires 10-18 months to get fully mature. Sugarcane is used in production of sugar, tomato ketchup, pickles, ethanol, biofuel, bioplastic, and furthermore, biomass is used for electricity production. The top producers are Brazil, India, China, Thailand, and Pakistan. The average worldwide sugarcane yield of sugarcane is approximately 60 t/ha and almost 20 to 40% of crops is destroyed by insect pest annually. Common pests of sugarcane includes *Emmalocera depressella*, *Chilo infescatellus*, *Scirpophaga excerptalis*, *Chilo Saccharifagus Indicus*, *Melanaspis glomerata*, *Pyrilla purpusilla*, *Aleurolobus barodensis*, *Colletotrichum falcatum*, *Ustilago scitaminea*, *Cephalosporium sacchari*, *Phytoplasma*, *Xanthomonas albilineans*, *Odontotermes obesus*, *Cavalerius excavatus*, *Saccharioccus sacchari*, *Hilotrachia consanguinea* and *Dysdercus cingulatus* etc. To control specific pests various synthetic chemicals are excessively implemented and although these chemicals have contributed to increased agricultural productivity, they also possess potential clastogenic and cytotoxic properties at the same time^[1-9].

Therefore, target of present research execution is to control common pest of sugarcane by using water and ethanolic extract of few medicinal plants including *Aegle marmelos*, *Calotropis procera* and *Datura stramonium*. The adult termite samples were collected from nearby sugarcane field and acclimatized to controlled conditions of temperature 25 ± 2 °C and relative humidity 40-60 % for 24 hours. Subsequently, adults were exposed to water and ethanolic extract separately, with respective control, for 24 hours under controlled conditions of insectory. Thereafter, mortality was recorded in each exposed stock, and was compared with control stocks. Each experiment was repeated thrice. The procured data was scrutinized by applying Z-test.

$$Z = \frac{p_1 - p_2}{\sqrt{p(1-p)\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

$$p = \frac{n_1 p_1 + n_2 p_2}{n_1 + n_2}$$

MATERIAL AND METHODS :

Test Organism: *Hospitalitermes monoceros* was selected for present work, as it is considered as major pest of sugarcane crop. The Adult organism are cream coloured with a black head (Figure 1 and 2).

Figure 1 :
Termite Sample



Figure 2 : Termite Infected Plant Sample



The Complete development require 32-90 days and life span is upto 7 to 12 months. Generally, termite attacks shoot and root regions or enter through cut ends of sugarcane stalk. Then it feeds on the inner tissue causing the plant to become flaccid and ultimately results in wilting of plant. Furthermore it affects the germination of crop and reduces the sugarcane production.

Processing of Medicinal Plants : For our study leaves, roots and fruits of selected medicinal plants were collected from the herbal garden of Lovely Professional University, Phagwara. Plant parts were washed with distilled water and shade dried for 20-25 days. After drying, a fine powder was made using electric grinder. The finely powdered product was used further for preparation of water and ethanolic extract (Figure 3-5).

Ethanol extract preparation from selected medicinal plants seeds: Dried powder of *Aegle marmelos*, *Calotropis procera* and *Datura stramonium* were used separately for preparation of 1% ethanolic extract. Dried plant powder was boiled in soxlet for eight hours continuum, thereafter extract

was dried using vacuum rotar, set at 45 °C and 151 RPM, for 2 hours. Finally, obtained extract was used as stock solution, which was stored at 4 °C for further use^[10].

Figure 3 : Dried Plant Parts of *Datura* and Processing



Figure 4 : Dried Plant Parts of *Calotropis* and Processing



Figure 5 : Dried Plant Parts of *Aegle Marmelos* and Powder



Water extract preparation from selected medicinal plants

leaves: 1% water extract had been prepared from dried plant parts powder of *Aegle marmelos*, *Calotropis procera* and *Datura stramonium* separately. The medicinal plants had been boiled in distilled water for 8 hours continuously in Soxlet apparatus and thereafter was dried with vacuum rotar at set at 45 °C and 151 RPM, for 2 hours. The obtained water extract had been stored in refrigerator and had been used to carry out further experiments (Figure 6-7).

Figure 6 :**Preparation of Botanical Extract by Soxlet Apparatus for Extract****Figure 7 : Processing of Extract by Vacuum Rotar****Exposure of termites to water and ethanolic extract :**

Acclimatized termite adults were exposed to water and ethanolic extract, separately along with respective control. After 24 hours, mortality was reported in each experimental set up along with respective control. The obtained data was scrutinized with Z-test, in each experimental set up, with respective control.

RESULTS AND DISCUSSION :

It was observed that ethanolic extract of medicinal plants induce greater mortality than water extract, for each selected medicinal plant. Furthermore, it was observed that water and ethanolic extract induce statistical significant mortality in exposed termite samples (**Table 1**), in comparison to control samples, when analysed through Z-test ($p < 0.05$).

Various environmental factors including affect distribution and infestation of pests on agricultural crops. Further, many scientific studies are available,

which deal with finding suitable control measures for specific pests. It has been reported that top borer *Scirpophaga novella*, infestation is affected by various factors like morphological, physiological and chemical factors^[11-12], whereas in another report it was observed that high level of silicon in soil increases the level of attack of insects on sugarcane crop^[13]. In one exploration, it was found that in *Dysdercus cingulatus*, eggs which were kept in salt water slightly take longer time to develop to nymph of the insect. It had reported that red cotton bug exhibit differential toxicity to certain specific synthetic chemical insecticides^[14]. Similarly, Cowie, et al., reported that termites are more susceptible to organochlorine insecticides^[15]. Few reports are available in scientific literature about insecticidal properties of selected medicinal plants as in one report, Aegle plant possess insecticidal properties^[16], whereas Begum, et al., observed that Calotropis induced toxic effect on Against *Musca domestica*^[17]. Jawalkar, et al., reported that Datura is responsible for causing mortality in Sitophilus oryzae in stored wheat grain. Similarly during present exploration, influence of medicinal plants on control of Hospitalitermes monoceros had been carried out^[18]. The concerned pest had been exposed to water or ethanolic extract of medicinal plants for continuum of 24 hours. It was observed that water and ethanolic extract induced statically significant mortality in treated pests than control population. It was further scrutinized that ethanolic extract is more effective than water extract.

CONCLUSION :

The use of pesticides is beneficial to agriculture, as pesticides are helpful in increasing food production and amelioration of vector borne diseases. Therefore, use of such agrochemicals is inevitable in agriculture. Although there are benefits of using pesticides, yet their use resulted in serious health problems for living organism including humans as immune suppression, cancer, hormone disruption, bioaccumulation, biomagnifications, genetic resistance and various abnormalities. Therefore, it is indispensable that alternatives of pesticide should be investigated by implementing medicinal plant extracts and integrated pest management.

Table 1 : Statistical Analysis of Termite Exposed to Medicinal Plants, Water and Ethanolic Extracts for Continuum of 24 Hours, Separately.

Medicinal plant	Termite exposed to water extract	Mortality in treated stock	Mean	Termite in control stock	Mortality in control stock	Mean	Z * Value	Termite exposed to ethanolic extract	Mortality in treated stock	Mean	Termite in control stock	Mortality in control stock	Mean	Z * Value
Aegle	180	138	46	180	02	1	14	180	143	48	180	08	3	18.7
Calotropis	180	128	43	180	09	3	16.5	180	130	43	180	03	1	14
Datura	180	145	48	180	04	1	6	180	148	49	180	05	2	14

ACKNOWLEDGEMENT :

Authors are very thankful to management of Lovely Professional University, Phagwara, for providing all facilities required to execute present research objectives.

REFERENCES :

1. Chaudhry A, Anand PK, Geeta, Singh S. and Lovleen. Ectopic pairing of the intercalary heterochromatin in the organophosphate pesticide treated mosquito chromosomes (Culicidae: Diptera). Cytologia 2006; 71(4): 431-437.
2. Chaudhry A, Lovleen. Evaluation of mutagenic potential of carbaryl by dominant lethal test on *Culex quinquefasciatus*. Journal of Cytology and Genetics, 2008; 9: 37-44.
3. Chaudhry A, Lovleen. Chromosomal aberrations and dominant lethal test based genotoxicity assessment of dicofol by using mosquito genome. Journal of Cytology and Genetics, 2010; 11: 11-20.
4. Lovleen. In vivo mutagenicity assessment of ethion pesticide using polytene chromosomes of *Anopheles culicifacies* (Diptera: Culicidae). Asian Journal of Pharmacology and Toxicology, 2015; 3(9): 1-6.
5. Lovleen, Asma J. In vivo genotoxicity evaluation of thiamethoxam using *Drosophila melanogaster*. International Journal of ChemTech Research 2017; 10(4) :481-488.
6. Lovleen, Khan A, Rani A, Devi L, Angurana R, Amin DS. Reprotoxicity profiling of Bispayribac Sodium, Pinoxaden and Spinosad Pesticides on *Drosophila melanogaster*. Toxicology International 2017a; 24(3): 233-239.
7. Lovleen, Angurana R, Amin DS. Clastrogenic Instruct of Sodium Bispayribac and Pinoxaden Herbicides on *Drosophila melanogaster*. Toxicology International, 2017b; 24(2): 178-184.
8. Marwaha L, In vivo Genotoxicity Evaluation of Carbaryl Pesticides Using Polytene Chromosomes of *Anopheles culicifacies* (Diptera: Culicidae). Toxicology International 2016; 23(1): 4-11.
9. Marwaha L. Genotoxicity Assessment of an Organophosphate Insecticide, Ethion Employing Mosquito Genome (Diptera: Culicidae). Toxicology International 2015; 22(2): 25-33.
10. Gidado A, Zainab AA, Hadiza MU, Serah DP, Anas HY, Milala MA. Toxicity studies of ethanol extract of the leaves of *Datura stramonium* in rats. African Journal of Biotechnology 2017; 6 (8): 1012-1015.
11. Khalio A, Ashfaq M, Akram W, Choi JK, Lee JJ. Effect of Plant Factors, Sugar Contents, and Control Methods on the Top Borer (*Scirpophaga nivella* F.) Infestation in Selected Varieties of Sugarcane. Entomological Research 2007; 35(3): 153-160.
12. Kvedaras OL, Keeping MG. Silicon impedes stalk penetration by the borer *Eldana saccharina* in sugarcane. Entomologia Experimentalis et Applicata. 2018; 125(1): 103-110.
13. Zera AJ, Denno RP. Physiology and ecology of dispersal polymorphism in insects. Annual Review of Entomology, 1997; 42: 207-230.
14. Sontakke H, Baba I, Jain SM, Saxena RC, Bhagel AK, Jadhav VB. Fecundity and fertility control of red cotton bug (*Dysdercus cingulatus*) by the extract of *Psoralea corylifolia*. International Journal of Research in Pharmaceutical and Biomedical Sciences 2013; 4 (2): 633-635.
15. Cowie CC, Rust KF, Ford ES, Eberhardt MS, Byrd-Holt DD, Li C, et al. Full accounting of diabetes and pre-diabetes in the U.S. population in 1988-1994 and 2005-2006. Diabetes Care. 2009; 32(2): 287-94.
16. Samarasekera JK, Khambay BP, Hemalal KP. A new insecticidal protolimonoid from *Aegle marmelos*. Nat Prod Res. 2004; 18(2): 117-22.
17. Begum N, Sharma B, Pandey RS. Evaluation of insecticidal efficacy of *Calotropis Procera* and *Annona Squamosa* ethanol extracts against *Musca domestica*. J Biofertil Biopestici. 2011; 1: 101.2-6.
18. Jawalkar N, Zambare S, and Zanke S. Insecticidal property of *Datura stramonium* L. seed extracts against *Sitophilus oryzae* L. (Coleoptera: Curculionidae) in stored wheat grains. Journal of Entomology and Zoology Studies 2016; 4(6): 92-96.

Original Research Paper

Determination of Sex from the Length of Mesosternum (Body of Sternum) in Indore Region, Madhya Pradesh

1. **Pankaj Nema**, Assistant Professor, Department of Forensic Medicine & Toxicology, Index Medical College, Indore
2. **Priyanka K. Nema**, Senior Resident, Department of Ophthalmology, Index Medical College, Indore
3. **S.K. Dadu**, Professor & Head, Department of Forensic Medicine & Toxicology MGM, Medical College, Indore
4. **B.K. Singh**, Associate Professor Department of Forensic Medicine & Toxicology MGM, Medical College, Indore
5. **A.K. Rastogi**, Assistant Professor, Department of Forensic Medicine & Toxicology, AIIMS Patna (Bihar)
6. **Rahul Agrawal**, PG Resident, Department of Forensic Medicine & Toxicology MGM, Medical College, Indore
7. **Devesh Pataria**, PG Resident, Department of Forensic Medicine & Toxicology MGM, Medical College, Indore
8. **Mohit Shrivastava**, PG Resident, Department of Forensic Medicine & Toxicology MGM, Medical College, Indore

ABSTRACT :

Introduction : Skeletal remains or decomposed bodies are an important initial step in forensic investigation. Determination of skeletal Sex from unknown mutilated and decomposed bodies has been a critical issue in medico legal cases and the accuracy depends on the nature and types of material available and methods applied. Sternum is a bone which is easily retrievable even from the advance decomposed, mutilated body and also from the bundle of bone so it become very important bone for age and sex determination.

Material and Methods : In our study Total 632 subject for sex having 350 male and 282 female of age >25years was taken. Data analysis was done by using SPSS software and relevant statistical test was applied.

Results : Bisexual variation in relation to length of mesosternum was found conclusive.

Corresponding Author :

Dr. Priyanka K. Nema

Senior Resident,

Department of Ophthalmology, Index Medical College, Indore

Contact : +91-99264-48595

E-mail : k22priya@gmail.com

Article History:

Received: 29 September 2018

Received in revised form: 20 November 2018

Accepted on: 21 November 2018

Available online: 1 June 2019

KEYWORDS : Anthropometry, Bisexual variation, sex determination, Mesosternum (Sternum Body) length.

INTRODUCTION :

The cranial and pelvic bones, in addition to the long bones, are important in estimating age and identifying the sex of an individual etc. Forensic experts are left with no choice but to depend on less sexually dimorphic elements of the human skeleton such as the sternum. Thus, studies focusing on sternum have provided important information to forensic experts^[1].

Identification of an individual is very important in criminal cases like assault, murder, rape, disputed paternity, impersonation etc. and in civil cases like marriage inheritance; disputed sex etc. "Article 6 of the Universal Declaration of Human Rights" states that everyone has the right to recognition everywhere as a person before the law^[2].

Identification of dead body and proof of "corpus delicti" is essential and integral part of any criminal and civil justice delivery system throughout the world. The main part of corpus delicti (i.e. the body of the offence; the essence of crime) is the establishment of the identity of the dead body^[3,4,5].

This study is done in various region of India but is not done in central India Indore (M.P.) region. It is well known fact that study of anthropometry result, standards vary according to different races and different region, and it is advised that one should not used the study data of one place to other, that is why this study is undertaken. Another purpose of study to derived a formula for this central Indian population in medico legal cases.

Sternum is a bone which is easily retrievable even from the advance decomposed, mutilated and also from the bundle of bone, so it become very important bone for sex determination..

MATERIALAND METHOD :

Present observational Cross sectional study was done at mortuary of Department of Forensic Medicine and Toxicology M.G.M. Medical College Indore over a period of 12 month from July 2016 to June 2017. Total 632 subject for sex having 350 male and 282 female of age >25years was taken. Data analysis was done by using SPSS software and relevant statistical test was applied.

Sternum showing any pathology, fracture, gross deformity or any missing part and body with unknown age was excluded.

As routine protocol for opening the thoracic cavity during autopsy, the sternum was removed from the body by sectioning the costal cartilages just beside the costo-chondral junction. For estimation of sex the length of mesosternum was examined.



Figure 1 : Length of mesosternum : Distance from Manubrio-mesosternal junction to mesosternoxiphoidal junction in midline

RESULTS :

In females the mean length of mesosternum was 76.93 ± 7.51 , while in the male it was 92.28 ± 9.78 . The difference was found to be statistically significant ($P < 0.05$), showing a longer length of mesosternum in the males in comparison to the females.

The length of mesosternum was found to be statistically significant ($P < 0.05$), showing that length of mesosternum will have meaningful addition to the model as changes in the predictor's value are related to changes in the response variable. Thus, length of mesosternum is having importance for this model.

The regression equation obtained was:

Gender = $-0.862 + 0.02831$ Length of mesosternum in mm

If the equation value nears 1, then the gender is female and if the equation value nears 2, the gender is male. (Table 1)

Table 1 : Regression analysis: Gender versus length of mesosternum in mm

	Coefficient	't' value	P value
Constant	-0.862	-7.75	0.000*
Length of mesosternum	0.028	21.91	0.000*

Regression analysis done. F value = 480.17, P value = 0.000*

Discriminant analysis : By this method we were able to correctly identify 249 females and 282 males. The proportion of correct identification was 88.3% in females and 80.6% in males. The overall accuracy of this method was 84%. (Table 2)

The length of mesosternum was able to correctly identify the gender in 80.6% (Sensitivity) while it was able to correctly

Table 2 : Discriminant Analysis of Gender based on the Length of Mesosternum in mm

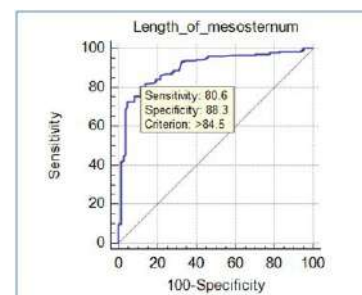
Length of Mesosternum	True Group	
	Female	Male
Female	249	68
Male	33	282
Total	282	350
N correct	249	282
Proportion	83.3%	80.6%
Overall proportion correct	84.0%	

negate the gender in 88.30% (specificity). This variable is having a good sensitivity and specificity, also according to the ROC curve, this variable is a very strong predictor of the gender as the curve is quite near to the left hand border and left top border. (Table 3)

Table 3 :

ROC Curve

[Length of Mesosternum]



Variable	Length_of_mesosternum
Classification variable	SEX_1
Sample Size	632
Positive group (Male)	350(55.38%)
Negative group (Female)	282(44.62%)

Area under the ROC curve (AUC)

Area under the ROC curve (AUC)	0.899
Standard Error ^a	0.0128
95% Confidence interval ^b	0.873 to 0.922
z statistic	31.117
Significance level P (Area=0.5)	<0.0001

^a DeLong et al., 1988

^b Binomial exact

Youden index

Youden index J	0.6887
Associated criterion	>84.5
Sensitivity	80.57
Specificity	88.30

The cut-off value of length of mesosternum is 84.5, i.e. if the length of mesosternum is >84.5, then it is a male, else it is a female.

Table No. 4 : Showing Comparison of Length of Sternum (Mean, Regression Value, Difference in Mean) Done by Various Workers

Name of Worker	Specimen		Manubrium (M) in mm		
	Sex	No.	Mean	Regression Equation Value	Difference
Dwight (1881)	M	30	105.9	2.13	16.50
	F	26	89.4	1.66	
Dwight (1890)	M	142	110.4	2.26	18.50
	F	86	91.9	1.73	
Paterson (1904)	M	310	103.7	2.07	12.70
	F	126	91	1.71	
Ashley(1956)African	M	85	96.5	1.86	13.60
	F	13	82.9	1.48	
Ashley(1956) European	M	378	104.7	2.10	13.90
	F	168	90.8	1.70	
Jit et al(1980), North India	M	312	95.35	1.83	16.75
	F	88	78.60	1.34	
Mahajan et al(2009),	M	98	115.19	2.39	21.34
	F	55	93.85	1.79	
Gautam et al(2003), Ahamadabad	M	56	95	1.82	19.00
	F	44	76	1.28	
Dahipale et al(2002), Maharastra	M	96	94.43	1.81	24.23
	F	47	70.19	1.12	
Puttabanthi et al(2012), Andra Pradesh	M	57	92.36	1.75	3.41
	F	22	88.95	1.65	
Adhvaryu Ankit et al(2013), Saurashtra region	M	45	92.11	1.74	13.83
	F	55	78.28	1.35	
Present Study(2017)	M	350	92.28	1.75	15.35
	F	282	76.93	1.31	

DISCUSSION :

The observations of various workers regarding the sexual dimorphism in length of mesosternum is shown in the **Table 4**.

In the present study, mean length of mesosternum In male and female sterna is 92.28 mm and 76.93 mm respectively, in accordance to the observations of other Indian workers, Adhvaryu et al¹², Jit et al¹³, Dahipale et al.¹⁴, Gautam et al.⁴ and Puttabanthi et al.¹⁵

The difference in mean length of mesosternum of male and female sterna is 15.35 mm which is statistically highly significant ($P < 0.05$) showing a longer length of manubrium in the males in comparison to female and in accordance with the value of Adhvaryu Ankit et al¹², Ashley¹⁶ and Jit et al¹³. Dahipale et al¹⁴ found the length of mesosternum extremely useful in determining the sex, which consistent with present study.

Mean value of length of mesosternum of different observer of male having more than present study is Dwight¹⁷, Dwight¹⁸, Paterson¹⁹, Ashley¹⁶ African, Ashley¹⁶ European, Jit et al.¹³, Mahajan et al²⁰, Gautam et al⁴, Dahipale et al¹⁴ and Female

having more value is Dwight¹⁷, Dwight¹⁸, Paterson¹⁹, Ashley¹⁶ African, Ashley¹⁶ European, Jit et al¹³, Mahajan et al²⁰. Mean value of length of mesosternum is less then present study in male is Adhvaryu et al¹² but in female it is, Dahipale et al¹⁴.

Regression analysis of present study of Mesosternum is 1.75 and 1.31 male and female respectively, which shows that if value is more then 1.5(near 02) it is male and if less than 1.5(near 01) it is female, on applying regression formula of present study on mean length of mesosternum of different observer shows Ashley¹⁶, Jit et al¹³, Gautam et al⁴, Dahipale et al¹⁴, Adhvaryu Ankit et al¹² consistent with present study.

CONCLUSION :

For Sex : The mean length of mesosternum in relation to gender Shows in the female the mean length of mesosternum was 76.93 ± 7.51 mm, while in the male it was 92.28 ± 9.78 mm. The difference was found to be statistically significant ($P < 0.05$), showing a longer length of mesosternum in the males in comparison to the females.

The discriminant analysis in finding out the gender based on the length of the mesosternum (B) shows correct identification

was 88.3% in females and 80.6% in males hence, overall accuracy 84%. and its regression analysis chart was found to be statistically significant ($P < 0.05$) hence concluded by regression equation that if the equation value nears 1, then the gender is female and if the equation value nears 2, the gender is male while its ROC curve correctly identify the gender in 80.57% (Sensitivity) while it was able to correctly negate the gender in 88.30% (specificity) and resulted that The cut-off value of length of mesosternum is 84.5 mm, i.e. if the length of mesosternum is > 84.5 mm, then it is a male, else it is a female.

SUMMARY :

- The mean length of mesosternum in relation to gender Shows in the female the mean length of mesosternum was 76.93 ± 7.51 mm, while in the male it was 92.28 ± 9.78 mm.
- The difference was found to be statistically significant ($P < 0.05$), showing a longer length of mesosternum in the males in comparison to the females.
- The cut-off value for length of mesosternum was calculated and observed 84.5 mm, i.e. if the length of mesosternum is > 84.5 mm, then it was a male, else it is a female.
- Regression equation formula for gender identification from length of mesosternum was derived as:-

$$\text{Gender} = -0.862 + 0.02831 \text{ Length of mesosternum (B) in mm.}$$

If the equation value nears 1, then the gender is female and if the equation value nears 2, the gender is male.

REFERENCES:-

1. Bongiovanni R, Spradley MK. Estimating sex of the human skeleton based on metrics of the sternum. *Forensic Sci Int.* 2012;219:2901-7.
2. The Universal Declaration of Human rights United Nations. Available from : <http://www.un.org/en/universal-declaration-human-rights/index.html>. Accessed on 10-Aug-2016.
3. Kaneriya D, Umarvanshi B, Patil D, Mehta C, Chauhan K, Vora R. Age determination from fusion of the sternal elements. *Int J Appl Basic Med Res.* 2013;3:22-9.
4. Gautam R, Jadhav G, Gohil B. The human sternum – as an index of age & sex. *J Anat Soc India.* 2003;52(1):20-3.
5. Jit I, Bakshi V. Time of fusion of the human mesosternum with manubrium & xiphoid process. *The Indian journal of medical research.* 1986 Mar;83:32231.
6. Singh J, Pathak RK, Singh D. Morphometric sex determination from various sternal widths of Northwest Indian sternums collected from autopsy cadavers: a comparison of sexing methods. *Egyptian Journal of Forensic Sciences.* 2012 Mar 31;2(1):18-28.
7. M. Wadhawan *, A. Murari ** & G. Murali ***at el Estimation of age from the fusion of mesosternum with manubrium and xiphisternum in Delhi: A comparative study *J Indian Acad Forensic Med*, 2009; 31(2): 103-6
8. Sobhan k. Das, is ossification of sternum at all a valuable guide for determination of age at middle age group? *JIAFM*, 2005 : 27 (1). ISSN 0971-0973
9. Bardale R, Principle of forensic medicine and toxicology, 1st edition .Jaypee Brothers Medical Publishers 2011.P.103
10. Waghmare1, Rupali B. Gaikwad2, Vaibhav Sonar3. Determination of the age with the help of ossification of the sternum. *Int J Pharm Biomed Res* 2012; 3(1): 5-6.
11. Chandrakanth HV, KanchanT, Krishan K, Arun M, Pramod kumar GN. Estimation of age from human sternum : An autopsy study on a sample from south india . *Int J legal medicine.* 2012 nov;126(6):8638
12. Adhvaryu AV, Adhvaryu MA, Rathod SP, Chauhan PR, Joshi HG. A study of sexual dimorphism in human sterna. *International Journal of Medical Research & Health Sciences.* 2013;2(3):577-81.
13. Jitindar I, Jhingan V, Kulkarni M. Sexing the human sternum. *American Journal of Physical Anthropology.* 1980;53:217-24.
14. Dahiphale VP, Baheete BH, Kamkhedkar SG. Sexing the human sternum in Marathwada Region. *J. Anat. Soc. India* 2002; 51(2):162-7.
15. Puttabanthi S, Velichety D, Padi D, Boddeti D, Priyanka J. Sexing of unknown adult human sterna by metrical analysis. *Int J Biol Med Res* 2012;3(2):1516-9.
16. Ashley G. A comparison of human and anthropoid mesosterna. *Am J Phys Anthropol.* 1956;14(3):449-65.
17. Dwight T. The sternum as an index of sex and age. *Journal of anatomy and physiology.* 1881 Apr;15(Pt 3):327.
18. Dwight T. The sternum is an index of sex, height and age. *Journal of Anatomy.* 1890;24:527-35.
19. Paterson A. The human sternum. London: University Press of Liverpool; 1904.
20. Mahajan A, Batra APS, Khurana BS, Sharma SR. Sex determination of human sterna in north Indians. *Journal of Punjab Academy of Forensic Medicine And Toxicology.* 2009;9(1):12-15.

Original Research Paper

Determination of Sex from Upper end of Right Radius in Western Rajasthan Sample

1. Sanjana Devi, Assistant Professor, Department of Anatomy, Adesh Institute of Medical Sciences and Research, Bathinda

ABSTRACT :

Introduction : Sex determination from human skeletal remains plays a very important role for establishing the identity of the subject

Aims & Objective: Present study was conducted to determine sex by using measurements of the upper end of Radius among Western Rajasthan Population.

Matrerial & Method: Fifty adult human radii of known sex available in the Department of Anatomy, Dr. S. N. Medical College & associated groups of hospitals, Jodhpur, Rajasthan were used for the present study. Out of 50 bones 25 were of male and 25 were of female. Five measurements of radius were statistically analyzed.

Results: A highly significant relationship ($p < 0.0001$) was observed in the mean value of all parameters between male and female long bones studied. Among the five variables, highest relative significance was shown by Maximum Head Diameter ($t = 9.986$). Accuracy of the five Radial measurements ranged from 86% to 92%. The highest percentage of accuracy were recorded in the maximum head diameter of the radius (92%), followed by vertical radial head height and circumference at radial tuberosity (90%).

Corresponding Author :

Mrs. Sanjana Devi,

Assistant Professor,

Department of Anatomy, Adesh Institute of Medical Sciences and Research, Bathinda (Pb.)

Contact : +91 97796-21147

E-mail : sanabansal20@gmail.com

Article History:

Received: 3 October 2018

Received in revised form: 10 October 2018

Accepted on: 10 October 2018

Available online: 1 June 2019

KEYWORDS : Forensic Medicine, Radius, Sex determination

INTRODUCTION :

Determination of sex from skeletal region is generally the first step in the establishment of a demographic profile of the deceased, as knowledge of a person's sex¹ and skeletal remains play a very important role for establishing the identity of the subject². The general anatomical regions used for sex determination are the pelvic girdle, the skull, and long bones but many times the pelvis, skull or other bony parts that point to fairly accurate conclusions regarding sex may be absent³. Therefore it is necessary to formulate functions for other bones especially the long bones like Radius that is frequently found in skeletal remains.

The Radius is the lateral bone of the forearm. It has a rounded proximal head, prominent radial tuberosity and expanded distal end with a large articular surface. The distal end of the radius articulates with the lunate and scaphoid bones of the wrist. Epiphyseal union of the head of the radius occurs at 14-15 years and distal radius at 18-19 years.

Sex determination formulae for the radius and ulna, however, have been developed by Steel (1972) using a small English sample of known age and sex⁴. Also, when the whole of the long bones becomes unavailable in many situations, some methods have been employed for the usefulness of the

fragments of long bones⁵.

Therefore, in the present study an attempt is made to determine the mean values and accuracy of right sided long bone of forearm in Western Rajasthan sample and compare with other populations which would assist in various medico legal and archaeological studies.

MATERIALAND METHOD :

The present work was conducted on 50 Radius (25 Male and 25 Female) which belonged to individuals of Western Rajasthan population. These bones were obtained from the Department of Anatomy, Dr. S. N. Medical College & associated groups of hospitals, Jodhpur, Rajasthan in 2012. The bone samples were extracted from adult cadavers and differentiated into male and female bones. Samples with any pathological changes, fractures, or the non union of the epiphysis of the head were excluded. Only right sided radii were considered for this study.

Measurements of the Radius - Five radial measurements were obtained. All measurements were taken with a digital sliding calliper & a measuring tape and recorded in millimetres. Each measurement was as follows (**Figure 1-6**):

- **Vertical radial head height** - It is the height of the radial head measured directly above the radial tuberosity.

- **Minimum head diameter** - It is taken while rotating the digital caliper around the radial head, the smallest diameters were recorded.
- **Maximum head diameter** - It is taken while rotating the digital caliper around the radial head, the largest diameters were recorded.
- **Circumference of the radial head** - It is taken by placing the tape measure around the radial head.
- **Circumference at the tuberosity** - It is taken by placing the tape measure around the radial tuberosity.

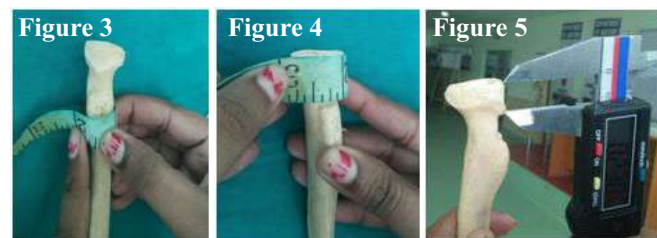
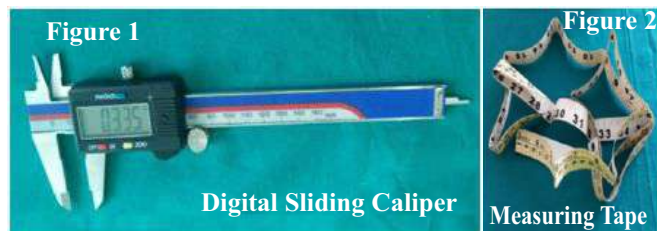


Figure 3 : Circumference at the Radial tuberosity

Figure 4 : Circumference of radial Head

Figure 5 : Vertical Radial Head Height



Statistical Analysis:

The data obtained for various parameters were subjected to statistical analysis. The student's t test was used to determine the mean and Standard deviation for each variable in both the sexes. The t and p value were estimated.

Accuracy- It is the degree of closeness of a measured or calculated quantity to its actual (true) value.

Accuracy- $\frac{\text{Correctly identified bones (males + females)}}{\text{Total sample (50)}}$

$$\frac{TM + TF}{TM + TF + FM + FF}$$

TM = No. of true identified male bones in the sample

TF = No. of true identified female bones in the sample

FM = No. of false identified male bones in the female sample

FF = No. of false identified female bones in the male sample

RESULT & DISCUSSION :

50 bones were studied, out of which 25 (50%) were of male while rest 25 (50%) were female bones. **Table 1 & 2** shows mean values of different measurements of the bones.

Table No. 1 : Mean values of Measurements of Circumference of Radial Head and Tuberosity (in mms)

Group	Radial head Circumference Mean± S.D.	Radial Tuberosity circumference Mean± S.D.
Male	66.88±4.024	52.56±3.697
Female	57.72±3.221	44.40±2.723
p value	<0.0001 (HS)	<0.0001 (HS)
t value	8.886	8.886

Table No 2 : Mean values of Measurements of Maximum Head Diameters, Minimum Head Diameters And Vertical Radial Head Height (in mms)

Group	Maximum Head Diameter Mean± S.D	Minimum Head Diameter Mean± S.D.	Vertical Radial Head Height Mean± S.D.
Male	22.4±1.36	20.28±1.45	9.744±0.87
Female	18.87±1.13	17.51±1.11	7.94±0.77
p value	<0.0001 (HS)	<0.0001 (HS)	<0.0001 (HS)
t value	9.986	7.594	7.814

Table 3 shows Percentage of accuracy of radial measurements. All the measurements were significantly larger among males than the females ($p < 0.001$). Highest relative significance was shown by Maximum Head Diameter ($t = 9.986$). The standard deviations denoted that males exhibited more variability than females in all the variables. Accuracy of the five Radial measurements ranged from 86% to 92% (**Table 3**).

Table No 3 : Accuracy of Correct Sex-determination from Radial Measurements:

Variables	Males (25) %	Females (25) %	Accuracy %
Radial head circumference	23 (92)	20 (80)	86
Radial tuberosity circumference	21 (84)	24 (96)	90
Maximum head diameter	22 (88)	24 (96)	92
Minimum head diameter	23 (92)	21 (84)	88
Vertical radial head height	23 (92)	22 (88)	90

The present study deals with the accuracy for sex determination of long bone of upper limb. The result reported here suggests that it is possible to determine the sex of skeletal remains using metric traits of long bone of upper limb.

The level of accuracy however is variable and is dependent on the preservation of the skeletal material and the experience of the observer. It has been demonstrated that skeletal elements associated with joints have shown the most sexual dimorphism due to the greater stresses applied to them with physical activity, presumed to be greater in males, which accentuates the dimorphism associated with their normal greater robusticity⁶.

Radial head circumference:

In present study the mean value for this variable was 66.88 ± 4.024 and 57.72 ± 3.221 in males and females respectively. A statistically highly significant relation ($t=8.886$; $p=0.0001$) was observed with lowest accuracy rate of 86% (males 92% and females 80%). In present study this variable was found less dimorphic which correlate with the study of Singh et al⁷.

Radial tuberosity circumference:

In present study the mean value of circumference at radial tuberosity was 52.56 ± 3.697 and 44.40 ± 2.723 mm, in male and female bones respectively with highly relative significance ($t=8.886$; $p<0.0001$) and accuracy rate of 90% (males 84% and females 96%). Safont and Tomohito also had used the Circumference at radial tuberosity for sex determination in Japanese population and could correctly identify the sex in 92.8% and 81.0% of bones respectively^{8,9}.

Waghmare et al reported that after, antero-posterior diameter at mid-shaft, circumference at radial tuberosity was the next best parameter ($t=10.27$) which had classified 79.8% of the bones¹⁰.

Maximum and minimum head diameter:

The mean value of Maximum head diameter in male and female bones was 22.4 ± 1.36 and 18.87 ± 1.129 mm,

respectively, with the highest relative significance ($t=9.986$; $p<0.0001$) and the highest accuracy rate of 92% (males 88% and females 96%). Mean minimum head diameter was 20.28 ± 1.447 and 17.51 ± 1.108 in males and females respectively with highly relative significance ($t=7.594$; $p<0.0001$) and accuracy rate of 90% (males 92% and females 84%). Berrizbeitia¹¹ demonstrated that the diameter of the radial head is an accurate sex discriminator for human remains in black and white North Americans. When the maximum diameter was 23 mm or the minimum was 22 mm, the subject was more likely male; when the maximum diameter was 22 mm or the minimum was 21 mm, the subject was more likely female. Also Mall and collaborators¹² demonstrated much higher percentages of correct classification (86%) for this variable. Whereas Barrier IL et al¹³ demonstrated a classification accuracy of 80% and 82% for males and females maximum head diameter respectively in a modern South African sample.

Our finding was in concordance with these findings that maximum diameter of the radial head is best sex discriminator for human remains with highest rate of accuracy (92%).

Vertical radial head height:

In present study mean value of Vertical radial head height was 9.74 ± 0.867 and 7.94 ± 0.744 mm, in males and females respectively. A statistically highly significant relation was observed ($t=7.81$; $p<0.0001$) with accuracy rate of 90% (males 92% and females 88%). In black South Africans mean value for this variable was 10.46 ± 1.20 and 9.49 ± 1.26 in males and females respectively¹³.

From above discussion it must be noted that the patterns and the distribution of sexual dimorphism differ between populations and that the results which were obtained, Could not have similar accuracy if they were applied to different Ethnic groups. Therefore, most skeletal biologists agree that the inter population difference necessitates the development of regionally specific standards for the identification of sex. This supported the hypothesis that human variation is diverse, and population difference should be taken into account when osteometric standards are applied to others.

CONCLUSION:

In conclusion, sex determination was made, in different rates of accuracy, in the human skeleton and the sex of an unknown individual can be detected by fragments of long bone. This could be of immense help to detect the sex of an unknown subject in the medico-legal cases, which is an important task for forensic science, anthropology and anatomy.

REFERENCES :

1. Loth SR, İşcan MY. Sex Determination. In: Siegel JA, Sauko PJ, Knufper GC, editors. The Encyclopedia of Forensic Sciences. 2000; Vol 1: 252-260.
2. Krogman WM. The Human Skeleton in Forensic Medicine. 3rd ed. Springfield, Illinois: Charles C Thomas. 1986; p. 35-41.
3. Kelley MA. Sex determination with fragmented skeletal remains. J Forensic Sci. 1979; 24(1):154-158.
4. Steele FLD. The sexing of long bones, with reference to the St. Bride's series of identified skeletons: J Royal Anthropol Inst. 1962; 92:212-22.
5. Mysorekar N L, Verma PK, Nandekar AN, Sharma TSR. Estimation of stature from parts of bones: lower end of femur and upper end of radius. Med Sci Law. 1980; 20 (4): 283-286.
6. Albanese J, Cardoso HFV, Saunders SR. Universal methodology for developing the univariate sample-specific sex determination methods: An example using the epicondylar breadth of the humerus. Journal of Anthropological Science. 2005; 32: 143-152.
7. Singh G, Singh S, Singh SP. Identification of sex from the radius. J Indian Acad For Sci. 1973; 13(1): 10-16.
8. Safont S, Malgosa A, Subirà ME. Sex assessment on the basis of long bone circumference. Am J Phys Anthropol. 2000; 113: 317-28.
9. Nagaoka T, Hirata K. Reliability of metric determination of sex based on long-bone circumferences: Perspectives from Yuigahama-minami, Japan. Anat Sci Int. 2009; 84:7-16.
10. Waghmare JE, Deshmukh PR, Waghmare PJ. Determination of Sex from the Shaft and Tuberosity of Radius- A Multivariate Discriminant Function Analysis: Biomedical Research. 2012; 23 (1): 115-118.
11. Berrizbeitia EL. Sex determination with the head of the radius. J Forensic Sci. 1989; 34(5): 1206-1213.
12. Mall G, Hubig M, Büttner A, Kuznik J, Penning R, Graw M. Sex determination and estimation of stature from the long bones of the arm. Forensic Sci Int. 2001; 117: 23-30.
13. Barrier IL, Labbe EN. Sex determination from the radius and ulna in a modern South African sample: Department of Anatomy, Faculty of Health Sciences, University of Pretoria. 2008; 179(1): 85.e1-7.

Original Research Paper

Traditional Versus Analytical Examination of Addition as Tampered Documents

1. **Shipra Rohatgi**, Research Scholar, Amity Institute of Forensic Sciences, Amity University, Noida, Uttar Pradesh
2. **Shruti Gupta**, Assistant Professor, Amity Institute of Forensic Sciences, Amity University, Noida, Uttar Pradesh
3. **Madhulika Sharma**, Ex-Director, State Forensic Science Laboratory, Govt. of NCT of Delhi, Madhuban Chowk, Sector 14, Rohini, New Delhi-110085

ABSTRACT :

Additions are the communal category among tampered documents which are frequently encountered in the division of questioned document. Additions are accomplished with an intention to gain self-benefit. In this research article the samples were prepared by add-ons of letters, words and figures on various forms of questioned documents such as cheques, bank deposit slips, memo, receipt, certificates etc. by using ball, gel and ink pen of different colors. The mere objective of the research is to trace out the best suitable non – destructive analytical technique for the detection and decipherment of the additions performed on different type of papers using different type of pens and of different colors. Samples were examined from basic instruments such as handlens, illumination sources (UV, Oblique light), stereomicroscope to advanced instruments like docubox HD, Projectina NIRVIS docucenter, docubox dragon. On examining the samples under different traditional and innovative prevailing techniques it was observed that the decipherment was easy and precise under Projectina NIRVIS docucenter, docubox HD and docubox dragon without detrimental effect on the samples in less duration of time. Infrared ranging from 715-735 and Lumi ranging from 665-830 proved to be paramount wavelength for decipherment irrespective of paper surface.

Corresponding Author :

Shipra Rohatgi,
Research Scholar,
Amity Institute of Forensic Sciences,
Amity University, Noida, Uttar Pradesh, India
Contact : +91- 8586878289
E-mail : shiprarohatgi8@gmail.com

Article History:

Received: 27 August 2018
Received in revised form: 4 September 2018
Accepted on: 4 September 2018
Available online: 1 June 2019

KEYWORDS : Additions, Projectina NIRVIS Docucenter, Infrared, Lumi, Stereomicroscope

INTRODUCTION :

Tampered documents are the most complex types of questioned document obtained in the field of forensic science. The most common types of cases encountered by the investigators are of Alterations, Additions, Erasures, Obliteration, invisible writings and charred documents. The examination of tampered document is problematic and time consuming due to various types of papers and the writing instrument used. Cases of additions include adding of any letter, figure, signature etc. are performed on any document needs to be verified whether they are genuine or forged. The main aim of the forensic document examiner is to find the authenticity the fakeness in the addition performed by the individual. In the early eras the traditional methods were employed for the analysis of addition which includes hand lens, stereomicroscopes and other illumination sources such as ultra violet, Oblique light, transmitted light. The degree of detection and decipherment by using the traditional methods is very limited and highly time consuming therefore there is a need for advanced analytical technique for a better and

accurate result as to aid in forensic questioned document cases. Mostly the documents are made of paper with the writing instruments such as ball pen, fountain pen, pilot pen, gel pen etc. It is very important to understand the property of paper as well as inks used. Writing surfaces can be butter paper, bond paper, construction paper, copier papers etc. whereas the inks used are mainly from liquid or semi liquid material used for writing. Chemically ink consist of dyes immersed in solvent with resins, fluoresces, lubricant etc. The ball pen ink is oil based which provides it thickness and contains synthetic dyes dissolved in glycol. Chelated dyes are also used which makes them highly stable towards light. The modern fountain inks contain pigmented dyes like copper pthalocyanine which makes the ink everlasting whereas the gel inks are water based and consist of insoluble color pigments. Therefore there is an urgency to identify inks of different colors used for addition in order to restore the authenticity of document being questioned^[1-8].

METHODOLOGY:

- Sample Size – Sixty (60)
- Writing instrument used- ball, gel and ink pen
- Color of inks used – Random (blue, black and red)
- Questioned samples obtained- cheques, bank deposit slips, memo, receipt, certificate and other relevant documents as mentioned in **table no.1**.
- Traditional techniques employed-handlens, illumination sources (UV, Oblique light), stereo microscope,
- Advanced Analytical techniques used- docubox HD, Projectina NIRVIS docucenter, docuboxdragon.

Objective to be achieved for the present research :-

1. To find out the best non-destructive method for the detection and decipherment.
2. To find out the technique to achieve easy and accurate result with less time consumption.

Table 1: Showing Some of the Samples of Additions (Moving from Left to Right Includes Bill Receipts, Bank Deposit Slips, Medical Prescriptions, Rent Receipts and Cheques)

**OBSERVATIONS AND RESULTS:**

The samples were subjected to hand lens, illumination sources (UV, Oblique light), stereomicroscope, docubox HD, Projectina NIRVIS docucenter, docubox dragon for analysis and the observations were concluded in **Table No. 2 and 3**. According to the examination of samples it was found that docubox HD, Projectina NIRVIS docucenter, docubox dragon proved to be fruitful in terms of accuracy of detection and decipherment and time consumption. The following are the observation seen in some of the selected samples mentioned in the tables in accordance to the type of pen (ball, gel or ink).

The random sampling were done and some of the peculiar results observed in the ball pen ink samples were compiled in **table no. 2**. The samples vary great in their paper surface and the ball pen company. Sample no. 1A, A bill receipt where 0 was added after 7000 which was detected and deciphered at Infrared 760. In sample 2A add on of 3 was performed using different blue ball pen ink which was easily deciphered under Lumi 830. Sample 3A reveals about signature and zero being added with the same tint of blue ball pen at Lumi 630. Sample 4A Sharma was added with different blue ball pen which was detected at Lumi 630 whereas in sample 5A tampering was done in date, 2 was changed to 3 using black ball pen which was detected at IR 715.

Table no.3 depicts the observation of gel and ink pens samples. Sample 1B depicts the addition of teen after four with blue gel pen which was detected at IR 735. In sample 2B - 8x100 and 800 was added with blue gel pen which was detected at IR 715. Sample 3B shows the addition of 2 prior to 5000 which was detected at IR 715. In sample 4B Sign is added over stamp with different ink, identified at IR 735. In sample 5B 7 has been changed to 9 with blue pilot pen at two places easily detected at Lumi 665. For the remaining samples undertaken for the research is being compiled in **table no. 4** showing the overall light source useful for the addition samples. Conversing about the traditional methods, stereomicroscope ascertained to be the best among them with a higher success rate in the detection and decipherment of additions as a tampering in the samples. Out of ten samples mentioned in **table no.2 and 3** it can be easily witnessed that only three of the samples were identified positive in the terms of decipherment whereas the rest seven showed negative results. Therefore, advanced analytical techniques serve as a better mode for add on samples, ascertaining a non-destructive procedures.

CONCLUSION:

Examination of tampered documents as 'addition samples' subjected to primitive and advanced techniques revealed significant results. Bulk of the samples, irrespective of paper surface and pen used were easily detected and deciphered

Table 2 : Results of Addition Samples (Ball Pen) Under Projectina NIRVIS Docucenter, Docubox HD and Docubox Dragon and Stereomicroscope [Addition Visible (+), Addition Not Visible (-)]

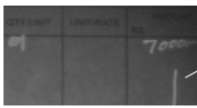
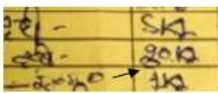
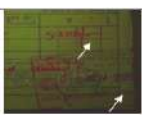
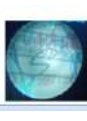
Sample No.	Sample	Best deciphered observations	
		Best analytical method (docubox HD, Projectina NIRVIS docucenter , docubox dragon)	Stereomicroscope Addition visible (+) Addition not visible (-)
Sample 1B			(-ve) 
Sample 2B			(+ve) 
Sample 3B			(-ve) 
Sample 4B			(-ve) 
Sample 5B			(+ve) 

Table 3 : Depicting the Results of Addition Samples (Gel and Ink Pen) Under Projectina NIRVIS Docucenter, Docubox HD and Docubox Dragon and Stereomicroscope [Addition Visible (+), Addition Not Visible (-)]

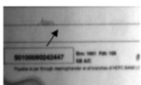
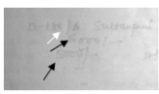
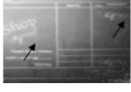
Sample No.	Sample	Best deciphered observations	
		Best analytical method (docubox HD, Projectina NIRVIS docucenter , docubox dragon)	Stereomicroscope Addition visible (+) Addition not visible (-)
Sample 1B			(-ve) 
Sample 2B			(-ve) 
Sample 3B			(-ve) 
Sample 4B			(-ve) 
Sample 5B			(+ve) 

Table 4 : Depicting the Results of Sixty (60) Addition Samples (Ball, Gel and Ink Pen) Under Projectina NIRVIS Docucenter, Docubox HD and Docubox Dragon and Stereomicroscope

Sr. No.	Light Source	Wavelength	No. of Samples	Total No. of Samples
1.	LUMI	610	3	29
		630	12	
		665	2	
		735	2	
		760	2	
		780	2	
		830	5	
		850	1	
2.	IR	715	18	31
		735	13	

under two major light sources namely infrared and Lumi. Both the lights sources have high emerging success rate in the forensic sample analysis especially the tampered documents which is commendable. Projectina NIRVIS docucenter, Docubox HD and Docubox dragon showed similar results for every sample analyzed under them without damaging the samples. Infrared proved to be the most successful in deciphering the maximum no. of samples i.e. 31, among them the wavelengths - 715 and 735 has successfully identified 18 and 13 samples respectively. The second light source which showed magnificent results was Lumi ranging from 610 to 850 where Lumi 630 attained the maximum sample detection and decipherment. In some of the cases stereomicroscope also proved to be beneficial in the identification of additions. The research objectives were established and Projectina NIRVIS docucenter, docubox HD and docubox dragon can be used for the tampered document analysis as a non destructive technique as an effortless and accurate method with less time depletion.

Acknowledgement :

The authors are grateful to the authorities of State forensic Science Laboratory, Govt. of NCT of Delhi, Madhuban Chowk, Sector14,Rohini , New Delhi-110085),India

Conflict of interest :

The authors declare no conflict of interest.

REFERENCES :

1. Osborn AS. Questioned Documents. New York: Boyd Printing Co; 1929.
2. Harrison W R. Suspect documents, their Scientific Examination. New York:Frederick A. Praeger Publishers; 1958.103 p.

3. Mitchell CA. Documents and their scientific examination: with especial reference to the chemistry involved in cases of suspected forgery, investigation of disputed documents, handwriting,etc.London :Charles Griffin;1922.83p.Available from: URL: <https://archive.org/details/cu31924032589313>
4. Abd-ElZaher MA. Different types of inks having certain medicolegal importance: Deciphering the faded and physically erased handwriting. Egyptian Journal of Forensic Sciences. 2014 Jun 1;4(2):39-44. Available from: URL:<http://dx.doi.org/10.1016/j.ejfs.2013.09.002>.
5. Rohatgi S, Gupta S, Sharma M, Shukla SK. A Study to Focus on Augmentation of Pre-existing Methodologies for Tampered Document Examination. Research Journal of Pharmacy and Technology. 2017 Nov 1;10(11):4085-9.
6. Souder W. Composition, properties and behavior of ball pens and inks. The Journal of Criminal Law, Criminology, and Police Science. 1955 Mar 1;45(6):743-7.
7. Wright RA, Sheridan C, Del Carmen RV. Textbook adoption and other landmark decisions: The coverage of court cases in criminal justice textbooks, 1986 to 1995. Journal of Criminal Justice Education. 1997 Nov 1;8(2):145-62.
8. Godown L. New nondestructive document testing methods. J. Crim. L. Criminology & Police Sci.. 1964;55:280.

Original Research Paper

**Amel Gene Profiling from Toothbrush for Sex Determination
Among Tanzanians in Surabaya, East Java.**

1. **Simon Martin Manyanza Nzilibili**^{a,b}, M Sc. Forensic Science Student
2. **Ahmad Yudianto**^{a,c,d}, Lecturer
 - a. Forensic Science Program, Post Graduate School, Airlangga University, Surabaya – Indonesia.
 - b. Ministry of Health, Community Development, Gender Elderly and Children, Dodoma – Tanzania.
 - c. Department of Forensic and Medico-legal, Faculty of Medicine, Airlangga University, Surabaya – Indonesia
 - d. Human Genetic Laboratory, Institute of Tropical Disease, Airlangga University, Surabaya – Indonesia.

ABSTRACT :

Introduction : Forensic identification depends significantly on evidence detected at the crime scene. Toothbrush continues to prove its usability to aid the identification for sex determination. Its contributory power continues to be confirmed to the different population at the varied time.

Materials and Methods : The present study reports the use of toothbrush in determining sex through Amel gene method by DNA band contrast at 106bp and 112bp among Tanzanians on ten volunteers.

Results : Electrophoresis band contrast confirmed six samples to be of male origin and three sample for the female. One sample presented undetectable DNA band contrast by showing the blank image on F1 sample profiled.

Conclusion : Toothbrush as source of biological samples and Amelogenin gene through DNAband contrast analysis is helpful in sex determination among Tanzanians. This finding not only evidently proposes but also contributes to bench marking applicability of Amel gene analysis as a successful and reliable method of sex determination to localized population (Tanzanians) for strengthened evidential data for forensic service.

Corresponding Author

Simon Martin Manyanza Nzilibili

Forensic Science Program, Postgraduate School, Airlangga University, 4-6 Airlangga Rd., 60286 Surabaya, Indonesia
Contact : +62 81334326515
E-mail : nzilibili@gmail.com

Article History:

Received: 18 September 2018
Received in revised form: 18 November 2018
Accepted on: 18 November 2018
Available online: 1 June 2019

KEYWORDS : Amel Gene, Toothbrush Evidence, Sex Determination

INTRODUCTION :

Sex determination remains an essential parameter to the revelation of unknown live or dead^[1]. Gene profile through DNA analysis has proved a reliable technique for this purpose^[2]. Toothbrush like any other evidence that might be found at a crime scene, can help in identification of an individual by analysis of the harbored material^[3-6]. This technique is based on Locard's exchange principle, that anything in violent contact may be retained or taken back and can later used as trace evidence^[7]. To attain sex determination of such recovered sample, a number of methods are studied one of which is through the use of Amel gene (categorized in a molecular group of methods)^[8]. AMEL X gene (female sex-determining DNA chromosomal marker) is present in 106 bps (base pairs) and AMEL Y (male sex-determining DNA chromosomal marker) is present in 112bps of the DNA hence homologous (XX) in female and heterogonous allele in male. This gene form Amelogenin which become detected as protein

when individualizing sex^[8-9]. The method is widely accepted in forensic sex identification with a tolerable percentage error^[10]. If the results are doubtful, it is suggested to run the SRY gene (sex region gene on the Y chromosome that is necessary and sufficient for male sex determination) or/and Y-STR markers (specialized class of short tandem repeats on the male Y-chromosome often used in forensics, paternity, and genealogical DNA testing) as parallel methods in order to confirm and resolve ambiguity^[11-12]. Despite the marginalized ambiguity, its usefulness remains profound into varied populations and continue to be suggested among techniques in sex determination^[8,13,14].

Social offenses, crime and disasters casualties accelerate immensely in costing and endangering global life among Tanzania inhabitants. Solving the mystery, forensic identification responds to involved individual, couple, family or mass in terroristic, catastrophic or destructive events. Forensic scientists at the front line are obliged to bring the

meaning of all thought “evidence” in identification to sexing gender of the involved for the law-suit purpose or resolving trepidation of the grieving^[15,16]. In mutilated, destructed, decomposed or burned cases, toothbrush substantiates DNA profile of genetic materials logged with cells worn during brushing. Therefore; this study aimed at exploring the usefulness of toothbrush and Amelogenin in determining sex among Tanzania population. This will mark continued efforts in deriving experience from a different population and strengthening and improving forensic services and human resource personnel in line with tremendous changes that Tanzania is taking recently^[17].

MATERIALS AND METHODS :

After taking approval from Ethics committee and consent from Ten Tanzanians (six male and four female) participants, the present study was conducted at the Human Genetic Laboratory of the Airlangga University. Toothbrushes were collected after being used (as instructed) regularly for one week and sent to the laboratory for DNA analysis.

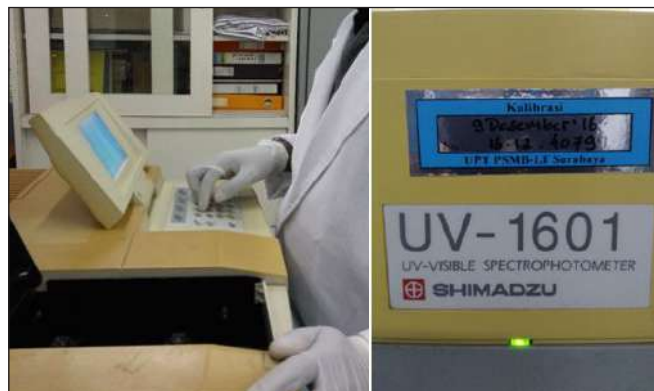
Experimentation Process :

Analytical process started by soaking of the toothbrushes into distilled water for an overnight in order to recover the logged biological samples (worn cells) from bristles at room temperature. Recovered biological samples were extracted by an organic method using DNA zol (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) at alternated Voltex, incubation and centrifugation^[18-19]. Resulted pellet was resuspended to form 50 µl final volume which was then measured by Ultraviolet-visible Spectrophotometer (UV-1601, PC, Shimadzu, Japan) to obtain concentration and purity. Amplification of sex fragment was by PCR mix (Promega Corporation, Madison, USA) using common amelogenin gene (Promega Corporation, Madison, USA) at a set of (forward: 5'-CCCTGGGCTCTGTAAAGAA-3' and reverse: 5'-ATCAGAGCTTAACTGGGAAGCTG-3') primers as recommended^[20]. Amplified DNA of Amel genes (106bp and 112bp) were visualized as band contrast using Gel Polyacrylamide 6% gel (cat. K562, GoTaq®, Green Master Mix, Promega Corp, Madison, USA) formed by electrophoretic migration.

A : DNA extraction process using DNAzol



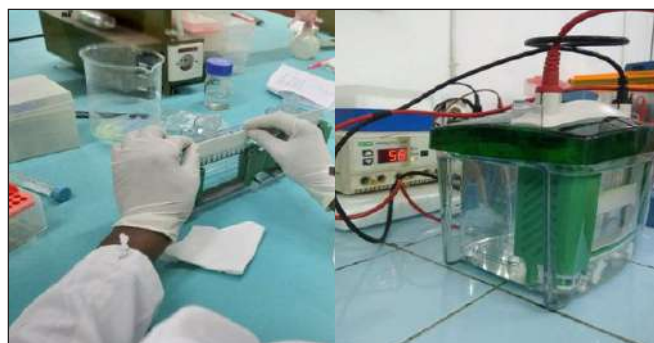
B: Spectrophotometer machine used to MEASURE extracted DNA



C: PCR machine used to polymerase targeted STR loci... thermal cycler



D: Electrophoretic reaction by the use of Vertical electrophoresis (acrylamide gel) to contrast AMEL gene



E: Toothbrushes used Colgate toothpaste used





G: Centrifuge machine used



RESULTS :

Amel Gene profile sex detection :

The Polymerase Chain Reactions (PCR) through electrophoresis visualization of the profile identified correctly nine out of ten (Table 1).

Amelogenin gene detection presented two bands for male samples and one band for female samples (Figure 1). However one sample didn't respond to any of the sex gene categories during electrophoresis ionic segregation (no band appearance). Based upon it, participants were divided into three categories :

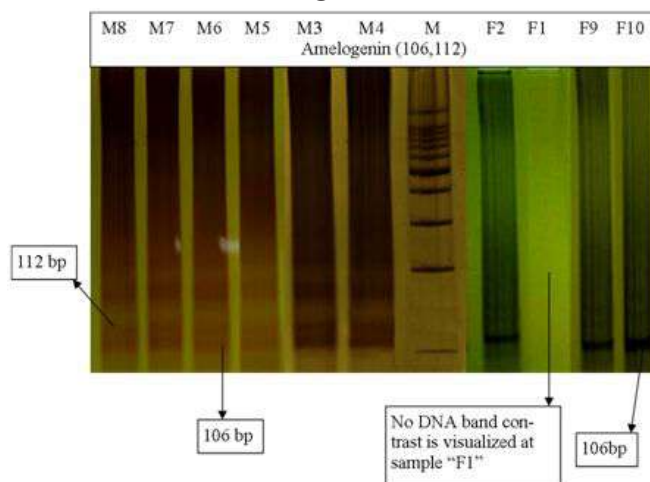
- **True positive male** (M3 – M8) : Amelogenin identified by 106 and 112bp in reference to 100bp
- **True positive female** (F2, F9 & F10) : Amelogenin identified by 106bp in reference to 100bp
- **Null** (F1) : no observed band presented on a profile

The null presenting sample (F1) category on further examination was found accumulated with green toothpaste remains of Sodium Monofluoro phosphate (Compound constituted in toothpaste) which is supported and proven to have contributory damage and degradation effect to DNA molecules^[21-24].

Table 1: Determined Sex by Amel Gene Profile of 106bp and 112bp DNA Band

Sample code	Amel gene	Sample Sex
F1	Null (-)	Null (-)
F2	106bp	Female
M3	106bp & 112bp	Male
M4	106bp & 112bp	Male
M5	106bp & 112bp	Male
M6	106bp & 112bp	Male
M7	106bp & 112bp	Male
M8	106bp & 112bp	Male
F9	106bp	Female
F10	106bp	Female

Figure 1: Determined Male sex on M3 – M8, Female on F2, F9 & F10 with the blank F1 sample with reference to Amel gene-sex determinants.



DISCUSSION :

Amelogenin gene is scientifically proved as a marker for sex determination. Present study has shown its applicability to Tanzania population which will help in solving forensic inquiries. Our findings are similar to studies by Indian authors^[8,13,14]. The sample profiled from toothbrush logged cells successfully responded to the testing of Amelogenin as similar to study by Kashyap et al^[10]. The finding is also supported by the fact that Amel profiling still work better except in

destructive and inhibitory media (toothpaste in this case).

In this study, all toothbrushes used responded to amelogenin sex determination except one that presented an undetectable DNA band on the electrophoresis profile as portrayed in **Figure1**.

The undetectable sample implies and agrees with the effect of fluorine ingredient in the toothpaste as established by similar other studies^[21-22]. Otherwise, the usage of toothbrush despite the predisposed and potential degradation, contaminants and inhibitory factors, remain reliable and preferred physical evidence at crime scene for recovering DNA samples. Toothbrush like many other sources of collecting evidence weighs similar importance and value in terms of usefulness, reliability, and applicability among Tanzanians.

CONCLUSION :

The toothbrush continue to be an important source to solve forensic inquiries especially related to identification and individualization. It has yielded enough quality DNA that successfully resulted in good DNA profiling. Amelogenin gene analysis has presented a convincing and promising finding for sex determination.

REFERENCES :

1. Souza DHD, Kiran J, Harish SS. Determination of Sex by Shape and Size of Hyoid Bone. *J Indian Acad Forensic Med.* 2013;35(2):145-7.
2. Roewer L. DNA fingerprinting in forensics: past, present, future. *Investig Genet.* 2013;4(1):22.
3. Jobim M.R, Gamio F, Ewald G, Jobim M, Jobim L.F. Human identification using DNA purified from residues in used toothbrushes. *Int Congr Ser.* 2004;1261:491-3.
4. Reddy VS, Sriram G, Saraswathi T, Sivapathasundharam B. Isolation of epithelial cells from toothbrush and gender identification by amplification of SRY gene. *J Forensic Dent Sci.* 2011;3(1):27.
5. Riemer LB, Fairley D, Sweet D. DNA Collection From Used Toothbrushes as a Means to Decedent Identification. *Am J Forensic Med Pathol.* 2012;33(4):354-6.
6. Tanaka M, Yoshimoto T, Nozawa H, Ohtaki H, Kato Y, Sato K, et al. Usefulness of a toothbrush as a source of evidential DNA for typing. *J Forensic Sci.* 2000 May [cited 2017 Aug 17];45(3):674-6.
7. Locard E. Locard's Exchange Principle - Forensic Handbook. *Forensic Handbook.* 1910.
8. Ramakrishnan K, Sharma S, Sreeja C, Pratima DB, Aesha I, Vijayabanu B. Sex determination in forensic odontology: A review. *J Pharm Bioallied Sci.* 2015 Aug [cited 2017 Aug 17];7(Suppl 2):S398-402.
9. Haas-Rochholz H, Weiler G. Additional primer sets for an amelogenin gene PCR-based DNA-sex test. *Int J Legal Med.* 1997;110(6):312-5.
10. Kashyap VK, Sahoo S, Sitalaximi T, Trivedi R. Deletions in the Y-derived amelogenin gene fragment in the Indian population. *BMC Med Genet.* 2006;7:37.
11. Kao LG, Tsai LC, Lee JC, Hsieh HM. Controversial cases of human gender identification by amelogenin test. *J Forensic Sci.* 2007;6(2):69-71.
12. Thangaraj K, Reddy AG, Singh L. Is the amelogenin gene reliable for gender identification in forensic casework and prenatal diagnosis? *Int J Legal Med.* 2002 ;116(2):121-3.
13. Sivagami A V., Rao AR, Varshney U. A simple and cost-effective method for preparing DNA from the hard tooth tissue, and its use in polymerase chain reaction amplification of the amelogenin gene segment for sex determination in an Indian population. *Forensic Sci Int.* 2000;110(2):107-15.
14. Steinlechner M, Berger B, Niederstätter H, Parson W. Rare failures in the amelogenin sex test. *Int J Legal Med .* 2002;116(2):117-20.
15. Pretty IA, Sweet D. A look at forensic dentistry – Part 1: The role of teeth in the determination of human identity. *Br Dent J.* 2001;190(7):359-66.
16. Basnaker M, Moolrajani C. Determination of Sex by Dental Pulp Tissue - A Single Blind Study Bhavnagar University's Journal of Dentistry. 2016;6(1):1-6.
17. GCLA. Government Chemist Laboratory Act, Tanzania. 2016;
18. Chen H, Rangasamy M, Tan SY, Wang H, Siegfried BD. Evaluation of five methods for total DNA extraction from western corn rootworm beetles. *PLoS One.* 2010;5(8).
19. Chomczynski P, Mackey K, Drews R, Wilfinger W. DNAzol: A reagent for the rapid isolation of genomic DNA. *Biotechniques.* 1997;22(3):550-3.
20. Sullivan KM, Mannucci A, Kimpton CP, Gill P. A rapid and quantitative DNA sex test: fluorescence-based PCR analysis of X-Y homologous gene amelogenin. *Biotechniques.* 1993;15(4):636-8, 640-1.
21. Nzilibili SMM, Ekodiyanto MKH, Hardjanto P, Yudianto A. Concentration and Purity DNA Spectrophotometer: Sodium Mono fluorophosphate forensic impended effect. *Egypt J Forensic Sci.* 2018;8(1):34.
22. Yiamouyiannis J. Consumer Health Articles: fluoride, the silent killer. 1998
23. Adams SE, Arnold D, Murphy B, Carroll P, Green AK, Smith AM, et al. A randomised clinical study to determine the effect of a toothpaste containing enzymes and proteins on plaque oral microbiome ecology. *Sci Rep.* 2017;7:43344.
24. Alfadaly N, Kassab A, Al Hedaithy F. Determination of DNA profiling of siwak and toothbrush samples used in Kingdom of Saudi Arabia. *Egypt J Med Hum Genet.* 2016;17(4):383-7.
25. Bandhaya A, Panvisavas N. Optimaization of DNA recovery from toothbrushes. *Forensic Sci Int Genet Suppl Ser.* 2008;1(1):9-10.

Original Research Paper

Adequacy of Dental Education and Environment Health

1. **Sonu Gupta**, Assistant Professor, Dasmesh Institute of Research and Dental Sciences, Faridkot
2. **Ravinder Nath Bansal**, Deputy Medical Superintendent, GGS Medical College Hospital, Faridkot
3. **Surender Pal Singh Sodhi**, Principal, Dasmesh Institute of Research and Dental Sciences, Faridkot
4. **Samriti Bansal**, Professor, Pedodontics, Swami Devi Dayal Hospital and Dental College, Barwala, Panchkula

ABSTRACT :

Introduction: Healthcare institutions have been growing in proportion to the increased affordability and awareness among public. Advances in techniques has helped dentist perform complex procedures like treatment of fractures, reconstruction surgeries, dentures, implants and bone grafting. To fulfill these dental requirements, dentists need to use variety of materials, consumables and implants. All these activities lead to generation of wastes including bio-medical waste (BMW) which is inevitable. Such healthcare waste has potential to cause injury and spread infection, if not handled and managed appropriately.

Material and Methods: A cross sectional study was performed at two dental colleges of Punjab which were selected by choice being easily accessible to the researchers. Questionnaire was specifically framed to assess the clarity on basic concepts behind the Biomedical Waste Management and Handling (BMW/MH) rules. Questionnaire was distributed to final year students and interns of these dental colleges.

Results: Significant differences were found between young graduates of the two colleges. It can be concluded this dental curriculum is deficient in providing and ensuring due training and logical understanding to the young graduates.

Corresponding Author

Dr. Ravinder Nath Bansal

Deputy Medical Superintendent,
GGS Medical College Hospital, Faridkot
Contact : +91 9217666699
E-mail : rnbansal@gmail.com

Article History:

Received: 29 December 2018
Received in revised form: 28 January 2019
Accepted on: 28 January 2019
Available online: 1 June 2019

KEYWORDS : Biomedical Waste, Dental Education, Dental Council of India, Dental Curriculum

INTRODUCTION :

Healthcare institutions have been growing in proportion to the increased affordability and awareness among public. Enhanced affordability and urbanization has increased the demand for dental care services even for minor ailments and including for cosmetic reasons. Also there has been an increased demand for dental services by elderly to be able to maintain appropriate aesthetics even at old age. Advances in techniques has helped dentist perform complex procedures like treatment of fractures, reconstruction surgeries, denture implants and bone grafting. To accomplish the advanced dentistry dental sciences have pronged into many specializations leading to formation of various departments at dental colleges. To fulfil these dental requirements, dentists need to use variety of materials, consumables and implants. All these activities lead to generation of wastes including bio-medical waste (BMW) which is inevitable. Such healthcare waste has potential to cause injury and spread infection, if not handled and managed appropriately. Any generated waste is a threat to environment, so it is equally important to reuse wherever possible but with care to prevent the risk of

iatrogenic infection which has been found to be significant in dental practice.¹

Health-care waste at dental institutions includes all types of waste generated within dental facilities like treatment clinics, denture preparation lab and pathological laboratories along with the waste generated by attached medical care units. Such waste could be general waste or BMW. Erratic handling and un-scientific disposal of BMW has been identified as a source of avoidable infection.²

Increasing focus of the Government and implementation efforts of designated agencies now ensures that managers and medical staff take more responsibility towards the healthcare wastes they produce during their activities.² It requires scientific motivation of medical teams by their managers³ to ensure adequate compliance. While the role of managers cannot be underlined, equally rather more important is foundation by teachers which is similar to importance of strong foundation of any building. In a research conducted earlier on nurses, gaps were identified in their education and curriculum on BMW.⁴ Adequate training during graduation studies and rigorous training during internship can sensitize the young

minds on importance of scientific handling of BMW and inculcate the same in their behaviour and conduct. Even Dental Council of India now requires compliance to BMW guidelines and rules^{5,6} by dental colleges and is checked during inspections⁷ and is similar to the requirement by National Accreditation Board of Hospitals⁸ and under The Clinical Establishments (Registration and Regulation) act, 2010.⁹

This study aims to identify sufficiency of current dental education in creating awareness among young dental graduates and building their confidence levels.

Material and Methods: A cross sectional study was performed at two dental colleges of Punjab which were selected by choice being easily accessible to the researchers. Identities of these dental colleges have been masked as the study pertains to education system rather than being specific institutes. Both the dental colleges had been more than 5 yrs old when the study was undertaken. Study was performed in the year 2015-2016. Dental college 'A' had an annual intake 100 BDS students and College 'B' had an intake of 50 BDS student. Both the colleges were private dental colleges. Following NULL hypothesis was formed H_0 : *that is there is no significant difference in knowledge and awareness levels between young graduates of two dental colleges*. Based on the available literature^{4-6,10}, available guidelines and applicable rules a structured questionnaire was formed. Questionnaire was specifically framed to assess the clarity on basic concepts behind the Biomedical Waste Management and Handling (BMW/MH) rules.⁵ Questionnaire was got validated by subject experts and pilot study was conducted. Consent was obtained from the respective colleges. Questionnaire consisted of two parts namely: respondent's demographic profile and awareness on BMW/MH rules.⁵

Principals of both the colleges were requested to gather the final year students and interns in batches and to provide their lecture halls. Pre-defined questionnaire was distributed to all the attendees and study objectives were explained. They were requested for their voluntary participation and confidentiality of responses was ensured. Following this an interactive session on BMW/MH as per the existing rules was conducted. The same questionnaire was then distributed again to the participants after the session. It is possible that some of the participants joined the session after the pre-test was over. Survey responses were fed in spreadsheet. Further assessment was done applying mean, median, t-test using statistical package (SPSS, Ver.20).

Results: In total 196 participants responded to pre-test and there were 199 participants for post-test. Three participants left before post test at college 'B' while 6 had joined at College 'A' after pre-test (**Table 1**). Wherever not specified, analysis has

been done on pre-test responses only. For pre-test there were 29 (15%) male and 155 (79%) female respondents, while 12 of them did not disclose their gender (**Table 2**).

Table 1: Count of Respondent's Pre-test and Post-test

College	Pre	Post	Total
A	150	156	306
B	46	43	89
Total	196	199	395

Table 2 : Gender Distribution of Respondent's (Pre-test)

College	Male	Female	No Response	Total
A	20	125	5	150
B	9	30	7	46
Total	29	155	12	196

Respondents were asked when the last class on BMW was held for them (**Table 3**). Only 39.3% (n=77) respondents had session/class on BMW in the last one year, for 33.2% (n=65) session was held more than a year ago, 21.9% (n=43) never had any session on BMW. Further it was enquired as to who was taking sessions on BMW for the respondents at dental colleges. It was found that 74.5% (n=146) were taught by faculty (**Table 4**).

Table 3: Timeline when class on BMW was held (pre-test)

College	Never	>1 Year	< 1 Year	No Response	Total
A	41	37	63	9	150
B	2	28	14	2	46
Total	43	65	77	11	196

Table 4: Who Had Taken Class on BMW (pre-test)

College	BMW Coordinator	Faculty/Dr. /HOD	Nurse	No Response	Total
A	3	105	1	9	150
B	2	41	1	2	46
Total	5	146	2	11	196

Efforts were made to investigate if respondents understood the logic behind need for managing and handling BMW. Multiple choice question was asked which had in all seven options (**Appendix -1**). Difference in responses were analysed between two colleges (**Table 5**). It was found that while there was no significant difference ($p=0.112$) in correct responses between two colleges, while there was significant difference in incorrect responses ($p=0.005$) between the two colleges.

Respondents were then required to respond on twenty healthcare waste items and the respective colour coding for their segregation. Data was analysed comparing results between the two colleges (**Table 6**). It was found that while there was no significant difference ($p=0.109$) in correct

responses between two colleges, there was significant difference in incorrect responses ($p < 0.0005$) between the two colleges.

Table 5: Differences (t-test) in Responses on Logical Understanding on Need for BMWH (pre-test)

		Levene's Test for Equality of Variances		t-test for Equality of Means		
		F	Sig.	t	df	Sig. (2-tailed)
correct responses	Equal variances assumed	7.332	.007	1.415	194	.159
	Equal variances not assumed			1.604	93.558	.112
incorrect responses	Equal variances assumed	23.093	.000	2.155	194	.032
	Equal variances not assumed			2.857	135.255	.005*

*Statistically significant at 0.05 level of significance

Table 6 : Differences (t-test) in responses on colour coding for BMW segregation (pre-test)

		Levene's Test for Equality of Variances		t-test for Equality of Means		
		F	Sig.	t	df	Sig. (2-tailed)
correct responses	Equal variances assumed	1.439	.232	-1.451	194	.148
	Equal variances not assumed			-1.617	90.347	.109
incorrect responses	Equal variances assumed	51.265	.000	5.013	194	.000001
	Equal variances not assumed			4.053	57.543	.000154*

*Statistically significant at 0.05 level of significance

Table 7b: Differences (t-test) in response on treatment options exercised by CBMWTF (pre-test)

		Levene's Test for Equality of Variances		t-test for Equality of Means		
		F	Sig.	t	df	Sig. (2-tailed)
Incineration	Equal variances assumed	4.673	.032	-.965	194	.336
	Equal variances not assumed			-.995	78.401	.323
Autoclaving	Equal variances assumed	.800	.372	-.459	194	.647
	Equal variances not assumed			-.443	70.968	.659
Shredding	Equal variances assumed	5.929	.016	1.083	194	.280
	Equal variances not assumed			1.126	79.567	.264
Chemical disinfection	Equal variances assumed	26.128	.000	2.193	194	.030
	Equal variances not assumed			2.282	79.730	.025*
Burial	Equal variances assumed	.046	.830	-.105	194	.917
	Equal variances not assumed			-.105	74.473	.917
Mutilation	Equal variances assumed	12.286	.001	-1.776	194	.077
	Equal variances not assumed			-1.399	56.175	.167

*Statistically significant at 0.05 level of significance

Young graduates were asked to specify treatment options which are exercised by CBMWTF. Responses (**Table 7a and Table 7b**) were analysed; wide variation was found and statistically significant ($p = 0.025$) variation was found for response on chemical treatment. For other options though there was no significant difference in responses, but young graduates of both the colleges erred uniformly and responses were away from expected.

Table 7a : Response to treatment options exercised by CBMWTF (pre-test)

Treatment	College	No	Yes	Total	No %	Yes %
Incineration	A	54	96	150	36.0%	64.0%
	B	13	33	43	28.3%	71.7%
	Total	67	67	196	34.2%	65.8%
Autoclaving	A	122	28	150	81.3%	18.7%
	B	36	10	43	78.3%	21.7%
	Total	158	38	196	80.6%	19.4%
Shredding	A	98	52	150	65.3%	34.7%
	B	34	12	43	73.9%	26.1%
	Total	132	118	196	67.3%	32.7%
Burial	A	60	90	150	40.0%	60.0%
	B	18	28	43	39.1%	60.9%
	Total	78	118	196	39.8%	60.2%
Chemical disinfection	A	77	73	150	51.3%	48.7%
	B	32	14	43	69.6%	30.4%
	Total	109	87	196	55.6%	44.4%
Mutilation	A	144	6	150	96.0%	4.0%
	B	41	5	43	89.1%	10.9%
	Total	185	11	196	94.4%	5.6%

Respondents were then asked multiple choice questions on treatment of various coloured coded bags and sharps by CBMWTF. Significant differences were found in responses for both colour coded bags and sharps (**Table 8a and Table 8b**) among the graduates of two colleges.

Null Hypothesis H_0 is thus rejected due to significant difference in responses between the graduates of two colleges.

Table 8a: Difference (t-test) in Responses on Treatment of Different Category of Waste by CBMWTF (pre-test)

		Levene's Test for Equality of Variances		t-test for Equality of Means		
		F	Sig.	t	df	Sig. (2-tailed)
Yellow Bags						
incinerated	Equal variances assumed	26.662	.000001	-2.273	194	.024
	Equal variances not assumed			-2.366	79.749	.020*
Autoclaved	Equal variances assumed	3.068	.081	-.881	194	.380
	Equal variances not assumed			-.723	58.365	.473
Crushed and autoclaved	Equal variances assumed	1.988	.160	-.712	194	.477
	Equal variances not assumed			-.628	63.060	.532
Disinfected with hypochlorite and then buried	Equal variances assumed	115.410	.000	3.625	194	.0004
	Equal variances not assumed			4.393	108.244	.00003*
Blue Bags						
Incineration	Equal variances assumed	96.716	.000	3.423	194	.001
	Equal variances not assumed			4.425	126.726	.00002*
Autoclaved	Equal variances assumed	.283	.595	.264	194	.792
	Equal variances not assumed			.277	80.769	.782
Chemical disinfection	Equal variances assumed	3.971	.048	.961	194	.338
	Equal variances not assumed			1.132	101.477	.260
Deep burial	Equal variances assumed	1.304	.255	-.622	194	.535
	Equal variances not assumed			-.608	72.231	.545
Shredding	Equal variances assumed	16.392	.00007	1.813	194	.071
	Equal variances not assumed			2.246	114.184	.027*
Mutilation	Equal variances assumed	.413	.521	-.323	194	.747
	Equal variances not assumed			-.300	67.409	.765
Sharps						
Incineration	Equal variances assumed	2.559	.111	.712	194	.477
	Equal variances not assumed			.721	76.182	.473
Autoclaved	Equal variances assumed	41.822	.000	-3.079	194	.002
	Equal variances not assumed			-1.888	47.287	.065*
Chemical disinfection	Equal variances assumed	13.535	.0003	1.700	194	.091
	Equal variances not assumed			2.385	156.387	.018*
Deep burial	Equal variances assumed	6.883	.009	-1.385	194	.168
	Equal variances not assumed			-1.245	64.535	.218
Shredding	Equal variances assumed	29.173	.000	2.246	194	.026
	Equal variances not assumed			2.684	104.824	.008*
Mutilation	Equal variances assumed	23.298	.000003	2.165	194	.032
	Equal variances not assumed			3.916	149.000	.0001*

* Statistically significant at 0.05 level of significance

Discussion: Dental graduates regularly attend patients and participate in patient treatment especially in the final year of the graduation and subsequently independently treat patients during their internship. Generation of BMW during such treatment procedures is inevitable. Thus it becomes imperative that they are taught and made fully aware of applicable rules and regulation on handling and disposal of BMW. Session on BMW is part of dental education curriculum and as such it is assumed that graduates would have been taught adequately on BMW rules and regulation.¹¹ Induction training is a regular feature in accredited hospitals. Further rules⁶ require induction training as a well as yearly training on BMW. With only 39.3% (n=77) respondents having training session on BMW in the last one year and 21.9% (n=43) having no training on BMW; it

is evident that either the dental education curriculum is lacking or the dental colleges are not serious on ensuring adequate training on BMW. Thus lack of adequate training was common to both the institutions. Further it was found that 74.5% (n=146) of the graduates were taught by faculty members (**Table 4**). This means that there are no dedicated BMW coordinators at dental colleges and all is dependent on their faculty. It is possible that with colleges having multiple specialty departments; it is the respective heads who are entrusted to ensure compliance to BMW in their respective departments. This can lead to dissimilarities in procedures being followed and consequent confusion to subordinates. It is not understood how colleges ensure compliance to BMW with no defined BMW coordinators/managers so as to ensure

Table 8b : Difference in responses on treatment of different category of waste by CBMWTF (pre-test)

Yellow Bags						
Bags & their treatment	College	No	Yes	Total	No %	Yes %
Incinerated	A	174	76	150	49.3%	50.7%
	B	14	32	46	30.4%	69.6%
Autoclaved	A	147	3	150	98.0%	2.0%
	B	44	2	46	95.7%	4.3%
Crushed and autoclaved	A	144	6	150	96.6%	4.0%
	B	43	3	46	93.5%	6.5%
Disinfected with hypochlorite and then buried	A	88	62	150	58.7%	41.3%
	B	40	6	46	87.0%	13.0%
Blue Bags						
Incinerated	A	99	51	150	66.0%	34.0%
	B	42	4	46	91.3%	8.7%
Autoclaved	A	142	8	150	97.7%	5.3%
	B	44	2	46	95.7%	4.3%
Crushed and autoclaved	A	137	13	150	91.3%	8.7%
	B	44	2	46	95.7%	4.3%
Deep burial	A	102	48	150	68.0%	32.0%
	B	29	17	46	63.0%	37.0%
Shredding	A	124	26	150	82.7%	17.3%
	B	43	3	46	93.5%	6.5%
Mutilation	A	145	5	150	96.7%	3.3%
	B	44	2	46	95.7%	4.3%
Sharps						
incinerated	A	89	61	150	59.3%	40.7%
	B	30	16	46	65.2%	34.8%
Autoclaved	A	149	1	150	99.3%	0.7%
	B	42	4	46	91.3%	8.7%
Crushed and autoclaved	A	135	15	150	90.0%	10.0%
	B	45	1	46	97.8%	2.2%
Deep burial	A	130	20	150	86.7%	13.3%
	B	36	10	46	78.3%	21.7%
Shredding	A	110	40	150	73.3%	26.7%
	B	41	5	46	89.1%	10.9%
Mutilation	A	136	14	150	90.7%	9.3%
	B	46	0	46	100%	0.0%

uniformity in segregation and handling procedures. Bansal⁴ (2018) highlighted the importance of dedicated BMW coordinator and importance of BMW training at graduation level.

Logical understanding of the defined rules for managing and handling BMW is important as it makes one aware of the possible consequences and subsequent self consciousness (integrated regulation and motivation) is important to ensure due handling of BMW. Significant difference in incorrect responses ($p=0.005$) between the young graduates of two colleges signify the lack of due training and teaching on BMW rules and regulations.

Crux of BMW management and handling at hospitals lies in

segregation of generated BMW in designated colour coded bags and bins. Even if one does not understand the logic behind BMWMH and defined colour coding, it is expected that young graduates with or without due logical understanding are well aware or have mugged up the colour coding such that institutions comply to all the requirement of BMWMH rules and regulations. On analysis of responses to twenty BMW items and their respective designated colour coded bags / bins, it was found that while there was no significant difference ($p=0.109$) in correct responses between two colleges, there was significant difference in incorrect responses ($p<0.0005$) between the two colleges. As such this difference will lead to faulty segregation at dental colleges which is probably getting unchecked and ignored at their respective colleges. Percentages of correct and incorrect responses have been highlighted in **table 9**. Even though the difference may be insignificant yet any single episode of non compliance to BMWMH may lead to serious consequences to the environment, institution and spread of infection to the innocent. Similar study on graduates of dental college found moderate to poor awareness levels.¹¹

As per the existing guidelines solid BMW generated by healthcare facilities (HCF) is to be collected by common BMW treatment facilities (CBMWTF) for further treatment, processing and disposal. Segregation is to be done by HCF in colour coded bags as per the pre-defined criterion on further treatment and processing by CBMWTF. One way to remember the colour coding is to repeatedly read and mug up without understanding the colour coding which has the limitations of the memory and items which can be remembered. Another way is to understand the conceptualisation of colour coding and then follow the same. In either scenario with BMW rules being taught to young graduates, they were asked to specify treatment options which are exercised by CBMWTF. No similar study was found comparing two institutions. Wide variation (Table 7a) was found in responses for various treatment options exercised by CBMWTF which highlight the lack of rigorous training leading to variation or possibly due to deficient training sessions.⁴

Segregation of BMW is done to ensure minimal handling and that waste generated undergoes designated treatment and disposal process. Understanding of final treatment and disposal is one of the way, one can ensure that even in an uncommon situation; items are segregated into correct bins/colour coded bags. Table 8b depicts variation in responses of graduates of two dental colleges. Such variation becomes a source of error and depicts that training sessions are deficient in providing thorough understating to dental

graduates. It may mean that teaching on BMW is to the minimal extent with guidance only on colour coding rather than making them understand the underlying logic. Lower awareness was also found in another study.¹⁰

One may argue that BMW rules and regulations are very confusing and it is not possible to provide complete clarity to graduates. This may not be acceptable in lieu of the strict penal provisions under the act where there is no scope for even a single error. Participating graduates after pre-test were provided with a dedicated interactive training session trying to provide conceptual clarity on BMWMH rather than just a theoretical session. Following this post session same questionnaire was again substituted to the volunteers. Remarkable improvement in responses were found but responses were not 100% correct, which essentially signify the need for repeated sessions^{4,12} with innovation and making graduates understand rather than mug up the applicable guidelines.

Conclusions: To conclude this study it can be said the dental curriculum is deficient in providing and ensuring due training and logical understanding to the young graduates and that there are significant difference on imparting training between two dental colleges indicating the lack in uniformity in education and training. Study showed a significant improvement after a dedicated training program.

Recommendations: Strict implementation of biomedical waste management rules is the need of the hour. Dental curriculum needs to be redefined and refined to enable uniformity and to ensure adequacy of training. Healthcare facilities must appoint specifically trained healthcare personnel. Training sessions must not be occasional and casual ones, rather training on BMW should be a continuous process with regular competency assessments of knowledge levels.

Study limitation: The study was limited to two colleges only. The sample size might not be the exact representatives of the national scenario so as to generalize the findings of the study.

Acknowledgment: The authors acknowledge participation and feedback of participants and the Principals and faculty members of both the dental colleges.

Financial support and sponsorship: Nil

Conflict of interest: Nil

REFERENCES :

- Gupta S, Sodhi SP, Brar GK, Bansal RN. Risk factors for Hepatitis C: A clinical study. J Med Sci 2018;39:1-7 DOI:10.4103/jmedsci.jmedsci_155_17
- World Health Organization (WHO). Wastes from healthcare activities. Fact sheet No. 253, Geneva. 2009.
- Bansal RN, Malhotra M. Motivating Human Resources for Health at Govt Medical Colleges: The leaders' Way. Online J Health Allied Scs. 2016;15(3):2. Available at URL: <http://www.ojhas.org/issue59/2016-3-2.html>
- Bansal RN, Gupta S. Gaps in Nursing Training on Biomedical Waste Management and Handling: Situational analysis at a Tertiary Care Public Hospital. J Punjab Acad Forensic Med Toxicol 2018;18(1):28-32 DOI:10.5958/0974-083X.2018.00006.7
- Government of India. Ministry of Environment and Forests Gazette Notification No.460, dated 27th July 1998, New Delhi. Available at: <http://envfor.nic.in/legis/hsm/biomed.html>
- Government of India ministry of Environment, Forest and Climate change, Notification dated 28th March, 2016, New Delhi, pp.1-37. Published in the Gazette of India, Extraordinary, Part II, Section 3, Sub-section (i).
- Dental council of India . http://www.dciindia.org.in/Inspection_pdf/dci%20inspection%20proformas%20modified-1,%2020.12.2011/for%20DCI%20Public%20Health%0-%20Modified,%202001.12.2011/starting%20mds%20Public%20Health.doc
- National accreditation Board of Hospitals. <http://www.nabh.co/dental-Standard.aspx>
- The Clinical Establishments (Registration and Regulation) ACT, 2010 <http://clinicalestablishments.gov.in/WriteReadData/147.pdf>
- Singh RD, Jurel SK, Tripathi S, Agrawal KK, Kumari R. Mercury and Other Biomedical Waste Management Practices among Dental Practitioners in India. BioMed Research International. 2014; Article ID 272750, 6 pages. <https://doi.org/10.1155/2014/272750>
- Kahar AR, Arora A, Radk e U, Joshi J. Assessment of awareness regarding biomedical waste management among students and interns of dental institute. Indian J Multidiscip Dent. 2017;7:65-70
- Mannapur BS, Dorle AS, Ghattargi CH, Kulkarni KR, Ramdurg UY, Hiremath LD, Suma N. "Impact of Educational Intervention on the Knowledge of Bio-Medical Waste Management among Health Care Workers in a Tertiary Care Hospital at Bagalkot City". Journal of Evolution of Medical and Dental Sciences. 2014;19:5076-5082. DOI: 10.14260/jemds/2014/2556

Name _____ Gender _____

Qualification _____

Graduation Completion year/month _____ Internship completion yr/mth _____

Questionnaire to assess level of awareness on:

Biomedical Waste (management and handling) Rules

* This feedback and assessment shall be kept confidential for the identity of person filling this form

* This feedback form may be used for statistical analysis

Please mark the correct options (there may be more than one correct option)

1 Bio-medical waste awareness training was provided to you

☐ never ☐ Within last 1 year ☐ More than 1 year ago

2 Who had taken training session on BMW for you

☐ Administrator ☐ Nurse of that area ☐ BMW Coordinator
☐ Faculty/Doctor/HOD ☐ Infection control Nurse

3 BMW management, segregation and proper disposal is required for

☐ To prevent reuse of consumable items
☐ To prevent cross infection through reuse of items
☐ To prevent smell from common garbage
☐ To prevent animals from swallowing plastics
☐ To prevent burning of plastics
☐ To prevent injury to waste handling worker
☐ To prevent infection to waste handling workers

4 Segregation of BMW (multiple options)

☐ must be done at point of generation ☐ at central treatment facility
☐ by persons generating waste ☐ by nurses
☐ at central collection point ☐ by class 4 staff
☐ By waste collecting workers

5 What are modes of disinfection used by outsourced contractors (multiple options)

☐ Incineration ☐ Shredding ☐ Burial
☐ Autoclaving ☐ Chemical disinfection ☐ Mutilation

6 what is done to waste is yellow bags by outsourced agency

☐ incinerated ☐ Crushed and autoclaved
☐ Autoclaved ☐ Disinfected with hypochlorite and then buried

7 what is done to waste is Blue bags by outsourced agency (multiple options)

☐ incineration ☐ Chemical Disinfection ☐ Shredding
☐ Autoclaved ☐ Deep burial ☐ Mutilation

8 what is done to sharps by outsourced agency (multiple options)

☐ incineration ☐ Chemical Disinfection ☐ Shredding
☐ Autoclaved ☐ Deep burial ☐ Mutilation

9 Mark the respective color coded bag for segregation of BMW

		White jar puncture proof	Yellow Bag	Blue bag	Black bag with BMW mark	Bag for Municipal Waste
a	Facemask					
b	Bandages					
c	Blood soaked plaster					
d	Plastic Test Tubes					
e	Broken Test Tubes					
f	Slides					
g	Amputated foot/ resected Mandible					
h	Eatables/food					
i	Biopsy					
j	Gloves					
k	Half filled blood bag					
l	Empty Blood Bag					
m	Burnt and disinfected needles					
n	Surgical Blades					
o	Cut Syringes					
p	Cut IV sets					
q	Torn/cut gloves					
r	Uncut Gloves					
s	Broken Ampoules					
t	Removed tooth					

Table 9: Responses on colour coding for segregation of specified hospital waste items

	Pre-Test							Post-Test						
	n	NR	WH	YE	BL	MW	Other	n	NR	WH	YE	BL	MW	Other
Face mask	196	40	0	126	27	0	3	199	10	1	176	11	0	1
Bandages	196	53	0	119	20	0	4	199	16	0	175	7	0	1
Blood soaked plaster	196	63	6	115	11	0	1	199	25	4	157	12	0	1
Plastic Test Tubes	196	81	33	9	63	3	7	199	19	19	4	156	0	1
Broken Test Tubes	196	66	93	7	21	3	6	199	18	138	5	37	0	1
Slides	196	74	87	10	19	2	4	199	31	127	11	29	0	1
Amputated foot/resected Mandible	196	88	14	76	7	5	6	199	27	6	149	9	0	8
Eatables/food	196	78	3	10	1	0	104	199	28	1	17	3	0	150
Biopsy	196	101	9	63	17	3	3	199	33	7	147	10	0	2
Gloves	196	54	4	30	108	0	0	199	18	4	29	148	0	0
Half filled blood bag	196	98	5	71	18	3	1	199	27	7	42	123	0	0
Empty Blood Bag	196	103	8	40	35	0	10	199	24	9	23	137	0	6
Burnt and disinfected needles	196	85	88	3	16	1	3	199	33	133	12	18	0	3
Surgical Blades	196	73	105	6	10	1	1	199	29	143	11	14	0	2
Cut Syringes	196	70	90	7	24	1	4	199	26	94	8	70	0	1
Cut IV sets	196	90	59	11	33	0	3	199	34	36	14	115	0	0
Torn/cut gloves	196	68	6	24	97	1	0	199	25	4	22	148	0	0
Uncut Gloves	196	80	1	26	85	1	3	199	34	4	26	130	0	5
Broken Ampoules	196	90	74	12	15	0	5	199	32	128	21	16	0	2
Removed tooth	196	73	10	107	2	1	3	199	22	6	159	10	0	2

n=no of respondents, NR=No response, WH=White, YE=Yellow, BL=Blue, MW=Municipal Waste,

Original Research Paper

Estimation of Range of Natural Variations Contemplating Time Interval in Handwritten Scripts

1. **Shruti Gupta**, Assistant Professor, Amity Institute of Forensic Science, Amity University, Noida, India.
2. **Rakesh K. Garg**, Ex Professor and Head, Department of Forensic Science, Punjabi University, Patiala, India.
3. **S K Shukla**, Director, Amity Institute of Forensic Science, Amity University, Noida, India.
4. **Tamanna Jaitly**, Research Scholar, Amity Institute of Forensic Science, Amity University, Noida, India.

ABSTRACT :

Handwritten scripts contemplates a great difference in range of natural variations every time an individual executes the process of writing. The purpose of the present study is to focus on the attempt made to extract out natural variations with time interval. The task involved collection of 540 samples (60 samples from each age group- 30 samples each from males & females) from 9 different age groups, that is two handwriting samples in present handwriting (with an interval of 5-10 minutes between the two handwritings) with content same as that of previous written script (old handwriting) which is also taken as a sample for comparative study with time interval ranged from 2-33 years between present and old handwriting. It has been found that connectivity between letters, alignment and spacing are highly significant characteristic along with those which remain unaffected and intact by showing consistency in skill, tremors and speed in due course of age and time. The findings of the present study will assist in the cases which are lacking in contemporary handwritings and can be undertaken with ease by taking into account the handwriting characteristics which are prone to variations.

Corresponding Author :

Tamanna Jaitly

Research Scholar, Amity Institute of Forensic Science,
Amity University, Noida, India.

Contact : +91-07838409054

E-mail : tamannajaitly@gmail.com

Article History:

Received: 7 August 2018

Received in revised form: 14 August 2018

Accepted on: 14 August 2018

Available online: 1 June 2019

KEYWORDS : Asynchronous Writing, Natural Variations, Time Interval, Contemporary, Consistency.

INTRODUCTION :

The unconscious act of both writing and speech varies in aspect of habits of the individual acquired by thousands of repetition. Writing regarded as a practically visible speech or the “talking paper” is the most personal and individual thing that a man does, leaving a record that can be seen and studied. The resultant matter of concern becomes the variation in any particular handwriting for identification and differentiation of two handwriting depending on the characters and permanency^[1]. The extent of variation differs among writers and consequently, natural variation forms an indispensable element in the identification of handwriting. Variation could be as slight as occurring in minor details with the attendant risk of confusing the mind of an individual or could vary as widely that will render a clear mechanical features about a master pattern^[2]. A complexity of such habitual patterns repeated within a typical range of variation around the model patterns constitutes an individual's handwriting. Consideration of natural variation is meticulously kept in mind so as to distinguish forged and genuine writing^[3]. Regardless of the class of problems, persistence of variation still exist and must be accurately evaluated. The evaluation of distinguished factor

“Variation” proves to be the basic part of the identification as each identifying characteristic itself^[4].

Natural variations are intended in a way due to acclaimed fact that an individual's brain working is not similar to that of computers. The variations are the imprecision with which the habits (discriminating elements) of the writer are executed on repeated occasions. The possibility of divergences took place with the writer's condition, the writing conditions and the nature of the document. At the point when conditions are controlled, least variety between executions is observed^[5].

Accordingly, the perceivable fluctuations in writing are said to constitute a writer's range of natural variations. The matter of natural variations too could be broad or narrow depending on following factors;

Skilled Writer v/s Unskilled Writer: Skillful writings constitutes less variations as compared to the one executed by unskilled writer. Greater quantities of standards particularly collected standards are collected in the latter case to properly portray the nature and the range of variation. The variables affecting writing have a greater influence on less skillful writing than in skillful writing^[6].

Age of the Writer: Within the two extremes of life i.e., childhood and old age of an individual, there are severally different handwriting patterns according to the fluency of their muscular movement and power of incoordination. Individuals with old age constitutes more variation in writing as compared to the individual's with less age. The outcomes makes it feasible to distinguish one person's writing from another^[7].

Time : Differences can be found in handwriting written by a person now and years before.

Dawson (1987) established an internal consistency in handwriting in his research^[8]. Followed by Shrihari (2002), on the basis of his study had concluded the significant role of individuality in handwriting along with variations within it^[9].

Bafra (1998) had worked on 'the effect of time on handwriting' the results concluded very less difference and writing was more or less fixed even in terms of the range of natural variations and that the changes found could mislead to a wrong answer^[10]. The most significant study incorporated in the field by Kapoor et al. (1985) for conducting research on the study of the form and extent of natural variations in the genuine handwriting with the maturity in age. The inference demonstrated the establishment of natural variation in writings but exclusion from some of the characteristics were also detected^[11].

Various researches have been carried out on different aspects of questioned documents like typewriters and type written documents, forgeries and its types, disguised documents, components of inks, age of documents, different instruments and pen tips involved in producing writings, influence of drugs or alcohol on handwriting, computed methods and software for signatures and handwriting verification and identification etc^[5]. 'Natural variations' are least explored area for research work and very little information is available on this aspect even though natural variations are regarded as basis and foremost criteria in establishing the authenticity of one's handwriting.

Document Experts are restricted to compare and form an opinion from the present and latest handwriting samples as admitted ones. It is expected that present research work will provide significant information to one step forward and aid to document experts in examining old handwritings as admitted for the purpose of comparisons especially for under those conditions who are unavailable or dead, by considering the aspect of natural variations and consistency in handwriting characteristics after an interval of time and age.

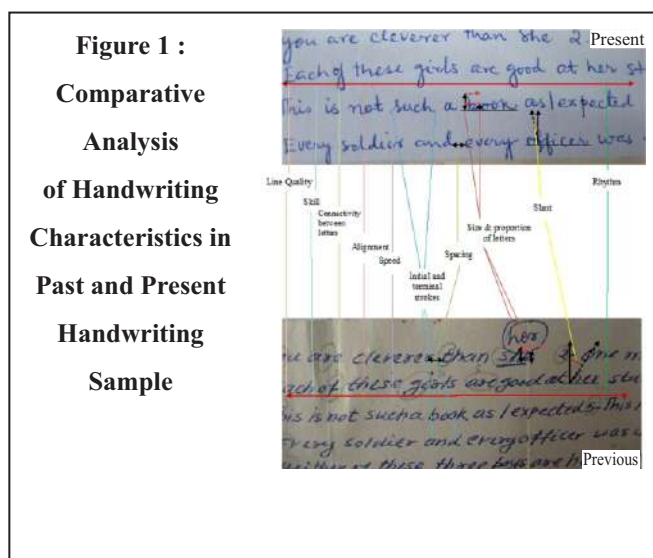
MATERIAL AND METHODS :

A total number of 540 (60 samples from each age group- 30 samples each from males & females), has been collected from 9 different age groups i.e.

- 20 to 25 years • 35 to 40 years • 50 to 55 years
- 25 to 30 years • 40 to 45 years • 55 to 60 years
- 30 to 35 years • 45 to 50 years • 60 above.

The exclusion method for continuous data classification has been adopted to classify above said age groups in this investigation^[12-13].

The samples were collected from individuals on **A-4 sized** plain paper sheet, two handwriting samples in present handwriting with content (a small paragraph consisting 4-8 lines) in English which was same as the previous written script which is also taken as a sample for comparative study (**Fig. 1**).



Three handwriting samples - two in present handwriting (with an interval of 5-10 minutes between the two handwritings) and one old or past handwriting sample were collected from each individual (interval ranged from 2-33 years between present and old handwritings). In another A-4 sized paper, handwriting sample of capital letters (A-Z), small letters (a-z) which was supposed to show significant variations were taken.

The samples were collected with '**045 Reynolds fine carbure**' ball point pen only for all samples.

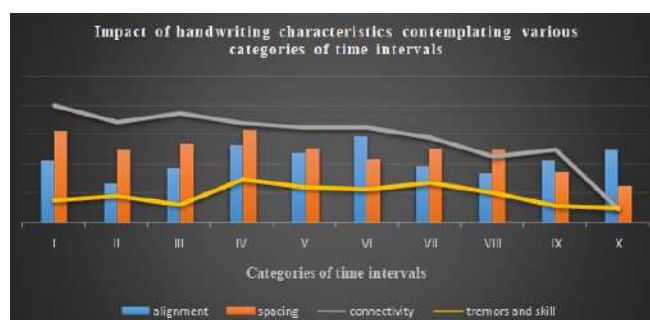
The effect of time interval between two handwritings and how this time interval will induce variations in handwriting characteristics have been examined. For this purpose the Past and Present handwriting samples were collected from different time intervals ranging from 2 years to 33 years. As the range of time interval from **2 years to 33 years** is quite large. Consequently, time intervals were categorized into 10 parts as follows – Ist category for 2 years gap, IInd category for 3 years, IIIrd category for 4 years gap, IVth category for 5 years gap, Vth category for 6 and 7 years of gap, VIth category for 8, 9 and 10 years of gap, VIIth category for 11, 12, 13, 14 and 15 years of gap, VIIIth category for 16, 17, 18 and 19 years of gap, IXth

have been noticed in Connectivity between letters and minimum in Rhythm. Followed by Vth (6 and 7 years) and VIth category, maximum variations have been found in Connectivity between letters and minimum in speed as at this point, actual variation in handwritings and considerable difference were majorly observed. Similar observations were depicted by VIIth category and VIIIth category (16, 17, 18 and 19 years) with maximum variations in Connectivity between letters and minimum in skill, tremors and speed. The significant result has been observed in VIII where skill has shown variation along with the other characteristics

As the time interval increases, the effect of natural variations contemplating Spacing, Alignment and tremors were found in majority compared to connectivity which is least affected (IXth category and Xth category). In overall maximum variations have been depicted by connectivity between letters in which variations were noticed since from category Ist i.e. at 2 years of time interval then rest of the handwriting characteristics. A minimum variation has been observed in Skill, which exhibited its existence in category VIIIth i.e. at 17 years of time interval.

The resultant impact of handwriting characteristics contemplating different categories of time intervals is demonstrated (Graph 1).

Graph 1: The resultant graph demonstrating the overall impact of handwriting characteristics contemplating various categories of time intervals.



The extent of connectivity gradually decreases with an increase in the time gap between the handwritings (from 2 years to 33 years). The rate of variation in spacing and alignment remains nearly consistent with the change in time interval. The minimum extent of variation within handwritings were observed collectively in the handwriting characteristic of "skill" as the foresaid element gets unaltered with time and age factor.

DISCUSSION :

Handwriting characteristics depicting variations in them with time interval is a new insight research so as to determine which

handwriting characteristic has depicted variations at which time interval. In the present research the time intervals ranging from 2 to 33 years were clubbed into 10 categories of the time intervals and the distribution of samples showing variations has been performed accordingly in the present investigation. The handwriting characteristics arranged in descending order of their variation on the basis of the findings, maximum variations have been reported in Connectivity between letters, Spacing and alignment, whereas minimum variations have been noted in skill, tremors and speed characteristics of handwriting. Bafra and Asicioglu (1998) have undertaken the research on the effect of time in handwriting. Collection of two samples from 50 individuals with a gap of 5 years were performed and observation revealed that size & proportion of letters, slant and writing quality, has more variations due to time than the rest of the characteristics, and these findings are in disagreement with the observations made from present research^[10]. Though this research is considered as recent and rare as the time interval ranged from 2 years to 33 years and the authors have not found much related studies or literature on the natural variations with this much of large time interval prior to this, therefore, no comparison can be made. And further authenticity using large of samples and in different populations can provide highly significant information.

CONCLUSION :

Contemporary writings are foremost requisite and unavailability of them due to infinite factors as if writer is no more alive or having poor physical/ health condition that resists the writer to reproduce handwriting in a similar manner or because of any accident cannot produce handwriting now with accustomed hand. In such cases, the present research provides useful information as being a new mode of investigation and will surely aid document experts to undertake these cases and to reach out a correct opinion. With the due effect of the findings gathered from the present investigation the cases lacking in contemporary handwritings can be undertaken with ease by taking into account the handwriting characteristics which are prone to variations (connectivity between letters, alignment and spacing) along with those which remain unaffected and intact by showing consistency (skill and speed) in due course or age and time. With throughout consistency observed in skill and speed, they are proven to be the most useful, promising and reliable handwriting characteristics in handwriting analysis with increasing passage of time. Connectivity between letters and alignment with increasing time interval has shown utmost natural variations in them and therefore they have established themselves as treacherous.

REFERENCES :

1. Osborn A. S. Questioned Documents. New York: The Lawyer's Co-operative Publishing Co.; 1910.
2. Kelly J.S., Lindblom B.S. Scientific Examinations of Questioned Documents. 2nd Ed. London: CRC Press, Taylor & Francis group; 2006. Pp 9-14;47-144.
3. Gupta S. "Estimation of Range of Natural Variations for Size and Proportion of Letters". Journal of Punjab Academy of Forensic medicine and Toxicology. 2017;17(02):20-24.
4. Hilton O. Scientific Examination of Questioned Documents. New York: Elsevier Science Publishing Co.Inc; 1984. Pp.56-67.
5. Jaitly T, Gupta S, Shukla S.K. "A Review Study to explore the dimensions of the Role of Size and Proportion of letters in Handwriting Examination". Anil Aggrawal's Internet Journal of Forensic Medicine and Toxicology [serial online]. 2016;19(2) (July - December 2018);5pp.
6. Huber A.R., Headrick A.M. Handwriting Identification Facts and Fundamentals. Boca Raton London: CRC Press; 1999. Pp.73-141.
7. Gupta S, Garg R.K., Nath S. "The influence of Natural variations on handwriting characteristics from different age groups with time gap". Turkish Journal of Forensic Science. 2012;11 (3): 11-20.
8. Dawson, G.A. "Internal Consistency in Handwriting". Canadian Society of Forensic Science Journal. 1987;20 (2): 57-60.
9. Shrihari, S. "Individuality of Handwriting". J. Forensic Sci. Soc. 2002;47(4):106-109.
10. Bafra J, Aşicioglu F. "Effects of Time in Handwriting". 1998. Proceeding 6th Indo Pacific Congress on Legal Medicine and Forensic Sciences. Kobe, Japan:890-893.
11. Kapoor T. S, Kapoor M, Sharma G.P. "Study of the Form and extent of natural variations in genuine writing with age". Journal of the Forensic Science Society. 1985;25 (5):371-375.
12. Gupta S.C, Kapoor V.K. Fundamental of Applied sciences (reprint). 3rd Ed. India: Sultan Chand & Sons; 1996. Pp.36-48.
13. Gupta S.P. Statistical Methods. 4th Ed. India: Sultan Chand & Sons; 2011. Pp.110-160.

Original Research Paper

A Study of Age Estimation through Epiphyseal Union of Medial End of Clavicle in an urban population of Haryana

1. **Varun Garg**, Senior Resident, Department of Forensic Medicine, North Delhi Municipal Corporation Medical College & Hindu Rao Hospital, Delhi
 2. **Ashish Singla**, Medical Officer, Government Hospital, Mandikhera Mewat, Haryana
 3. **Kunal Khanna**, Assistant Professor*
 4. **S K Dhattarwal**, Professor & Head, Department of Forensic Medicine, Pt. B D Sharma Post Graduate Institute of Medical Sciences, Rohtak, Haryana, India
 5. **Vijaypal Khanagwal**, Professor & Head*
- *Department of Forensic Medicine, Kalpana Chawla Government Medical College, Karnal (Haryana)

ABSTRACT :

Introduction: Age estimation has been considered a critical requirement in a number of medico legal settings and needs forensic expertise. A varied number of methods have been in practice with variable findings. Age determination with epiphyseal union of clavicle has its own benefits specially in identifying if the person has attained age of maturity.

Aim: To identify the stage epiphyseal fusion of medial end of clavicle for determination of age in a tertiary population of North India, along with any differences between epiphyseal fusion of medial end of clavicle in both sides.

Methods: 136 consenting volunteers of either sex between age group 16-25 years were radio graphed for medial end of clavicle bilaterally. The stage of ossification is identified according to Schmeling classification and age estimation is done.

Results: In this study no epiphyseal union is found in 22 participants ranging in age from 16-19 years. The first stage of fusion is seen at 16 years of age and extended upto 19 years of age. The stage of fusion on either side shows statistical significant association for age group indicating that stage of fusion is dependent upon the age of the person. The ossification stage is symmetrical on both side.

Conclusion: The present study concludes that epiphysis appeared in the range of 16-17 years and extended upto 19-20 years. The age of fusion of medial end of clavicle can be given as 22-24 years in the population of Haryana. Also, either side can be used for age

Corresponding Author :

Varun Garg,
Department of Forensic Medicine, Pt. B D Sharma Post
Graduate Institute of Medical Sciences, Rohtak, Haryana,
India, 124001.
Contact : +91 99911-66452
E-mail : garg42varun@gmail.com

Article History:

Received: 6 October 2018
Received in revised form: 7 October 2018
Accepted on: 7 October 2018
Available online: 1 June 2019

KEYWORDS : Anthropometry, Bisexual variation, Age estimation, Clavicle, Xiphi Sternum.

INTRODUCTION :

Age determination has been identified as a critical requirement in a number of settings including both civil and criminal proceedings. It becomes even more imperative for a country like India where majority lives in rural areas and awareness regarding registration of births and deaths were not needed until made mandatory by law. The role of medical jurist takes prime importance for age estimation to help the individual and government to proceed age appropriately. Height, weight, dental development, puberty stages and ossification centres appearance all are methods which aid in age identification. Determination of the age of an individual from the appearance and the fusion of the ossification centers is a well accepted fact

in the field of medical and legal professions^[1]. This is possible due to complex but dependable system by which the osseous framework of the body develops, grows and matures.

Examination of two main anatomic aspects teeth and hand^[2-4] wrist bones^[5] are traditionally used but their applicability in the forensic field is relatively limited due to biological variability, characters of the selected sample, racial, social and health differences and due to limited amount of information at key ages. Specially for discriminating a minor (i.e. 18 years) they are either fully developed as seen in the cases of hand wrist bones or are in the process of development as in third molars. Even so, at times third molars are missing for reasons of agenesis or extraction. Therefore and to confirm the dental

evaluation, skeletal parameters are also considered.^[6]

In recent years, there has been a renewed interest in forensic age estimation in living subjects by means of radiological methods. The post cranial skeleton begins to assume adult form between 11 and 20 years of age in majority of skeletal epiphysis with females typically commencing fusion a couple of years earlier than the males. The only exception is the clavicle, in which fusion occurs during the second decade and traces of its union sometimes extending up to late 20's^[7]. Singh and Chavali stated that the clavicle bone in comparison to other long bones in the body shows the longest time of growth activity in humans, so its predictive value can be considered as an effective and credible indicator for bone age assessment during the 25 years when other bones will have been inactive in human life^[8]. These changes can be studied by X-rays and thus, provide the medico-legal expert with a very strong tool. Extensive work on the determination of age of epiphyseal fusion has been carried out worldwide including some parts of our country. However, from the findings of various workers, it is evident that there is not only difference in the age of epiphyseal fusion in different countries but also in the different States in India. These differences may be on account of varying genetic, dietary and geographical factors. Reporting on medico-legal practice in India, a survey committee recommended to the Government of India in the year 1964 that a zone wise study for the problem of determination of age should be encouraged^[9]. Hence, radiological reference studies are needed to aid forensic age estimation in living individuals.

Traditional radiography classification systems differentiate four stages of clavicle ossification:

Stage 1 : ossification centre is not ossified

Stage 2 : ossification centre is ossified, and epiphyseal plate is not ossified

Stage 3 : epiphyseal plate is partly ossified

Stage 4 : epiphyseal plate is fully ossified

Schmeling et al. divided the stage of total epiphyseal fusion into two additional stages :

Stage 4 : epiphyseal plate is fully ossified, and epiphyseal scar is visible

Stage 5 : epiphyseal plate is fully ossified, and epiphyseal scar is no longer visible^[10].

Schmeling identified that stage 5 was noticed very far from legally relevant thresholds, as the minimum age is 26 for women and 26.7 for men. Subsequently, when the existence of stage 5 development is verified, the subject must have attained the age of 21 at least 5 years prior to the examination and the age of 18 at least 8 years prior to the examination. This stage

becomes therefore relevant in cases where there is a period of several years between the crime and the court proceedings.

From biological and forensic stand points, CT and MR have also proved to be extremely helpful in estimating age in subjects still undergoing the process of growth. CT scan with its added benefits of two- and three-dimensional reconstructions of high quality, with no spatial deformation of the features studied helping in better assessment of chronological changes over age^[11,12] but also has its disadvantages in use of contrast dye injections causing higher radiation as compared to that in X rays and also increased errors if slice thickness is increased above a certain maximum value. On the other hand, the MR examination has decreased exposure risk but is expensive, uses contrast materials at times and also can induce a claustrophobic panic state in certain individuals^[13]. Questions also exist regarding reliability and reproducibility, together with the same concerns over the effects of ethnicity, coupled with the range of normality for bone fusion. Keeping the crucial role of identification of age with easily accessible methods in a developing country like India where resources are difficult to get by, this study aimed to know the status of epiphyseal fusion of medial end of clavicle for determination of age in a tertiary population of North India, along with any differences between epiphyseal fusion of medial end of clavicle in both sides. Secondary objective was to find if there is any association with height and weight changes and stages of fusion.

MATERIALS AND METHODS :

The present study was conducted in the Departments of Forensic Medicine and Radio-diagnosis, Pt. B. D. Sharma Post Graduate Institute of Medical Sciences (PGIMS), Rohtak.

The study comprised of a total number of 136 subjects aged between 16-25 years, subdivided into nine subgroups (i.e. 16 - 17 years; 17-18 years; 18-19 years; 19-20 years; 20-21 years; 21-22 years; 22-23 years; 23-24 years; 24-25 years) on age basis. The volunteers are mostly the students from medical college, various paramedical courses, degree colleges and schools of Rohtak from the state of Haryana region. It included 73 males and 63 females. The purpose of study and procedure was explained to volunteers and informed written consent was taken. The age was verified from the birth certificate / date of birth entered in the admission records of school/matriculation certificate. The X-ray films of the medial ends of both clavicles were taken in posterior anterior view (PA) and Anterior Oblique view after general physical examination and detailed history was taken to rule out any chronic illness and skeletal deformity. Institutional ethics committee approval was taken to conduct the study.

Inclusion criteria:

1. Those subjects in whom the exact date of birth is known (duly verified from the birth certificates/school admission records/matriculation certificate) are included in the study.
2. Apparently normal and healthy individuals (after taking detailed history regarding any chronic illness and skeletal deformities etc. and the brief clinical examination for the same) are considered for the study.

Exclusion criteria:

1. Those cases in whom exact date of birth is not known and those having any chronic illness, nutritional deficiency, endocrine abnormalities and apparent skeletal deformity/malformation which affects the skeletal growth, are excluded from the study.

Proforma was devised to collect all relevant information from volunteers. The ossification status of medial end of clavicle was divided into five stages in accordance to Schmeling et al¹⁰ method which is as follows:

1. **Stage 1 (F1)** Non union – when the epiphyseal cartilage did not begin to decrease in thickness.
2. **Stage 2 (F2)** Commence of Union- when the thickness of epiphyseal cartilage was found to be reduced appreciably ($1/4^{\text{th}}$ united)
3. **Stage 3 (F3)** Incomplete Union- when the epiphysis has begun to fuse with the shaft and complete union was well underway ($1/2$ united)
4. **Stage 4 (F4)** Complete Union –when the epiphyseal cartilage was bony in architecture with its density indistinguishable from the epiphysis and diaphysis in its neighborhood but an epiphysis line called epiphysis scar could still be distinguished.
5. **Stage 5 (F5)** Complete Union –complete union with absence of epiphyseal scar.

The appearance and fusion of medial end of clavicle is evaluated radiologically and the results were compared with previous known standards. The age of epiphyseal fusion was taken as the youngest age group in which complete fusion was noticed in all (100%) of the subjects. The results are statistically analyzed using SPSS (Statistical Package for Social Sciences) statistical software version 20.0 and the results were analyzed using simple proportion and percentage and Chi-Square is used to test the significance.

RESULTS :

In the present study total 136 subjects are enrolled out of which 73 are males. **Table 1** depicts age and gender wise distribution among the 9 different age groups.

Table 1 :Age and Gender wise Distribution of Cases

Age groups (in years)	Total (N=136)	Male (73)	Female (63)
16-17	14	7	7
17-18	17	10	7
18-19	20	13	7
19-20	14	7	7
20-21	14	7	7
21-22	14	7	7
22-23	14	7	7
23-24	14	7	7
24-25	15	8	7

On both side there are 22 subjects each with no epiphyseal end extending upto age group 16-19 years. The F 4 stage that is the stage of complete fusion but with an epiphyseal scar is seen in 19 subjects on right side. This stage started in the age group of 20-21 and extended up to 22-23 but of these no stage F4 is seen in 22-23 years age group female as by that age all the medial epiphysis of clavicle has already fused with the diaphysis. The F 5 stage that is the stage of complete union with absence of epiphyseal union is seen in total of 38 subjects out of which 16 were males and 22 are females. (**Table 2 and 3**). The stage of fusion on either side showed statistical significant association for age group indicating that stage of fusion is dependent upon the age of the person.

DISCUSSION :

The importance of age estimation has been implicated over the last few decades in both civil reasons as well as those with more serious criminal consequences. Forensic expertise is frequently sought after in such scenarios. Considering that there are various methods of age estimation, a reliable and more accurate method is always looked up. Age estimation through clavicle gains an upper hand due to it being the last bone to fuse. Various studies both India and outside as shown in **Table 4** have time and again recorded the importance and reliability of age evaluation by this method. India with its cultural diversity and ethnic differences may lead to different values recorded in different parts of country and hence the need to verify the findings from every region so that standards can help in civil and criminal proceedings whenever its needed. Schmeling classification is used considering higher precision and X rays were used to radiograph clavicle as it is one of the most accessible, easily available cost effective and equally reliable tool for estimation in comparison to advanced radiographic techniques like CT scan and MRI for a developing country like India. In the present study, an attempt was made with the best available resources to identify the age estimation with clavicle radiographs and help in reaching a

Table 2 : The Stage of Fusion on Right Side with Age Group

Age Group	Right Side												Total	
	NA		F1 Stage		F2 Stage		F3 Stage		F4 Stage		F5 Stage			
	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent		
16-17 years	12	54.55%	2	8.33%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	14	10.29%
17-18 years	6	27.27%	11	45.83%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	17	12.50%
18-19 years	4	18.18%	10	41.67%	6	60.00%	0	0.00%	0	0.00%	0	0.00%	20	14.71%
19-20 years	0	0.00%	1	4.17%	3	30.00%	10	43.48%	0	0.00%	0	0.00%	14	10.29%
20-21 years	0	0.00%	0	0.00%	1	10.00%	6	26.09%	7	36.80%	0	0.00%	14	10.29%
21-22 years	0	0.00%	0	0.00%	0	0.00%	6	26.09%	7	36.80%	1	2.64%	14	10.29%
22-23 years	0	0.00%	0	0.00%	0	0.00%	1	4.35%	5	26.30%	8	21.06%	14	10.29%
23-24 years	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	14	36.89%	14	10.29%
24-25 years	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	15	38.47%	15	11.03%
Total	22	100.00%	24	100.00%	10	100.00%	23	100.00%	19	100.00%	38	100.00%	136	100.00%
							Chi Square		df		p			
							311.114		40		<0.001		Highly Significant	

Table 3 : The Stage of Fusion on Left Side with Age Group

Age Group	Left Side												Total	
	NA		F1 Stage		F2 Stage		F3 Stage		F4 Stage		F5 Stage			
	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent		
16-17 years	12	54.55%	2	8.33%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	14	10.29%
17-18 years	6	27.27%	11	45.83%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	17	12.50%
18-19 years	4	18.18%	10	41.67%	6	60.00%	0	0.00%	0	0.00%	0	0.00%	20	14.71%
19-20 years	0	0.00%	1	4.17%	3	30.00%	10	43.48%	0	0.00%	0	0.00%	14	10.29%
20-21 years	0	0.00%	0	0.00%	1	10.00%	6	26.09%	7	36.80%	0	0.00%	14	10.29%
21-22 years	0	0.00%	0	0.00%	0	0.00%	6	26.09%	7	36.80%	1	2.64%	14	10.29%
22-23 years	0	0.00%	0	0.00%	0	0.00%	1	4.35%	5	26.30%	8	21.06%	14	10.29%
23-24 years	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	14	36.89%	14	10.29%
24-25 years	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	15	38.47%	15	11.03%
Total	22	100.00%	24	100.00%	10	100.00%	23	100.00%	19	100.00%	38	100.00%	136	100.00%
							Chi Square		df		p			
							311.114		40		<0.001		Highly Significant	

possible standard of identification.

In the present study the appearance of ossification centre is first observed at the age 17 and extends up to age 18 years. Stage 1 fusion of epiphysis, when the epiphyseal cartilage did not begin to decrease in thickness first appeared at the age of 16-17 years and extended upto the age of 19-20 years. This finding is in keeping with several others studies where minimum age of appearance of ossification was noted around 16 years. In an Indian study by Inderjit²² the age was even younger as early as 11 years than seen in this study which could have been due to selected target population as well as due to methodological differences of radiology versus osteology.

The last stage when the epiphyseal cartilage is bony in architecture with its density indistinguishable from the epiphysis and diaphysis in its neighborhood and no epiphyseal cartilage is seen is first observed in the age group of 21-22

years and beyond 22-23 year age group all the female subjects had complete fusion of epiphysis whereas in the study conducted by Paterson²³ this stage was noted at the age of twenty five. Indian study by Singh et al had reported similar ages of epiphysis fusion appearance and complete fusion as this study postulating that since both the studies belonged to similar northern region of the country indicating ethnic similarities among population. Regional and role of ethnicity was even more validated when another study by Balasubramaniam et al in the southern region of our country reported a slightly higher age of complete fusion as compared to seen in this study. Also, in a recent study conducted in the district of Rawalpindi in Punjab in Pakistan also postulated that these figures where majority of cases showed complete epiphysis union between 24-27 years of age and earliest union occurred at the age of 22 years in males and 20 years in females

Image 1: Non Appearance of epiphysis of medial end of clavicle (Left side)

In the present study for non appearance of epiphysis of medial end of clavicle is 16-17 years and extends upto the age group of 18-19 years.



Image 2: Non Appearance of epiphysis of medial end of clavicle.(Right Side)

In the present study for non appearance of epiphysis of medial end of clavicle is 16-17 years and extends up to the age group of 18-19 years.



Image 3: Stage 1 of epiphysis of medial end of clavicle.

Stage 1 is stage of non union that is when the epiphyseal cartilage did not begin to decrease in thickness. In the present study for appearance of Stage 1 of epiphysis of medial end of clavicle is 16-17 years and extends up to the age group of 19-20 years



Image 4: Stage 2 of epiphysis of medial end of clavicle.

Stage 2 is stage of Commence of Union-when the thickness of epiphyseal cartilage was found to be reduced appreciably (1/4th united) .In the present study for appearance of Stage 2 of epiphysis of medial end of clavicle is 18-19 years and extends up to the age group of 20-21 years.



Image 5: Stage 3 of epiphysis of medial end of clavicle.

Stage 3 is stage of Incomplete Union-when the epiphysis has begun to fuse with the shaft and complete union was well underway (1/2 united) .In the present study for appearance of Stage 3 of epiphysis of medial end of clavicle is 19-20 years and extends up to the age group of 22-23 years.



Image 6: Stage 4 of epiphysis of medial end of clavicle.

Stage 4 is stage of Complete Union –when the epiphyseal cartilage was bony in architecture with its density indistinguishable from the epiphysis and diaphysis in its neighborhood but an epiphysis scar could still be undistinguished. In the present study for appearance of Stage 4 of epiphysis of medial end of clavicle is 20-21 years and extends up to the age group of 22-23 years.



Image 7: Stage 5 of epiphysis of medial end of clavicle.

Stage 5 is stage of Complete Union –when the epiphyseal cartilage was bony in architecture with its density indistinguishable from the epiphysis and diaphysis in its neighborhood with no epiphysis scar could be distinguished. In the present study complete union is first seen in the age group of 21-22 years and is complete till the age group 24-25 years.



Mean height and weight among different stages of fusion

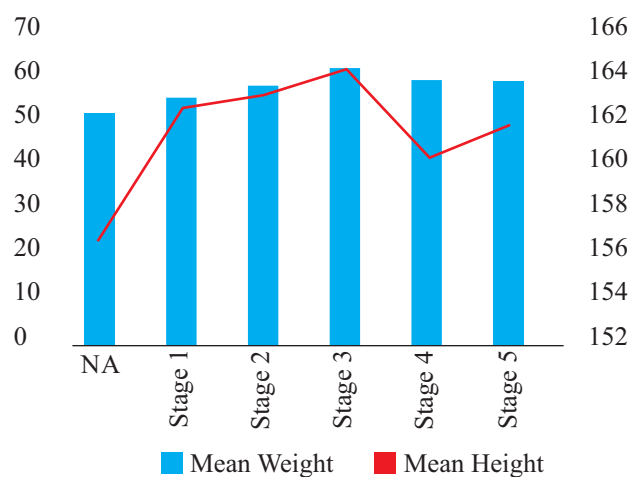


Figure 1 : Mean height and weight among different stages of fusion

Table 4: Different Studies on Age Estimation with Epiphyseal Changes at Medial end of Clavicle

Study/ year	Methodology	Conclusion
Mateen 2013; ¹⁴ Rawalpindi, Pakistan	Cross sectional study; 100 cases;18-27 years age group, radiological examination X rays,traditional stage classification	Complete union age from 20-21 years to 26-27 years
Hassan 2016; ¹⁵ Karachi , Pakistan	Cross sectional study; 624 subjects;19-24 years age group,radiological examination X rays,traditional stage classification(fused vs non fused)	Mean age of fusion of medial end of clavicle is 21 ±1.43 years
Ufuk 2016; ¹⁶ Denizli , Turkey	Cross sectional study; 354 subjects;10-30 years age group, Chest CT and pulmonary CT angiography, Schmeling classification	Epiphyseal ossification center appeared from 11 to 21 years of age. Complete fusion was first achieved at the ages of 18 - 19 years.
Ho Yoon 2016; ¹⁷ Seoul, Korea	Cross sectional study; 1151 subjects;16-30 years age group,radiological examination X rays, Schmeling classification	Stage V range from 18-30 years
Wittschieber 2014; ¹⁸ Munster, Germany	Cross sectional study; 572 subjects;10-40 years age group,radiological examination CT Scan, Schmeling classification	Stages 4 and 5 were found in the ages of 21 years and 26 years, respectively, as minimum ages for these stage
Singh & Chavali 2011; ⁸ Chandigarh, India	Cross sectional study; 343 subjects; post pubertal >17 yr age group, gross examination	Commencement of fusion was seen as early as 18 years of age but complete fusion was not seen until the age of 22 years and latest at 31 to 32 years. Advanced stages of clavicular epiphyseal union were seen in most after 23 years of age.
Balasubramanian 2016; ¹⁹ Chennai, India	Cross sectional study; 155 subjects; 15-30 yr age group, radiological examination X rays, traditional stage classification	In this study complete epiphyseal union was seen at the age of 25–26 years.
Pardeep Singh 2010; ²⁰ Punjab , India	Cross sectional study; 100 subjects; 16-25 yr age group, Chest X ray examination, traditional stage classification	Earliest union at 20 years of age and majority showed complete union by 22–23 years
Bhise 2012; ²¹ Mumbai, India	Cross sectional study; 131male and 68 female between 9-25 yr age group and 3-23 yr age group respectively, radiological X ray examination, Schmeling classification	Epiphysis appeared at 15–16 years and complete fusion occurred at 23–24 years in males and 21–22 years in females

still cannot be applied to the other races in criminal investigations as different races have different age of union of sternal end of clavicle. The present study also concurred with commonly held view that secondary centres of ossification appear and fuse in India earlier than the western countries, though recently, our southern counterpart found slightly different findings disputing this fact. The observations of the present study are found to be in accordance with the study on time frame for ossification of the medial epiphysis of the

clavicle as revealed by CT scans by Schulz, Muhler et al.²⁴The findings of present study are closely related to those in a study on development of the medial clavicular epiphysis in an Australian population using computed tomography as the imaging modality by Bassed, et al.²⁵ They observed that if an individual in this population has completely fused clavicles at stage 5, then for males they will be at least 18 years of age, with a 99% certainty of being at least 21, and for females they will be at least 20 years old.

In the present study there is no difference found in the time and stage of fusion in both the sides of clavicle in any subject that is ossification is found to be bilaterally symmetrically. This is in keeping with study by Ufuk et al where no significant differences between left/right clavicular epiphyseal stages was seen. Only 18 subjects had stage differences between the two sides and mainly seen in stages III B and IV.¹⁶ The rate of left/right difference in Ufuk study was comparable to 8.6% reported by Hillewig et al.²⁶ and 8% reported by Franklin et al.²⁷ In study by Brown et al ²⁸ 4.6% of the X-rays showed a developmental gap between the left and right clavicle but the difference was non significant. Therefore it was concluded that results from either left or right clavicle can be used during the forensic estimation of age.

Study by Brown et al concluded that poor nutrition which is related to low socio-economic status has been shown to generally retard linear growth and may also contribute to the delayed fusion.²⁸ Interestingly our study showed statistically significant difference between mean height and weight with stage of fusion hypothesizing the link between linear growth, socio economic status, stages of fusion and age estimation. Forensic specialist should always look into this aspect among other things before giving a final conclusion on age of any individual.

A small sample size in each group can be a limitation of this study as phase changes and differences could be more possible to identify in a larger series. Nutritional, developmental and endocrine abnormalities along with the socio economic status were not taken in which could affect the skeletal growth. This study could also have been correlated with anatomic and if feasible CT findings.

CONCLUSION :

With the present study it is concluded that epiphysis appeared in the range of 16-17 years and extended upto 19-20 years. The age of fusion of medial end of clavicle can be given as 22-24 years in the population of Haryana. Also, either side can be used for age estimation with reliable results. The variability seen in different areas globally as well as locally calls for further studies on large sample for standardization considering the ethnic and racial differences.

Source of Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of Interest : None

REFERENCES :

1. Thali MJ, Viner MD, Brogdon BG. Brogdon's Forensic Radiology. 2nd ed. Boca Rotona : CRC Press;2010.
2. Foti B, Layls L, Adalian P, Giustiniani J, Maczal P, Signoli

- M, et al. New forensic approach to age determination in children based on tooth eruption. Forensic Sci Int. 2003;132(1):49-56
3. Demirijan A, Goldstein H, Tanner JM. A new system of dental age assessment. Hum Biol. 1973;45:211-22
4. Cameriere R, Ferrante L, D Angelis D ,Scarpino F, Galli F . The comparison between measurement of open apices of third molars and Demirijan stages to test chronological age of over 18 years old in living subjects .Int J Legal Med. 2008;122:493-7
5. Cameriere R, Ferrante L. Age estimation in children by measurement of carpals and epiphysis of radius and ulna and open apices in teeth: A pilot Study. Forensic Sci Intl. 2008;174:60-3
6. Schmeling A ,Geserick G , Reisinger W , Olze A. Age estimation. Forensic Sci Int. 2007;165(2):178-81
7. Sheuer L, Black S, Cunningham C. Developmental Juvenile Osteology .New York: Academia Press ;2000
8. Singh J, Chavali K. Age estimation from clavicular epiphyseal union sequencing in a Northwest Indian population of the Chandigarh region. J Forensic Legal Med. 2011;18(2):82-7.
9. Survey committee report on medico-legal practice in India, Director General Health Services, Ministry of Health, New Delhi. 1964. [Online][cited on 2013 April 10]. Available from; URL : <http://www.forensic.to/.../survey%20committee%20report%20details.html>
10. Schmeling A, Schulz R, Reisinger W, Muhler M, Werecke KD , Geserick G. Studies on the time frame for ossification of the medial clavicular epiphyseal cartilage in conventional radiography. Int J Legal Med .2004;118(Feb;118):5-8
11. Kellinghaus M, Schulz R, Vieth V, Schmidt S, Schmeling A (2010) Forensic age estimation in living subjects based on the ossification status of the medial clavicular epiphysis as revealed by thin-slice multidetector computed tomography. Int J Legal Med 124:149–154
12. Cameriere R, De Luca S, De Angelis D, Merelli V, Giuliadori A, Cingolani M, Cattaneo M, Ferrante L. Reliability of Schmeling's stages of ossification of medial clavicular epiphyses and its validity to assess 18 years of age in living subjects . Int J Legal Med 2012. Published online. DOI 10.1007/s00414-012-0769-4
13. Ramsthaler F, Proschek P, Betz W, Verhoff MA (2009) How reliable are the risk estimates for X-ray examinations in forensic age estimations? A safety update. Int J Legal Med 123:199–204

14. Mateen A, Khan A, Rasheed A, Aziz K. Age Estimation from Medial End of Clavicle by X-Ray Examination. www.pjmhsonline.com/octdec2013/ (accessed on 10/Oct/2014)
15. Hassan Q, Memon MU, Khalil IU, Ashraf T, Arif MM, Iqbal AS. Radiological Mean Age of Fusion of Medial End of the Clavicle : A Parameter of Age. *J Coll Physicians Surg Pak.* 2016 Jan; 26 (1) : 18-22. doi : 01.2016/JCPSP.1822
16. Ufuk F, Agladioglu K, Karabulut N. CT evaluation of medial clavicular epiphysis as a method of bone age determination in adolescents and young adults. *Diagnostic and Interventional Radiology.* 2016;22(3):241-246. doi:10.5152/dir.2016.15355.
17. Yoon SH, Yoo HJ, Yoo R-E, et al. Ossification of the Medial Clavicular Epiphysis on Chest Radiographs: Utility and Diagnostic Accuracy in Identifying Korean Adolescents and Young Adults under the Age of Majority. *Journal of Korean Medical Science.* 2016;31(10):1538-1545. doi:10.3346/jkms.2016.31.10.1538.
18. Wittschieber D, Schulz R, Vieth V, Küppers M, Bajanowski T, Ramsthaler F, Püschel K, Pfeiffer H, Schmidt S, Schmeling A. The value of sub-stages and thin slices for the assessment of the medial clavicular epiphysis: a prospective multi-center CT study. *Forensic Sci Med Pathol.* 2014;10:163–169
19. Balasubramanian S ,Priyatharsini K. Determination of age from the appearance and fusion of medial clavicular epiphysis - An autopsy based study conducted at Govt. Stanley Medical College. *Indian J Forensic Community Med* 2016;3(3):187-193
20. Singh P, Gorea R.K., Oberoi S.S. Kapila A.K. Age estimation from Medial end of Clavicle by X-Ray Examination. *J Indian Acad Forensic Med.* 2010;32(1):28-30
21. Bhise S S ,Chavan GS ,Chikhalakar BG ,Nanadkar SD. Age determination from Clavicle: A radiological Study in Mumbai Region. *J Indian Acad Forensic Med.* 2012;34(1):7-9
22. Jit I, Kulkarni M. Times of Appearance and Fusion of Epiphysis at the medial end of Clavicle. *Indian J Medical Res.* 1976;64(5):773-83
23. Paterson RS. A Radiological Investigation of the Epiphyses of the Long Bones. *J Anat.* 1929;14(1):28-46.
24. Shulze R ,Muhler M, Mutze S , Schimdt S, Reisenger W ,Schmeling A. Studies on time frame for ossification of medial epiphysis as revealed by CT scan. *Intl J legal Med.* 2005;119(3):142-5
25. Bassed RB , Drummer OH , Briggs C, Valenzuela A. Age estimation and medial clavicular epiphysis :Analysis of the age of majority in an Australian Population using Computed Tomography. *Forensic Sci Med Path* 2011;7(2):148-54.
26. Hillewig E, De Tobel J, Cuhe O, Vandemaele P, Piette M, Verstraete K. Magnetic resonance imaging of the medial extremity of the clavicle in forensic bone age determination: a new four-minute approach. *Eur Radiol.* 2011;21:757–767
27. Franklin D, Flavel A. CT evaluation of timing for ossification of the medial clavicular epiphysis in a contemporary Western Australian population. *Int J Legal Med.* 2015;129:583–594.
28. Brown AA, Derkyi-kwarteng L, Amonoo-Kuofi HS. Study on the time frame for ossification of the medial clavicular epiphyseal cartilage by X-ray in Ghanaian students. *Int J Morphol.* 2013;31(2):491-6.

Original Research Paper

Medicolegal Study of Deaths Due To Hanging In Varanasi Region

1. **Satish Kumar Khalkho**, Junior Resident (JR-III), Department of Forensic Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh
2. **Manoj Kumar Pathak**, Professor and Head, Department of Forensic Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh

ABSTRACT :

Introduction : Asphyxia is a condition caused by interference with respiration or due to lack of oxygen in inspired air which causes the organs and tissue being deprived of oxygen causing unconsciousness or death. Hanging is one of the leading manners of suicide. In hanging, there is suspension of the body by a ligature material compressing the neck externally, the constricting force is weight of the body or weight of the head.

Materials and Methods : The present prospective study was conducted on 86 cases of hanging reported during October 2016 to March 2018. Detailed history from police personnel and eye-witnesses as well as relatives regarding scene of crime, position of body, ligature material used, etc. were taken. Ligature mark was observed meticulously for all parameters.

Results : Total 150 out of 2976 medico-legal deaths were identified to be due to violent asphyxia with incidence rate of 5%. Hanging was the most common method of asphyxial death constituting 57.33 % of cases. Most common age group involved was 21-30 years. Majority of the hanging cases took place at the residence with suicide being the preferred manner of death.

Conclusion : This study was done with the objective of assessing data on hanging cases and documenting the characteristics of deaths resulting from hanging.

Corresponding Author

Dr. Manoj Kumar Pathak,

Professor and Head, Department of Forensic Medicine,
Institute of Medical Sciences, Banaras Hindu University,
Varanasi, Uttar Pradesh,
Contact : +91 94501-79177
E-mail : drmanojpathak@gmail.com

Article History:

Received: 23 November 2018
Received in revised form: 23 December 2018
Accepted on: 23 December 2018
Available online: 1 June 2019

KEYWORDS : Mechanical Asphyxia, Violent Asphyxial Death, Suicide, Accidental Hanging.

INTRODUCTION :

The term "Asphyxia" commonly means "lack of oxygen" and literally means 'defective aeration of blood' due to any cause. However, the term has been translated from the original Greek, implying "pulselessness/ absence of pulsation"^[1] Adelson defined asphyxia, as "a state in living organism in which there is acute lack of oxygen available for cell metabolism associated with inability of body to eliminate excess of carbon dioxide."^[2]

Hanging is the suspension of a person by a noose or ligature around the neck.^[3] The *Oxford English Dictionary* states that hanging in this sense is "specifically to put to death by suspension by the neck", though it formerly also referred to crucifixion and death by impalement in which the body would remain "hanging". Hanging has been a common method of capital punishment since medieval times, and is the official execution method in numerous countries and regions. The first account of execution by hanging was in Homer's *Odyssey* (Book XXII).^[4]

Hanging is a form of mechanical asphyxia in which the force applied to the neck is derived from a gravitational drag of the weight of the body or part of the body.^[5] Hanging is always considered suicidal except accidental hanging in sexual pervers, homicidal hanging in lynching and justifiable judicial hanging. In England and Wales, hanging accounts for about 2000 deaths each year and is considered the most common method of suicide.^[6] In USA, 92.3% of all suicides were caused by firearms, hanging and poisoning.^[7] A report from Canada has also indicated hanging as the second most common method of suicide after suffocation.^[8] Many cultural and socio-economic factors are responsible for the causation of hangings. Rapid urbanization, industrialization and emerging family systems are resulting in social upheaval and distress.^[9]

Hence death due to hanging is one of the most complex and controversial areas in study of asphyxial deaths. To ascertain cause and manner of death in cases of hanging, meticulous examination of ligature mark, ligature material, neck dissection and other autopsy findings are much helpful. Also

visiting the scene of crime is pertinent in collection of circumstantial evidences and in examining the status of place of incidence.

MATERIALS AND METHODS :

After taking approval from Institutional Ethical Committee, the present study was conducted on 86 cases of hanging at Department of Forensic Medicine, Institute of Medical Sciences, Banaras Hindu University; Varanasi on bodies brought from the various Police Stations of Varanasi and surrounding areas during October 2016 to March 2018. Selected cases had died due to violent asphyxial death and confirmed to be so after the post-mortem examination. Additionally cases reported by police as not being the case of violent asphyxial death but came out so after post-mortem examination, were also included in present study.

RESULTS AND DISCUSSIONS :

Total number of autopsies conducted during the study period was 2976, out of which 150 were mechanical asphyxial deaths. (Table 1). Incidence of violent asphyxial death in our study was 5%.

Table 1: Incidence of Violent Asphyxial Deaths.

Case	No. of Autopsy	Percentage (%)
Asphyxial Deaths	150	5
Other	2826	95
Total	2976	100

Similar observations were reported by Sharma et al (5%)^[10], Amandeep et al (5.26%)^[11], Patel Ankur et al (5.63%)^[12], Mariam Arif (5.9%)^[13], Tirmizi S Z et al (7.08%)^[14], Murty OP et al (10%)^[15] and Ajay Kumar S et al (10.50%)^[16]. But in studies done by Reddy SP et al, and Azmac D et al, the incidences were 19.15% and 15.7% respectively.^{[17][18]}

Out of 150 asphyxial death cases, hanging was found to be the commonest of all, 86 cases (57.3%), followed by drowning 36 (24.0%), strangulation 7 (4.67%), throttling 2 (1.33%), smothering 1 (0.67%) respectively and accidental finding of relatively higher proportion of traumatic asphyxia, 18 (12%) number of cases. Similar findings were observed in hanging deaths the study done by Sharma et al (69%)^[10], Reddy SP et al (61.19%)^[17] and Chaurasia N et al (52.21%)^[19], Tirmizi SZ et al (36.48%)^[14] and Patel Ankur P et al (82.48%)^[12]. (Figure 1)

Figure 1:
Distribution of Cases of Violent Asphyxia Death on the Basis of the Type of Death

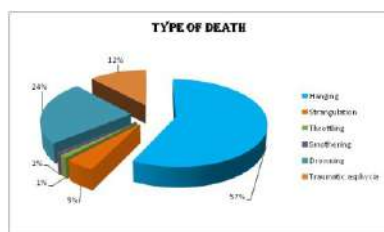


Table No. 2 provides the statistics about the *status of Place of Incidence*: Most cases happened in places locked room from inside 44 (51.16%), looked room from outside 10 (11.63%), places of hanging for 29 (33.72%) subjects were open space. The findings of present study are similar to study by Punitha R et al. indoor in 27 cases (79.4%) and outdoor in 04 cases (11.7%)^[20], Rawat V E J reported 72 (71.29%) indoor & outdoor sites 29 (28.71%)^[21]. Similar with the done by Uzunet al., (2007)^[22] Ambadeet al., (2007)^[23], Mohantyet al., (2007)^[24], Ankur P et al., (2013)^[12]. It can be seen that majority people from urban population of Gujarat State committed suicide by hanging within the closed secure places i.e. at their home rather than at open place like hanging from a branch of tree.

Table 2: Place of Incidence in Cases of Hanging:

Place of Incidence	No.	Percentage (%)
Locked Room from Inside	44	51.16
Locked Room from Outside	10	11.63
Open Space	29	33.72
Not Known	3	3.49

Table No.3: Highlights about the place of incidence due to hanging. 57 (66.28%) were found in home followed by hostel 8 (9.30%) and forest in 7 (8.14%) cases respectively. The findings of present study are similar with the study done by S H Bhosleet al.; most common place of hanging was home in 69.88%^[25], by Cooke et al., (71%)^[26], Uzunet al., (83.33%)^[22], Elfawalet al., (95.08%)^[27] and Bowen DA^[28]. Usually person prefers any secluded place which suit for his/her purpose. The victim being very well aware of the home and its surroundings, it suits his or her needs for hanging.

Table 3: Distribution of Cases of Hanging on the Basis of the Place of Incidence:

Places	Frequency	Percentage (%)
House	57	66.28
Hostel	8	9.30
Hotel	3	3.49
Working Place	5	5.81
Farm	3	3.49
Forest	7	8.14
Any other/ Unknown	3	3.49
Total	86	100.00

Table No. 4 compiles the statistics about points of Suspension used: The suspension point used by victims most commonly was ceiling fan 35 (40.70%) followed by ceiling of room 21 (24.42%), hook of door 12 (13.95%) and trees in 10 (11.63%) cases respectively. Vijyakumari^[29] reported ceiling fans, beams and grills as the common ligature points in

hanging deaths. Pal SK et al reported iron guider was the commonest ligature point in 23.77% cases followed by ceiling fan in 22.95% and trees in 18.55% cases^[30]. Ambade et al found trees were the commonest ligature points in hanging deaths followed by beams and ceiling fans.^[23]

Table 4 : Distribution of Hanging Cases on the Basis of Suspension of Body

Body was suspended from	Frequency	Percentage (%)
Ceiling of Room	21	24.42
Ceiling Fan	35	40.70
Hook of Door	12	13.95
Window	3	3.49
Beam	5	5.81
Trees	10	11.63
Total	86	100.00

Table No. 5: This table highlights the type of material used for hanging. Soft material was used in 45 (52.33%) cases followed by hard material in 41 (47.67%) cases. These observations were consistent with the study of Sharma B R et al (soft material 56.36% & hard 43.64%)^[10], Naik SK (soft 59.97% & hard 46.03%)^[31] and Patel AP et al, (soft 80% & hard 20%)^[12].

Table 5: Distribution of Hanging Cases on the Basis of Ligature Material

Material	No.	Percentage (%)
Soft	45	52.33
Hard	41	47.67
Total	86	100

Table No. 6: Most common material used was *Rope* 37 (43.02%) followed by *Saree* 14 (16.28%), *Bed Sheet* 12 (13.96%), *Dupatta* 10 (11.63%). Least used was *Electric Wire* in just 4 (4.65%) cases. These observations were more or less similar to the studies of Elfawal et al (85.41%)^[27], Sheikh et al (53.02%)^[34], S H Bhosle et al (53.01%)^[25], Dixit et al, Uzun et al and Cooke et al (reported rope as most common ligature material)^[35] [22][26]. Dupatta was the most common used ligature material in a study by Momin et al., 31.96% cases^[36] and Sharma et al., in 30.90% cases^[10]

Table No. 7 : This table depicts about the position of knot in hanging. Maximum cases presented with atypical type i.e 47 (54.65%) cases and typical type in 39 (45.35%) cases. These observations were similar to the studies of Jignesh B Patel et al and Naik SK et al^[31-32].

Table No. 8 : Maximum victims of hanging cases i.e 61 (70.93%) were found fully suspended followed by feet touching the ground in 22 (25.59%) cases and sitting 2 (2.32%)

cases and kneeling 1(1.16%) case. The observations were similar to the study by Vijaynath (reported 5.88% victims hanging partially and rest 94.12% were completely suspended)^[33]. Incidences were found lower for complete hanging by Suárez-Peñaranda et al i.e 62.4%^[37] but slightly higher by Sharma et al i.e 68%^[10]. Similarly, higher incidences of complete hanging were found in a study conducted by Naik and Patil, in 217 cases out of 232 cases of hanging^[31].

Table 6: Distribution of Hanging Cases on the Basis of Ligature Material and its Types

Material	Frequency	%age
Soft Material	Saree	14
	Dupatta (Chunni)	10
	Bed Sheet	12
	Lungi	5
Hard Material	Rope	37
	Electrical Wire	4
Total		86
		100.00

Table 7 : Gender-wise Distribution of Types of Hanging Cases on the Basis of Position of Knot

Position of Knot	Male		Female		Cases	
	No.	%	No.	%	No.	%
Typical	23	45.10	16	45.71	39	45.35
Atypical	28	54.90	19	54.29	47	54.65
Total	51	100	35	100	86	100

Table 8: Distribution of Hanging Cases on the Basis of Feet Touching the Ground

Feature of Suspension	Cases	Percentage (%)
Fully Suspended	61	70.93
Feet touching the ground	22	25.59
Sitting	2	2.32
Kneeling	1	1.16
Not known	0	0
Total	86	100.00

Figure 2 : Position of knot was observed at the occiput in 48 (55.81%) cases followed by positions of knot at right and left side of neck among 32 (37.20%) and 30 (34.89%) subjects respectively, while fixed noose in 34 (39.53%) cases and running noose 61(70.93%) cases. 46.25% had fixed knot in a study by Patel Ankur et al and 172 cases (53.75%) had running noose^[12]. Vijaynath V et al reported 44.45% fixed knot and 52.94% cases with running noose^[33]. Sharma B R et al reported 58.78% cases with fixed knot and 41.22% cases with slip knot^[10].

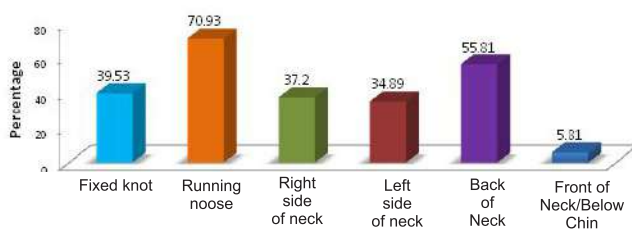
Figure 2: Incidences of Hanging Cases on the Basis of Noose of the Ligature used (n= 86):

Table No. 9 : Depicts distribution of cases according to the direction of ligature mark in hanging. Amongst 86 cases, discontinuous ligature mark was seen in 79 (91.86%) cases, however continuous ligature mark was present in remaining 7 (8.14%) cases. In 78 (56.0%) subjects, the mark was directed obliquely and horizontal mark was seen in 8 (9.30%) subjects. In 70 (81.40%) subjects, ligature mark was observed above thyroid cartilage, 12 (13.95%) subjects had the mark over thyroid cartilage while, 4 (4.65%) subjects had the mark below thyroid cartilage. Ligature marks were found sharply grooved in 26 (30.23%) and shallow grooved in 31 (36.05%) of cases. However mark was faint and just visible in 28 (32.56%) cases and not visible in 1 (1.16%) case. Parchmentisation of skin beneath ligature mark, ecchymosed margins and white glistening appearance of underneath tissue was present in 47 (54.65%), 54 (62.80%) and 39 (45.35%) cases respectively. The similar findings above the level of thyroid cartilage were reported by Rawat V e al (91.09%)^[21], Sharma et al (84.62%)^[10] and Mukharjee JB in 80% of cases^[38]. Ligature mark above level of thyroid cartilage was reported by Elfawal et al (65.57%)^[27] and Dixit et al (77%)^[35]. Ligature mark below the level of thyroid cartilage in significant percent was reported by Davidson et al (6.66%)^[39], Elfawal et al (24.59%)^[27] and Dixit et al (23%)^[35]. The high reporting may be because that, they had not differentiated mark at the level of thyroid cartilage or below it; but clubbed both as below the level of thyroid cartilage.

Table No. 10 : External findings: In our study cyanosis (evident as bluish discoloration of nails, mouth and lips) was seen in 77(89.53%) cases and congestion of face in 69 (80.23%) cases. Similar findings were observed by Saiyed and Modi (94.59%)^[40], Rawat and Rodrigues (66.33%)^[41] and Pal SK et al in 51.63% cases^[30]. Petechial haemorrhages, visceral congestion and dark fluid blood were noticed in 100% cases as also by Gambhir Singh et al, Patel et al and Sarangi et al^{[42][12][43]}.

Dribbling of saliva was reported in 64 (74.42%) cases. Dribbling of saliva is considered an important finding of ante mortem hanging. Similar findings were observed by Ankur P et al and recorded in 228 out of 320 cases (71.25%)^[12], Pal SK et

al in 64.75% cases and Sheikh et al in 38.37% cases^{[30][34]}.

Protrusion of tongue was observed in 59 (68.6%) cases. Similar findings were observed by Pal SK et al 79(64.75%)^[30], Rawat and Rodrigues 50.49% and Shao et al 31%.^{[41][44]}

Seminal fluids discharge was seen in 27 (31.40%) cases. These observations were similar to the studies of to Pal SK et al 37 (30.32%) cases^[30], Sahoo et al 17% cases^[44], Patel et al 17.5% cases^[12], Rawat and Rodrigues 10.89% cases.^[41]

Urinary incontinence was seen in 37 (43.02%) cases. Similar findings were observed by Pal SK et al 52 (42.62%) cases^[30], Patel et al 13.75%, Rawat and Rodrigues noticed only 0.99% cases.^{[12][41]}

Faecal incontinence was seen in 4 (4.65%) cases. Sengupta observed faecal discharge in 14.85% cases, while faecal discharge was not seen by Rawat and Rodrigues.^{[45][41]}

Table 9 : Features of ligature mark in Hanging

Feature	Number	Percentage (%)
Continuous	7	8.14
Discontinuous	79	91.86
Oblique	78	90.69
Horizontal	8	9.30
Above thyroid cartilage	70	81.40
At. Thyroid cartilage	12	13.95
Below thyroid cartilage	4	4.65
Single ligature mark	80	93.02
Multiple ligature marks	6	6.98
Sharply grooved	26	30.23
Shallow grooved	31	36.05
Just visible	28	32.56
Not visible	1	1.16
White Glistening appearance	39	45.35
Parchmentisation	47	54.65
Ecchymosis	54	62.80

Table no. 11: Observation of neck structures: 81 (94.19%) cases in hanging presented with congestion in their larynx and trachea while sterno-cleidiomastoid muscle contusion was observed in 5 (5.81%) cases. Hyoid and thyroid bone fracture was not found in our study. Similar findings were observed in the study done by Vijaynath et al. White glistening subcutaneous tissue below the mark (because of continuous pressure of ligature and haemorrhages in the neck muscles was seen.

CONCLUSION:

According to NCRB statistics (2014), distribution of suicide by means of hanging was 41.8% and by drowning was 5.6% out of all suicide cases reported all over India.^[47] At the same

Table 10: External Examination of Victims of Violent Asphyxial Deaths

Features	No.	%age
Bilateral closed eye.	23	26.74
Both partially open eye	40	46.51
Unilateral partially open eye	5	5.81
Bilateral open eye	18	20.93
Congested eye	75	87.21
Sub-conjunctival haemorrhages	10	11.63
Post-mortem Staining in lower limbs	59	68.60
Cyanosis	77	89.53
Petechial haemorrhage	48	55.81
Mouth closed	6	6.98
Partially open	55	63.95
Complete open	25	29.07
Tongue tip protruded	58	67.44
Oral dribbling of saliva	64	74.42
Oral bloody discharge	32	37.21
Nasal bloody discharge	29	33.72
Ear bleeding	0	0
Congestion of face	69	80.23
Pale face	17	19.77
La facie sympathique	2	2.32
Seminal emission	27	31.40
Urinary incontinence	37	43.02
Faecal incontinence	4	4.65
Hesitation (struggle mark)	3	3.49
Suicide note	7	8.13

Table 11: Observation of Neck Structures

Feature	No.	%age
Laryngeal Congestion	81	94.19
Tracheal Congestion	81	94.19
Fracture of hyoid bone	0	0
Sterno-cleidomastoid muscle contusion	5	5.81
Laryngeal contusion	0	0
Tracheal contusion	0	0
Fracture of tracheal rings	0	0
Nail marks / Abrasion	2	2.32
Contusion / Laceration wound	2	2.32

time, NCRB statistics (2014) also showed that in Uttar Pradesh hanging as a means of suicide, accounted for 1585 cases out of 53026 cases all over India while drowning accounted for 162 cases out of total 7401 cases. In our study, suicide by means of ligature (hanging) was 57.33% while drowning deaths were 24% out of all Asphyxial deaths.

Hanging is being considered as giving swift and painless death, with easily available ligature material & ligature points in secluded places without arising much suspicion. This method is increasingly adopted to commit suicide. Peak incidence of mortality due to hanging is in young age group i.e. 21-30 years. The present study highlights on the emerging trends that nylon rope is being increasingly used for committing suicidal hanging.

Attempt to commit suicide; under section 309 of IPC viz shooting, hanging and stabbing are a 'hard' way of committing suicide and typically a male choice; poisoning and drowning are 'soft' ways of committing suicide.

Previously there was punishment for attempt to suicide which was imprisonment which may extend to one year, with or without fine. Now according to Mental Health Act, 2017, Chapter XVI, Section 115, notwithstanding anything contained in Section 309 of the Indian penal Code any person who attempts to commit suicide shall be presumed, unless proved otherwise, to have severe stress and shall not be tried and punished under the said Code.

Now the study concludes that hanging is preferred methods for committing suicides (under Section 309 IPC) and abetment of suicide (under Section 306 of IPC) compared to poisoning and thermal burn.

ACKNOWLEDGEMENT:

Authors would like to thank faculty and staff of department of Forensic Medicine IMS, BHU; Varanasi for their valuable support and full help in data collection from the autopsied case and providing with adequate literature support.

REFERENCES:

1. Vij k. Textbook of Forensic Medicine and Toxicology. 5th edition. New Delhi: Reed Elsevier India Pvt. Ltd.; 2011. Pp. 71-72
2. Adelson L. The Pathology of Homicide. 1st edition. Springfield: Charles C Thomas Pub Ltd.; 1974. Pp. 555-557.
3. Oxford English Dictionary. Hanging as method of execution is unknown, as method of suicide from. 2nd edition. Oxford University Press; 2009. Pp. 1325.
4. Rayes M, Mittal M, Setti S. Mittal S. Hangmans's fracture: a historical and biomechanical perspective. ([http://www.zillanatomy.com/Reference article Hangman's Fracture Rayes JNeurosurg Spine 2011](http://www.zillanatomy.com/Reference%20article%20Hangman's%20Fracture%20Rayes%20JNeurosurg%20Spine%202011)). Pp. 198-208.
5. Reddy KSN. The essentials of Forensic Medicine & Toxicology. 19th edition. Jaypee Brothers Medical publishers; 2000. Pp. 283-295.

6. Bennewith O, Gunnell D, Kapur N, Turnbull P, Simkin S, Sutton L et al. Suicide by hanging: multicentre study based on coroner's records in England. *The Brit J Psychiatry*. 2005; 186:260-261.
7. U.S. Suicide Statistics. Suicide. Org. Suicide Prevention, Awareness and Support; 2001. Available from www.suicide.org/suicidestatistics.html. Accessed on Nov. 15th, 2018.
8. Hanging- New World encyclopaedia. [Online] 2009. [Cited 2009 Nov 11]. Available from www.newworldencyclopedia.org/entry/Hanging. Accessed on Nov. 14th, 2018.
9. Kamalakar D. A new trend of suicide in India, Counter currents. Org., July 2012. Available from www.countercurrents.org/kamalakar210712.htm. Accessed on Nov. 13th, 2018.
10. Sharma BR, Harish D, Sharma A, Sharma S, Singh H. Injuries to neck structures in deaths due to constriction of neck, with a special reference to hanging. *J Forensic Leg Med*. 2008; 15:298-305.
11. Singh A. A study of demographic variables of violent asphyxial death. *Journal of Punjab Academy of Forensic Medicine and Toxicology*. 2003; 3:32-34.
12. Patel AP, Bhoot RR, Patel DJ, Patel KA. Study of Violent Asphyxial Death. *International Journal of Medical Toxicology and Forensic Medicine*. 2013; 3(2):48-57.
13. Arif M. Ligature mark on the neck; How elucidative?. *Professional Med J*. 2015; 22:798-803
14. Trimizi SZ, Mirza FH, Paryar HA. Medicolegal investigation of violent asphyxial deaths – an autopsy-based study. *J Dow Uni Health Sci*, 2012; 6:86-90
15. Murty OP, Agnihotri AK. Homicidal Deaths in South Delhi. *J Ind Acad Forensic Med*. 2000; 22: 9–11.
16. Ajay K, Handan V, Rudresh YC, Govindaraju HC, Gouda S. Study of violent asphyxial deaths in Chitradurga district of Karnataka IJBAR. 2013 ;4(12): 868-871.
17. Reddy SP, Kumar R, Rudramurthy. Asphyxial deaths at district hospital, Tumkur: a retrospective study. *J Indian Acad Forensic Med*. 2012;34(2):146-147.
18. Azmak D. Asphyxial deaths: a retrospective study and review of the literature. *AM J Forensic Med Pathol*. 2006;(2):134-144.
19. Chaurasia N, Pandey SK, Mishra A. An Epidemiological Study of Violent Asphyxial Death in Varanasi Region (India) a Killing Tool. *J Forensic Res*. 2012; 3(10.6): 174.
20. Punitha R, Pradeep Kumar M.V, Jagadeesh N.H, Jayapakash. A cross sectional study on homicidal asphyxial deaths. *JKAMLS*. June 2017; Vol 26(1). 9-12.
21. Rawat V, Rodrigues EJ. Medicolegal study of hanging case in North Goa. *Int J Forensic Sci Pathol*. 2015; 3(5), 110–118.
22. Uzun I, BuYuk Y, Gurpiner K. Suicidal hanging: Fatalitirs in Istanbul Retrospective analysis of 761 autopsies. *J Forensic Leg Med*. 2007;14(7):406-409.
23. Ambade VN, Godbole Hv, Kukde HG. Suicidal and homicidal deaths: A comparative and circumstantial approach. *J Forensic Leg Med*. 2007; 14:253-60.
24. Mohanty S, Sahu G, Kumar M, Patnaik M. Suicide in India – A four year retrospective study. *J Forensic Leg Med*. 2007;14:185-9.
25. Bhosle SH, Batra AK, Kuchewer SV. Violent asphyxia death due to hanging: a prospective study. *J Forensic Med Sci Law*. 2014. 23 (1): 1-8.
26. Cooke CT, Cadden GA, Margolius KA. Death by hanging in Western Australia. *Pathology* Jul 1995; 27(3): 268-72.
27. Elfawal MA, Awad OA. Death from hanging in the eastern province of Saudi Arabia. *Med Sci Law*. Oct 1994; 34(4): 307-12.
28. Bowen DA. Hanging - a review. *Journal of Forensic Science international*. Nov-Dec 1982; 20(3): 247-9
29. Vijayakumarri N. Suicidal Hanging: A Prospective Study. *J Indian Acad Forensic Med*. October- December 2011; 33-35.
30. Pal SK, Sharma A, Sehgal A, Kaushik N, Rana A. Hanging Suicides in Himachal Pradesh: An Analysis of Forensic Case. *Int J Forensic Sci Pathol*. 2016; 4(11), 297-304.
31. Naik S K. A study of fracture of hyoid bone in cases of asphyxial deaths resulting from constricting force around neck. *Journal of Indian Academy of Forensic Medicine*. 2005; 27(3):149-153.
32. Jignesh B. Patel, Alpesh B. Bambhaniya, Kalpesh R. Chaudhari, Mehul C. Upadhyay; *International Journal of Health Sciences & Research* Vol.5; Issue:8. August 2015; 76-81.
33. Vijaynath V, Anitha MR, Rajan K. A Study of autopsy profile in cases of hanging. *Journal of Forensic Medicine and Toxicology*. 2009; 26(1): 34-36.
34. Shaikh MM, Chotaliya HJ, Modi AD, Parmar AP, Kalele SD. A study of gross post-mortem findings in cases of hanging and ligature strangulation. *J Indian Acad Forensic Med*. 2013; 35(1): 63-65.

35. Dixit PG, Mohite PM, Ambade VN. Study of Histopathological changes in thyroid, salivary gland and lymph nodes in hanging. Journal of forensic medicine and toxicology. July-Dec 2001; 18(2): 1-4.
36. Momin SG, Mangal HM, Kyada HC, Vijapur MT, Bhuvana SD. Pattern of ligature mark in case of compressed neck in Rajkot region: A prospective study. J Indian Acad Forensic Med. 2012;34(1):40-43
37. Suárez-Peñaranda JM, Alvarez T, Miguéns X, Rodríguez-Calvo MS, de Abajo BL, Cortesão M, et al. Characterization of lesions in hanging deaths. J Forensic Sci. 2008; 53:720.
38. Mukharjee JB. Forensic Medicine and Toxicology. R N Karmakar editor. Third edition New Delhi: Arnold Associates; 2007. Pp. 571-617.
39. Davidson A, Marshall TK. Hanging in Northern Ireland-a survey. Med Sci Law. 1986; 26: 23-28.
40. Polson CJ, Gee DJ, Knight B. The Essentials of Forensic Medicine. New York, NY: Pergamon; 1985. Pp. 357-388.
41. Saiyed MZG, Modi KK; Retrospective study of post-mortem cases of hanging-a method of suicide. NHL J Med Sci. 2013; 2(2): 48-50.
42. Singh G. A study of violent mechanical asphyxial deaths in homicide; Journal of Forensic Medicine and Toxicology. 2008; 25(2):34-35.
43. Sarangi M P. "Ligature marks" – in Forensic Pathologist's Perspective. Journal of Forensic Medicine and Toxicology. 1998; 15(1):99-102.
44. Sahoo N, Kumar N, Panda BB, Datta A Significance of external findings in hanging cases during autopsy. IJBAR. 2016; 7(3):119-122.
45. Sen Gupta BK. Studies on 101 cases of death due to hanging. J Indian Medical Assoc. 1965; 45 (3): 135-139.
46. Chand S, Rishi S, Aggrawal A, Dikshit PC, Ranjan R. Neck structures post mortem finding in asphyxial death, International Journal of Scientific Study. 2017; Vol 5; Issue 4. 248-256.
47. Crime Statistics 2014. National Crime Records Bureau (NCRB). Ministry of Home Affairs, 2014 Govt. Of India New Delhi. Available from <http://www.ncrb.gov.in/StatPublications/CII/CII2014/Compendium%202014.pdf>. Accessed on Nov. 10, 2018.



The Photographs showing "Lefasciesympathique"



Tongue protruded, clenched between jaws and bluish lips.

Dried Stain of dribbling of saliva



Photograph showing posterior view of partially hanged victim, feet touching the ground with partially bent knee. The place of ligature knot found at back showing their presence at alignment of posterior mid line with evident features of typical hanging

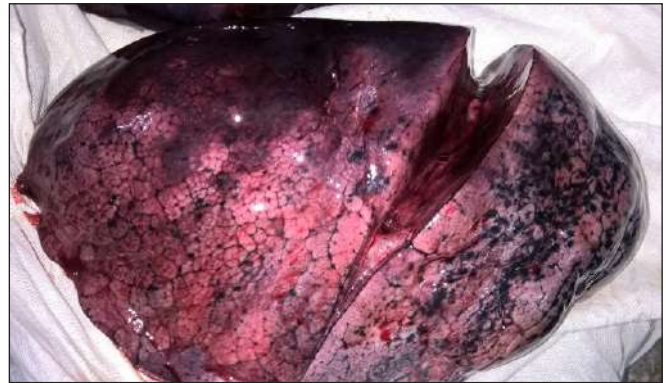
Photograph showing anterior view of partially hanged victim, touching feet, hyper stretched neck and ligature material used is cloth piece hanged with ceiling Fan.





Broad, prominent and parchmented ligature mark at mid line- front and adjacent both side of neck

Conjunctival Congestion & petechial hemorrhagic patches over face.



Presence of multiple Tardieu's spots on the surface of congested lungs, cut portion of lung with congested parenchymal layer.

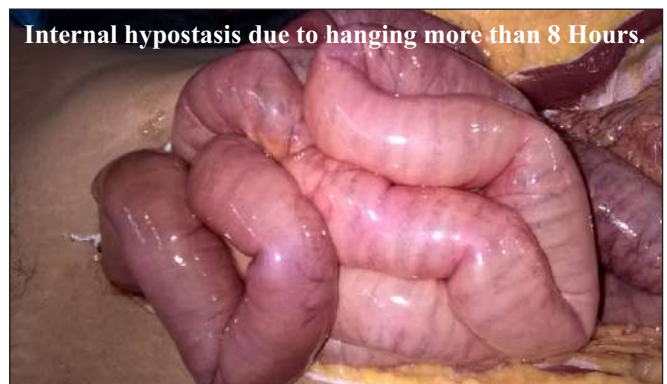


Double ligature mark caused due to use of nylon rope. Parchmentisation and grooves formed mostly at front and right side of neck also the lower placed ligature mark has evidence of upwards slipping

Double ligature mark found parchmented and grooves formed progressing left side of neck upward and back which is



Thin petechial hemorrhage present on the surface of Liver



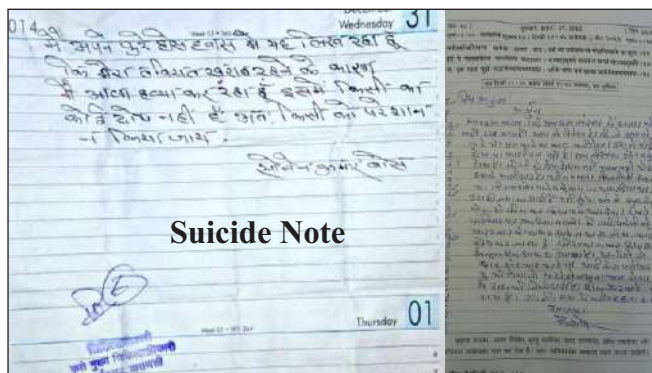
Internal hypostasis due to hanging more than 8 Hours.



Dissected skull with evidence of congested scalp, duramatter and brain with prominent fine blood vessels.



Cyanosed nails.



Suicide Note

Ecchymosis present at places subjacent to ligature mark.



Original Research Paper

Knowledge of Fresh Interns About Legal Aspects of Medicine

1. **Smitha Rani**, Assistant Professor*,
2. **Chinmayi Y**, Assistant Professor*,
3. **Arun M**, Professor*,

*Department of Forensic Medicine and Toxicology, J.S.S Medical College, JSS Academy of Higher Education and Research, Mysuru, Karnataka, India.

ABSTRACT :

Background: The aim of teaching Forensic Medicine and Toxicology to undergraduate medical students is to produce a physician who is well-versed about medicolegal responsibilities in practice of medicine, has ample knowledge of law in relation to medical practice and has due respect for codes of medical ethics.

Objective: The aim of this study was to assess the knowledge of fresh interns about legal aspects of medicine and in turn assess whether the programme educational objective of teaching Forensic Medicine to undergraduates is effectively met and programme outcome is achieved.

Methods: A cross sectional questionnaire based survey was conducted among 100 fresh interns on the first day of their internship training during the orientation programme held for them.

Results: Total of 100 fresh interns participated in the present study. 74% of them felt that present undergraduate teaching of Forensic Medicine is not sufficient for the Indian Medical Graduate to handle medicolegal cases independently and 53 % were of the opinion that they would be anxious to deal with medicolegal cases. 89% of the subjects knew that obtaining consent for medical procedure is not imperative in emergency situations. 82 % of the fresh interns were aware that when a victim of a sexual offence is a girl child, she has to be examined by a female doctor. More than half of the respondents were not aware of procedure of issuing cause of death certificate in medicolegal cases.

Conclusion: Reinforcement of what is taught in the Forensic Medicine Syllabus is the need of the hour, which could be achieved by teaching which is aligned and integrated both horizontally and vertically thus recognizing the importance of medico-legal and ethical issues as they relate to the practice of medicine.

Corresponding Author

Dr. Chinmayi.Y,

Assistant Professor,

Department of Forensic Medicine and Toxicology, J.S.S Medical College, JSS Academy of Higher Education and Research, Sri Shivarathreeshwara Nagar, Bannimantap, Mysuru-570015, Karnataka, India.

Contact : +91 97430-34560

E-mail : chinmayiy@jssuni.edu.in

Article History:

Received: 30 October 2018

Received in revised form: 28 December 2018

Accepted on: 31 December 2018

Available online: 1 June 2019

KEYWORDS :Medicolegal issues, Forensic Medicine & Toxicology, Knowledge, Indian Medical Graduate.

INTRODUCTION :

Graduate medical regulations put forth by Medical Council of India expects the medical graduates to identify the basic medicolegal aspects of hospital and general practice and define the medicolegal responsibilities of a general physician while rendering community service. They are supposed to be capable enough to make observations and logical inferences in order to initiate enquiries in criminal matters and medico legal problems^[1].

In the present undergraduate curriculum, Forensic Medicine toxicology is taught in the second phase (3rd to 5th semester),

along with the other three Para-Clinical subjects. The 9th semester is followed by one year of compulsory rotating internship^[2]. wherein, posting in department of Forensic Medicine & Toxicology is elective, if the graduate wishes to choose it; training period would be for a span of 15 days.

Medico-legal duties of a Registered Medical Practitioner are dealt in second year of M.B.B.S. and unfortunately afterwards nobody bothers about it till one faces some problem like compensation case or case of negligence^[3].

The present study was undertaken to assess the knowledge of fresh interns about legal aspects of medicine and in turn

evaluate whether the objective of teaching Forensic Medicine to undergraduates is effectively met and whether the outcome is satisfactory.

MATERIALS AND METHODS :

This study was undertaken at JSS Medical College, JSS Academy of Higher Education and Research, Mysuru, Karnataka during the Intern orientation programme for the fresh interns conducted by the Medical Education Unit, JSS Medical College on the first day of their internship training in March 2018. 100 interns participated in the present study. A self-administered questionnaire containing 10 items relating to knowledge of legal aspects of medicine was designed. The written responses to the provided questionnaire (**Table No.1**) were studied. The data obtained was tabulated and analyzed statistically for number and percentage using EPI-Info statistical software version 6.

RESULTS :

Responses given by 100 fresh interns were studied in the present study (**Table No.1**). Only 24 % of them knew that a

case of bee sting has to be registered as a medicolegal case. 76% were aware that the doctor should register a medicolegal case wherever indicated, irrespective of patient or relatives request and 72 of them had knowledge about brought dead cases with no available medical records to be labeled as medicolegal case. 89% of the subjects knew that the doctor can go ahead with required medical procedure without obtaining consent in emergency situations, when the patient is unconscious and no relatives are available for providing consent. When a victim of a sexual offence is a girl child, she has to be examined by a female doctor was known to 82 % of the interns. Regarding treatment protocol, maximum number of interns (96%) knew that any case of head injury has to be kept under observation irrespective of the severity. 56% of the respondents didn't know that the cause of death certificate should not be issued in medicolegal cases by the treating doctor. Regarding preservation of medical records of a medicolegal case, 36 % of the subjects felt that it has to be preserved till the case is judged and 44 % of them felt that it has to be kept forever. 74% of them felt that present undergraduate

Table No.1: Questionnaire to assess the knowledge of Fresh Interns about Legal Aspects of Medicine & responses obtained

Sl. No.	Questions	Responses obtained (Total No. of subjects – 100)
1	Is a case of Bee sting a medico legal case? (Yes/ No)	Yes – 24 No - 76
2	Mr. X, a gynaecologist was on call, when a girl child of 12yrs comes to the casualty with history of sexual assault by her neighbour. Can Mr. X examine her for the collection of the evidentiary materials? (Yes/ No)	Yes – 18 No - 82
3	For how many years hospital is legally bound to preserve the medical records in a medico legal case? (Encircle the correct response) a. 3yrs b. 5 yrs c. Till the case is judged d. Kept Forever	Option A – 03 Option B – 17 Option C – 36 Option D - 44
4	A person was brought to the casualty by public, who was found in an unconscious state on the road. After examination he was found to require an emergency laparotomy. When can he be taken up for surgery? (Encircle the correct response) a. Immediately b. Wait for Police c. Wait for relatives	Option A – 89 Option B – 08 Option C – 03
5	A victim of road traffic accident was diagnosed of head injury, while on treatment he succumbed to his injuries. Can the treating doctor issue a cause of death certificate? (Yes/ No)	Yes – 56 No - 44
6	Is Undergraduate teaching of Forensic Medicine sufficient enough for an Indian Medical graduate to handle medico legal cases independently? (Yes/ No)	Yes – 26 No - 74
7	Should the Casualty medical officer register a medico legal case irrespective of patient or relatives request? (Yes/ No)	Yes – 76 No - 24
8	Do you think you would be anxious if you are made to handle a medico legal case in the casualty? (Yes/ No)	Yes – 53 No - 47
9	Are brought dead cases with no available medical records to be made a medico legal case? (Yes/ No)	Yes - 72 No - 28
10	A case of head injury with GCS 15/15 was brought to casualty. What is your plan of action? (Encircle the correct response) a. Discharge with advice b. Observation for 24hrs	Option A – 04 Option B – 96

teaching of Forensic Medicine is not sufficient for the Indian medical graduate to handle medicolegal cases independently and 53 % were of the opinion that they would be anxious to deal with medicolegal cases.

DISCUSSION :

Knowledge of medicolegal aspects is of utmost importance in medical profession. In the present study, an attempt was made to assess the knowledge of fresh interns about legal aspects of medicine and in turn ascertain the effectiveness of teaching Forensic Medicine & Toxicology in the second phase of the medical course to the undergraduates. With the aim to assess the knowledge of when a medicolegal case has to be registered and how a medicolegal case has to be dealt with, the question no.1, 5 7 and 9 were designed (**Table No.1**). Knowledge of medicolegal issues was mediocre among the interns, which is in concordance with findings of studies conducted in two different medical colleges of Maharashtra, India^[4,5].

Majority of the interns knew that one need not wait for consent in emergency situations. As per Section 92 of Indian Penal Code, consent may be absolutely dispensed with when the circumstances are such as to render consent impossible or when, in case of person incapable of assenting, there is not one at hand whose consent can be substituted^[6].

As per chapter VI of The Protection of Children from Sexual Offences Act passed in the year 2012, whenever the victim is a girl child, she has to be examined by a female doctor^[7]. This information was known to more than 3/4th of the respondents of the present study.

Importance of maintaining medicolegal records was known to the majority of the subjects (80%) in the present study. As per MCI regulation dealing with the maintenance of medical records, every physician shall maintain the medical records pertaining to his/her indoor patients for a period of 3 years^[8]. Under Article 51 A(h) of the Constitution of India, there is a moral obligation on the doctor, and a legal duty, to maintain and preserve medical, medico-legal, and legal documents in the best interests of social and professional justice^[9]. As per the Director General of Health Sciences guidelines medicolegal records has to be kept for a period of 10 years^[10]. Medico-legal records to be kept for at least period of 30 years or up till the cases are decided in the court of law^[11].

Maximum number of fresh interns knew the importance of keeping any patient of head injury under observation for atleast 24 to 36 hrs^[12] as he may be in lucid interval.

Findings of present study reveal that with the present method of teaching, the graduates dint feel competent enough to handle medicolegal cases independently. Similar opinion was expressed in a study conducted in a southern state of India,

wherein 55 (45.83%) interns and 63 (78.75%) post graduate students were of the opinion that the present undergraduate teaching is not sufficient for them to tackle medico legal cases^[13]. Madras High Court Judge, Justice S Palanivelu opined in one of his order with regard to criminal original petition that the medico-legal curriculum in India is traditionally packed with a large quantity of theoretical knowledge. Once qualified, students are expected to blend this information and apply it while performing medico-legal duties. The knowledge in medico legal features, expected in a fresh undergraduate, should be considered in the curriculum itself which he/she has gone through during his/her course. He was of the opinion that to facilitate a better understanding of nuances of Forensic Medicine, the teaching of the subject may be taken up in the later part of the clinical years because in the early years of their study, they may not be in a position to know the importance & they may read this subject for the purpose of getting through the examination, but the real involvement therein could not be expected. He also suggested compulsorily posting during internship in the Department of Forensic Medicine for a reasonable period, for a better comprehension of the subject. This suggestion was made, viewing that the medical students would be sufficiently equipped at the later part of their studies and the niceties of the features in the subject would be appropriately appreciated^[14].

The suggestions made by the honorable justice are supported by the researchers worldwide. A study conducted in Ghana found that majority of respondents who had training about medicolegal issues in their first year in medical school had preferred training throughout their years of academic studies^[15]. This finding is consistent with findings of study conducted in Barbados, that medical schools do not place a lot of emphasis on training on ethical and medico-legal issues^[16]. The call for training throughout the medical training years is in line with the recommendation put forth by a Nigerian study^[17]. The same findings were found in an Indian study conducted among the practitioners of a medical college, wherein, majority of them (88 %) expressed that 15 days duty in the department of Forensic Medicine should be mandatory during internship to handle the medico legal cases appropriately^[18].

CONCLUSION :

Reinforcement and reorientation to the subject is the key, to equip the Indian medical graduate to handle medicolegal cases appropriately without any uncertainty. This could be achieved by teaching which is aligned and integrated both horizontally and vertically. Alternatively, shifting the subject to Final year would be a welcome change which would make the students to comprehend the applicability of the subject thus making them recognize the importance of medico-legal and ethical issues in

the practice of medicine .

Acknowledgement : Authors thank all subjects who volunteered to participate in the study.

Funding : This research did not receive any specific grant from funding agencies. Study was approved by the Institutional Ethics Committee

Conflict of Interest : Nil

REFERENCES :

1. Medical Council of India. Regulations of Graduate Medical Education. 1997.
2. Sharma BR, Harish D, Chavali KH. Teaching, training and practice of Forensic Medicine in India: An overview. *J Indian Acad Forensic Med.* 2005; 27(4): 247-251.
3. Nanandkar SD, Chavan GS. Assessment of medico legal awareness of practicing obstetricians & gynecologists. *J Indian Acad Forensic Med.* 2008; 30(3):136- 140.
4. Pratibha A. Mardikar, Arti A. Kasulkar. "To Assess the Need of Medicolegal Education in Interns and Residents in Medical Institution". *Journal of Evolution of Medical and Dental Sciences* 2015; 4(17): 2885-2889.
5. Giri PA, Chavan KD, Phalake DB. Knowledge about medicolegal problems amongst the interns and resident from rural tertiary care teaching hospital in western Maharashtra-A cross sectional study. *Indian journal of Forensic Medicine and Pathology.* 2010; 3(3): 89-93.
6. Vij K. Consent to and refusal of treatment. In, *Textbook of Forensic Medicine and Toxicology, Principles and Practice.* 4th Ed. New Delhi: Elsevier; 2008:483.
7. The Protection of Children from Sexual Offences Act, 2012. Available online at :
<http://wcd.nic.in/sites/default/files/childprotection31072012.pdf>.
8. Singh H, Vij K, Garg A, Dhatarwaal SK, Sandhu SS, Aggarwal D. Maintenance of records.... How vital? *J Punjab Acad Forensic Med Toxicol* 2012;12(2):107-10.
9. Rai JJ, Acharya RV, Dave D. Knowledge and awareness among interns and residents about medical law and negligence in medical college in Vadodara-A questionnaire study. *Journal of Dental and Medical Sciences.* 2013; 3(4): 32-38
10. Biswas G. Medical Jurisprudence and Ethics. In: Biswas G. *Review of Forensic Medicine & Toxicology.* 3rd Ed. New Delhi: Jaypee The Health Sciences Publisher; 2015: 25.
11. Pawar GS, Pawar JG. Facts of Medical Record Keeping - The Integral Part of Medical and Medico Legal Practice. *Indian Journal of Forensic Medicine and Pathology.* 2009; 2(2):57-60
12. Reddy KSN. Regional Injuries In, *The essentials of Forensic Medicine & Toxicology.* 33rd Ed. New Delhi: Jaypee The health Science Publishers; 2014:255.
13. G Venkat Rao, N Hari. Medico-legal knowledge assessment of interns and post graduate students in a medical institution. *IAIM,* 2016; 3(10): 105-110.
14. Muniammal vs. The Superintendent of Police, Kancheepuram District, Kancheepuram, Criminal Original Petition No.12582 Of 2007, in the High Court of Judicature at Madras, order Dated: February 16, 2008. Available online at : <https://indiankanoon.org/doc/1229644/>. (accessed 31 July 2018)
15. Barnie BA, Forson PK, Opere-Addo MNA, Appiah-Poku J, Rhule GP, et al. Knowledge and Perceptions of Health Workers' Training on Ethics, Confidentiality and Medico-Legal Issues. *J Clinic Res Bioeth,* 2015; 6: 205. doi:10.4172/2155-9627.1000205.
16. Hariharan S, Jonnalagadda R, Walrond E, Moseley H .Knowledge, attitudes and practice of healthcare ethics and law among doctors and nurses in Barbados. *BMC Med Ethics* 2006;7: E7.
17. Fadare JO, Desalu OO, Jemilohun AC, Babatunde OA. Knowledge of medical ethics among Nigerian medical doctors. *Niger Med J* 2012; 53: 226-230.
18. Dash SK. Medical ethics, duties and medical negligence awareness among the practitioners in a Teaching Medical College, Hospital-A survey. *J Indian Acad Forensic Med.* 2010; 32 (2): 153-156.

Original Research Paper

Firing Range Estimation of handguns (0.32 Revolver & 7.65 mm Pistol) by Studying Inorganic Constituents of Firearm Discharge Residue Particles Using Scanning Electron Microscope coupled with Energy dispersive X-rays and Atomic Absorption Spectrometer flame

1. **Puneet Puri**, Assistant Director(Ballistics), Forensic Science Laboratory, GNCTD of Delhi
2. **Preeti Grewal**, Scientific Assistant(Ballistics), Forensic Science Laboratory, GNCTD of Delhi
3. **R.K. Sarin**, Director , Forensic Science Laboratory, Andhra Pradesh
4. **S.K. Shukla**, Professor and Director, Amity Institute of Forensic Sciences, Amity University, Noida

ABSTRACT :

Though, a number of studies regarding the analysis of firearm discharge residue (FDR) particles have been conducted globally for range estimation by utilizing cartridges of various makes and origin, but in this paper, we have conducted several firings using ammunition manufactured by Indian Ordnance Factories and studied the presence of inorganic constituents contributed by priming mixture, cartridge case and bullet for this purpose. It has been found that there is atleast one element which can be used for range estimation irrespective of its origin from priming mixture, cartridge case or bullet.

Corresponding Author :

Puneet Puri,
Assistant Director (Ballistics),
Forensic Science Laboratory, GNCTD of Delhi
Contact : +91 9868141041
E-mail : puneetpuri71@gmail.com

Article History:

Received: 16 November 2018
Received in revised form: 16 December 2018
Accepted on: 16 December 2018
Available online: 1 June 2019

KEYWORDS : FDR, Priming Mixture, Propellant, Ammunition, Firearms, Range

INTRODUCTION :

In India , the firearms like pistols, revolvers , rifles , shotguns , country made firearms etc. are being increasingly employed by criminals in committing heinous crimes like murders, attempt to murders, robbery, decoity, suicide etc. but occasionally accidents do take place^[1]. The most common handguns used by criminals in India are pistol 7.65 mm calibre, pistol .30"/7.62mm/7.63mm, pistol 9mm calibre, revolver .32" calibre, revolver .380" calibre etc. These hand guns are used by criminal because these can be easily hidden and on provocation they take out these firearms and commit the crime. The estimation of range of firing is a significant problem. It helps in deciding whether a criminal case involving use of firearm is an act of homicide, suicide or accident. In case range of firing can be proved to be more than length of arm of victim , the theories of suicide or struggle can be disproved. It is considered, if the range of firing is close, people sometimes fabricate the injuries to implicate others and sometimes plea of encounter may be taken. Thus estimation of range of firing helps in revealing the truth.

The methods which are commonly employed for estimation of range of firing are:

- i) The effects of burning/scorching/charring/singeing, blackening, tattooing etc. produced by ejecta moving behind the bullet. The unburnt and partially burnt

propellant powders(Tattooing) move in a conical fashion. As the range increases, their spread on target increases until their velocity reduces and they fall on ground.

- ii) The spreading of pellets fired from the shotgun.
- iii) The quantity of elements (metals) like copper from bullet, cartridge case and lead, antimony and barium from priming mixture around gunshot hole.

The effects like burning/scorching/charring/singeing, blackening, tattooing etc produced by ejecta moving behind the bullet can be used for estimation of range of firing only when the distance between firearm and target is very close i.e. limited to few inches only but if we have to estimate range of firing beyond close range, then quantity of inorganic constituents like Cu, Pb, Sb and barium of firearm discharge residues is to be analysed around suspected gunshot hole.

In addition to smoke and/ unburnt propellant grains, elements contributed by cartridge case, priming mixture and bullet are also found around the gun shot hole and their behaviour have been studied by various workers^[1-3]. In case of firing from lead bullets, copper contributed by cartridge case is found around the gun shot hole. The priming mixture may consist of lead styphnate, barium nitrate and antimony sulphide. When the firing pin strikes the percussion cap, the priming mixture gets crushed in between the indentation caused by the firing pin and the anvil. The high explosive lead styphnate, gets crushed and

produces lot of heat which causes the fuel antimony sulphide to burn and barium nitrate supplies oxygen for burning of fuel. This results in the intense flame produced by primer which ignites the propellant. The propellant burns, the high pressure gases cause discharge of bullet. Along with the propellant gases, there are vapours of lead, barium and antimony etc are moving along with gases. When these gases come out of firearm, they solidify into spheroids of diameter ranging from less than 10 microns to about 100 micron^[1,2]. These spheroids are of three types^[4-5]:

- 1) **Regular Spheroids:** Particles of lead (Pb), Barium(Ba), Antimony (Sb).
- 2) **Nodular Spheroids :** Particles formed by joining of two smaller particles- Pb-Pb, Sb-Sb, Ba- Ba, Pb-Ba, Ba-Sb, Pb-Sb etc...
- 3) **Unique Particles :** Consisting of Pb on inner core of Ba and Sb. In case of revolvers, these spheroids may get deposited on the hands of shooter when propellant gases and vapours of these elements leak from the gap in between cylinder and barrel^[3]. The gases leaving the muzzle form a plume over the muzzle. The GSR consisting of spheroids of Pb, Sb, Ba are projected forward. This behaviour had been studied by various workers^[2-6]. These workers found that the spheroids were deposited around gunshot hole and their amount was found to decrease with distance.

Other Elements found in Priming Mixture :

Now, several countries have adopted lead free priming compositions resulting in deposition of titanium, zinc, strontium, potassium, aluminium, silicon, calcium, sulphur, copper, nickel, chromium, sodium, barium etc.

In this study we have estimated range of firing using 0.32" revolver and 7.65 mm pistol by analysing the quantity of inorganic constituents like Cu, Pb, Sb and Ba of firearm discharge residue around gunshot holes using SEM-EDX and AAS (Flame) instrumental techniques. The outcome of this study will be helpful in criminal cases where it is to be ascertained whether the case involving firearm is a case of homicide or suicide or accident.

MATERIALS AND METHODS :

Present Study was conducted at Forensic science laboratory, Govt. of NCT of Delhi to estimate the firing range of 0.32" revolver and 7.65mm semiautomatic pistol by studying inorganic constituents of firearm discharge residue particles using Scanning Electron Microscope coupled with Energy Dispersive X-rays (SEM-EDX) and Atomic Absorption Spectrometer (

AAS).

Calibre	Firearm	Ammunition	Clothing Material	Distance in inches
0.32"	0.32"revolver (Small Arms Factory, Kanpur)	0.32" K.F.	Markin	Contact to 48
7.65mm	7.65mm Semiautomatic Pistol (Indian Ordnance Factory)	7.65mm K.F.	Polyester	Contact to 30

Two shots with 0.32" revolver were fired on a mark on markin from each distance of 0" (contact) to 6", 12", 18", 24", 30", 36", 42" and 48 inches. The firearm discharge residues were lifted from each test sample with the help of stubs for analysis on SEM-EDX. Then from each test sample, a piece of cloth having gunshot hole was cut and dissolved in 5% nitric acid solution and 25ml solution was prepared after filtration for each sample for analysis on AAS. A control sample of 25 ml solution was also prepared using the same markin cloth. Similarly two test samples along with control sample from each distance of 0", 6", 12", 18", 24" and 30" were prepared by firing 7.65mm semiautomatic pistol on polyester.

Calibration of AAS: Standard solutions of copper, lead, antimony and barium were prepared in Millipore distilled water having concentrations 1PPM, 2PPM, 5PPM and 10 PPM. After calibration, the samples along with control sample were analyzed.

RESULTS AND DISCUSSIONS : 0.32" Revolver

(A) By Scanning Electron Microscope Coupled with Energy Dispersive X-rays:

- (i) In the case of Contact range (**Figure1-2**), the following elements were detected: (**Table 1, Graph 1, Chart 1**)

Figure 1 and 2 : Shows FDR Particles at Contact Range

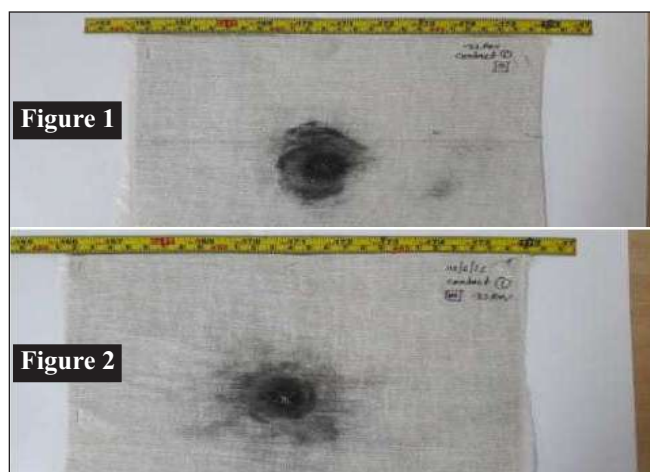
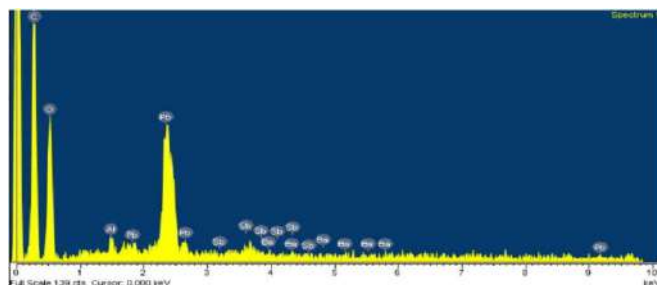
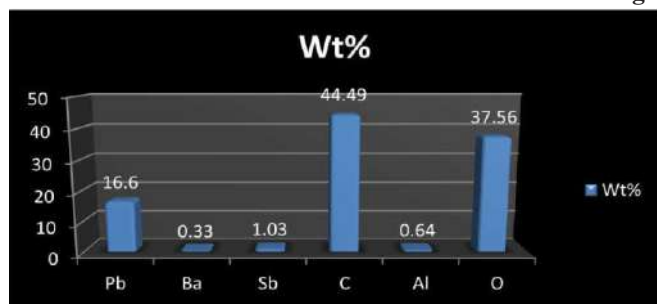


Table 1 : Shows Wt% of FDR Particles at Contact Range

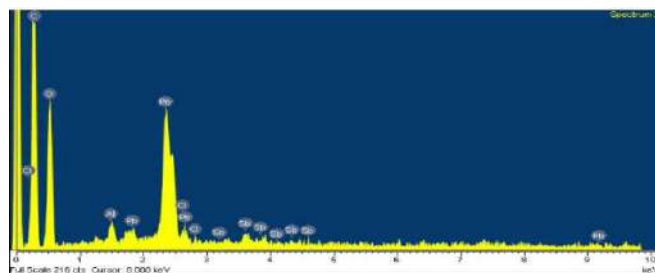
S. No	Elements	Wt %
1	Pb	16.6
2	Ba	0.33
3	Sb	1.03
4	C	44.49
5	Al	0.64
6	O	37.56

Graph1: Shows Scattering of FDR Particles at Contact Range**Chart 1: Shows wt% of FDR Particles at Contact Range**

(ii) In case, the range of fire at 6" (Figure 3-4), the following elements were detected. (Table 2, Graph 2, Chart 2)

Figure 3 and 4: Shows FDR Particles at 6 Inches**Table 2 : Shows Wt% of FDR Particles at 6 Inches**

S. No	Elements	Wt %
1	Pb	16.12
2	Sb	1.57
3	C	45.00
4	Al	0.83
5	O	36.00

Graph 2 : Shows Scattering of FDR Particles at 6 Inches**Chart 2 : Shows Wt % of FDR Particles at 6 Inches**

(iii) In case, the range of fire at 12", the following elements were detected. (Table 3)

Table 3: Shows Wt% of FDR particles at 12 Inches

S. No	Elements	Wt %
1	Pb	6.47
2	C	64.42
3	O	29.11

(iv) In case, the range of fire at 18", the following elements were detected. (Table 4)

Table 4: Shows Wt% of FDR particles at 18 Inches

S. No	Elements	Wt %
1	C	74.23
2	O	25.77

(v) The samples which were analysed for the detection of quantity of firearm discharge residue (FDR) under SEM-EDX showed that Pb, Ba and Sb were detected at contact range.

(vi) Pb, Sb were present whereas Ba was absent at 6 inches.

(vii) Pb was present whereas Ba, Sb both were absent at 12".

(viii) Pb, Ba, Sb were absent at 18".

(ix) As a result of above observations we can conclude that FDR particles could only be detected up to 12" only.

By Atomic Absorption Spectrometer(Flame):

The results of samples which were analysed for quantity

of each element of FDR particles around the gunshot hole are as follows: (Table 5)

Table 5: Shows Quantity of FDR Particles at Various Distances

S. No	Range (Inches)	Quantity (ppm) Pb	Quantity (ppm) Sb	Quantity (ppm) Ba	Quantity (ppm) Cu
1	Contact	76	10.7	0.15	0.24
2	6	33	2.9	0.26	0.16
3	12	13.3	0.58	0.09	0.10
4	18	3.6	0.32	0.08	0.09
5	24	2.4	0.19	0.08	0.09
6	30	0.57	0.14	0.04	0.07
7	36	0.94	0.18	0.10	0.11
8	42	0.82	0.16	0.13	0.12
9	48	1.2	0.20	0.06	0.11
10	Contact	0.0	0.18	0.06	0.11

(i) The total lead that may be released from primer, evaporated from bullet, rubbing of bullet against rifling and rubbing of bullet on cloth follows the curve as shown in graph 5.

Graph 5: Shows Quantity of Element Lead with Respect to Distance.

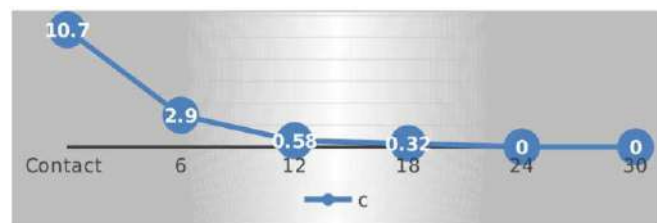


copper is released from the cartridge case.

(iii) Barium was detected up to 6 inches only. It may be released by primer.

(iv) Antimony was detected up to 18 inches only. (Graph 6)

Graph 6 : Shows Quantity of Element Antimony with Respect to Distance.



lead bullet when high temperature gases impact on bullet

during its motion inside the barrel (used for hardening of lead) or from clothing. The amount of antimony present at various distances i.e upto 18 inches were such that it also follows a pattern of decreasing amount as in case of lead which could also be used for range estimation

RESULTS AND DISCUSSIONS : 7.65 mm calibre Pistol

When 7.65mm pistol is fired, the ejecta consist of metals like copper, lead, antimony, barium. Copper is contributed by cartridge case and bullet. When the copper jacketed bullet passes through the barrel, copper particles gets cut from bullet. Depending upon the relative diameter of bullet and calibre of pistol, some particles may be of small size where as others may be of slightly larger size in firing different cartridges. So, the element copper, which is found on the target, comes from cartridge case, copper particles that gets cut from the bullet during its motion inside the barrel and the copper left by the rubbing of bullet on target. Element lead comes from priming mixture and vapours from behind the jacketed bullet. Similarly antimony comes from priming mixture and the bullet lead which is hardened by antimony as well as from clothes. Likewise, barium may be contributed by priming mixture.

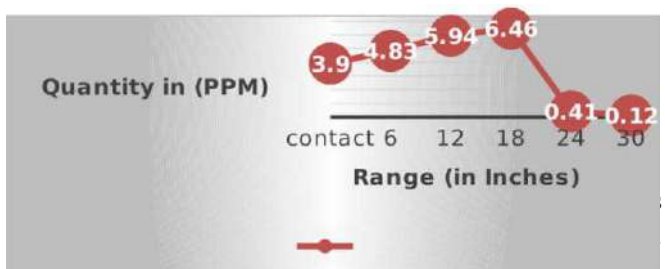
In the experiments conducted by the authors, the presence of elements copper, lead, antimony and barium were determined around the gunshot holes by AAS (Flame) only and the results obtained are as follows: (Table 6)

Table 6 : Shows Quantity of FDR Particles at Various Distances

S. No	Range (Inches)	Quantity (ppm) Pb	Quantity (ppm) Sb	Quantity (ppm) Ba	Quantity (ppm) Cu
1	0	19.6	0.58	4.25	3.90
2	6	13.4	0.43	6.18	4.83
3	12	5.81	0.06	3.81	5.94
4	18	1.86	0.11	0.89	6.46
5	24	1.04	0.11	0.69	0.41
6	30	0.33	0.07	0.25	0.12

(i) Copper : The amount of copper with distance is shown in graph 7.

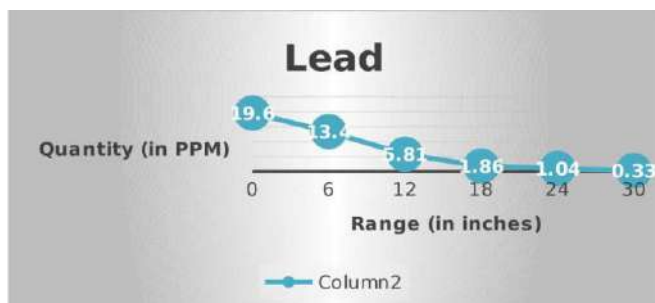
Graph 7 : Shows Quantity of Element Copper with Respect to Distance



But, in some firings these particles may be of small size and in other firings these particles may be of larger size. This depends on the relative diameters of bullet and calibre of firearm as well as other factors affecting pressure of gases and bullet velocity inside the barrel due to round to round variations. Most of the copper is found up to about 18" because the copper particles are light and cannot go to longer distances. Some particles of larger size may go to about 30" also because of their larger mass. These results shows that in routine case work, it may not be possible to estimate range of firing from the presence of copper.

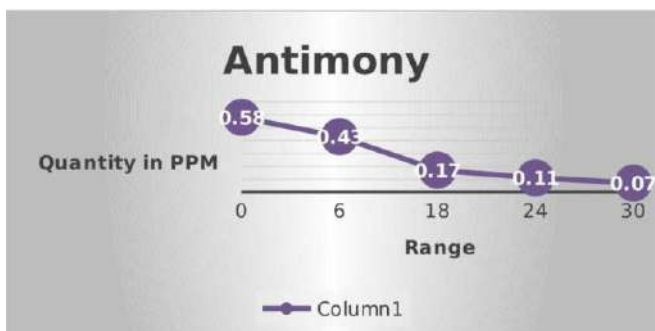
(ii) **Lead** : This amount of lead has been plotted with distance in graph 8. Here it is found that lead particles have gone to about 18"-24" because of their higher density and mass. This lead deposit also follows a graph that can be used to estimate range of firing.

Graph 8: Shows Quantity of Element Lead with Respect to Distance.



clothing was determined by instrumental technique AAS (Flame). The **graph 9** shows that the deposit of antimony around the gunshot holes also follows a graph which can be used to estimate range of firing.

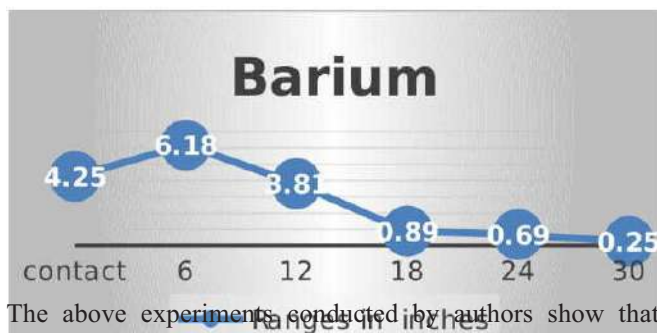
Graph 9: Shows Quantity of Element Lead with Respect to Distance



contributed by priming mixture was determined by AAS

(Flame) and **graph 10** shows the deposition of barium around the gunshot holes.

Graph 10: Shows the Quantity of Barium at Various Distances.



The above experiments conducted by authors show that elements copper, lead, antimony and barium are contributed by priming mixture, bullet, rubbing of bullet against rifling, evaporation of lead and antimony from behind the bullet when the high temperature gases make impact on the base of bullet during its passage inside the barrel, cartridge case and rubbing of bullet against clothes. Antimony may also be contributed by clothes. The experiments shows that these elements can be detected by SEM-EDX upto near contact distance only. There is no movement of GSR behind the bullet as concluded by Baumgartner^[2].

It is found that quantity of some of these elements may vary with distance in a specific manner such that the quantity of the elements with distance follows a certain graph. This graph could be used for range estimation. The quantities of these elements around the gunshot holes on the evidence clothes is determined. Now test firings using the suspected firearms and the ammunition recovered from accused are conducted from various distances, amounts of these elements are separately determined by AAS (flame) and their graphs are drawn. It is hoped that atleast one element may follow a graph. Now corresponding to quantity of this element as available on the evidence cloth, the range can be estimated^[7-9].

CONCLUSION:

In case of firing through .32 inch revolver, from the studies, it is found that range estimation may not be possible by using SEM-EDX due to its low sensitivity of detecting inorganic constituents of FDR particles. However by using AAS (Flame), lead could be detected upto 48 inches and antimony upto 18 inches. The amount of lead and antimony deposited around the gunshot holes follows a graph with decreasing amount and thus could be used for range estimation.

From the results of firing through pistol 7.65mm, it is

concluded that both lead and antimony can be used to estimate range of firing. It may not be possible on the basis of copper deposition around gunshot holes.

Hence from the results, it is concluded that Atomic Absorption Spectrometry is much better technique due to its higher sensitivity for detection of inorganic constituents of FDR particles and thus for range estimation.

REFERENCES :

1. Sellier K. Forensic Science Progress. s.l. : Springer-Verlag, 1967.
2. Baumgartner F, Stark H, Schontag A(1963).
3. Matricardi V, Kilty J. Detection of gunshot residue particles from the hands of shooter. J Forens Sci. 1977;22(4):725-38
4. Wolten G, Nesbitt R. On the Mechanism of Gunshot Residue Particle Formation. J Forens Sci. 1980;25(3):533-45
5. Basu, S. Formation of gunshot residues. Journal of Forensic Sciences.1982;27: 72e91..
6. Sellier K. Forensic Science Progress. s.l. : Springer-Verlag Berlin, 1991.
7. Andrasko J, Maehly A. Detection of gunshot residues on hands by Scanning Electron Microscopy. J Forens Sci. 1977;229(2):279-87
8. Schwoeble AJ, Exline DL. Current methods in forensic gunshot residue analysis. s.l. : Boca Raton: CRC Press LLC, 2000.
9. Chohra M, Beladel B, Ahmed L B, Mouzai M, Akretche D, Zeghdaoui A, Mansouri A, Benamar ME A. Study of 9. gunshot residue by NAA and ESEM/EDX using several kinds of weapon and ammunition. Journal of Radiation Research and Applied Sciences .2015; 404-10

Original Research Paper

Variant alleles observed in short tandem repeat (STR) analysis of random Indian population

1. **Monika Chakravarty**, Reporting officer, FSL Delhi
2. **Nidhi Singh**, LA Biology, FSL Delhi
3. **Damini Varshney**, SA Biology, FSL Delhi
4. **Dhruw Sharma**, AD Biology, FSL Delhi
5. **Naresh Kumar**, SSO Biology, FSL Delhi
6. **Prateek Pandya**, Assistant Professor, Amity University, Noida
7. **Anupama Raina**, Senior Scientist, All India Institute of Medical Sciences, Delhi.

ABSTRACT :

Short tandem repeat (STR) profiles generated from 1160 individuals generated by the Identifiler plus PCR amplification kit were screened for variant alleles not represented within the manufacturer's provided allelic ladder. A total of ten (10) distinct variants were observed at 2 of the 15 loci namely D13S317 & FGA. These alleles strengthen the genetic evidence in trouble some cases & increase the range of alleles.

Corresponding Author

Monika Chakravarty,

Forensic Science Laboratory, Home Department, Govt. of NCT Delhi, Rohini,

Contact : +91 9868931045

E-mail : monika42@gmail.com

Article History:

Received: 28 November 2018

Received in revised form: 25 January 2019

Accepted on: 25 January 2019

Available online: 1 June 2019

Keywords: DNA typing, STR, Allele, Variant, D13S317 & FGA

INTRODUCTION :

Copy number repeats in Short tandem repeats (STR's) are exploited by electrophoresis of amplicons to distinguish between alleles^[1]. Alleles in unknown samples are designated by comparison with the alleles in ladder. Most of the forensic & paternity cases involve comparison of amplified products/amplicons to alleles from commercially produced allelic ladder in order to designate the alleles correctly^[2]. Most of the DNA profiles comprise of Short tandem repeat (STR) alleles represented within the allelic ladder but a small number of individuals have alleles which are absent from the allelic ladder (Variant alleles). Allelic ladder is the combination of alleles based on the genetic variation in the population. To confirm the existence of an allele re- amplification of the sample containing the suspected variant has to be done. Identification of the variant is confirmed by re-amplifying the DNA. To draw proper conclusion from the casework, the forensic experts should be aware of the existence of rare alleles so that the variant can be identified. Correctly designated alleles can enhance the power of discrimination of STR analysis^[3].

METHODOLOGY : India is a highly diverse country in terms of culture & geography owing to its vast cultural heritage. STR profiles generated from 1160 individual data

taken from random routine case work performed at FSL, Delhi were screened for variant alleles not represented within the allelic ladder provided by the manufacturer.

DNA extraction & Quantification :

DNA from the samples was extracted by Phenol: Chloroform extraction method as well as by using Automate Express™ Forensic DNA extraction system. Extracted DNA samples were quantified by using quantifiler Duo DNA Quantification kit.^[4]

PCR amplification & STR typing :

PCR amplification was carried out using the Applied Biosystems Veriti & Proflex PCR system (By Thermo Fisher Scientific) according to manufacturer's recommendations. The AmpFISTR Identifiler plus PCR amplification kit was used to simultaneously amplify 15 STR loci: D8S1179, D21S11, D7S820, CSF1P0, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TP0X, D18S51, D5S818, FGA as well as the gender determination locus, Amelogenin.^[5]

Electrophoresis of the amplified product was performed on an Applied Biosystems 3500 and 3500xl Genetic analyzer using 36 cm capillary on Performance optimized polymer 4 (POP4). 0.5 microlitres of the amplified product was mixed with 9.5 microliter HiDi Formamide & 0.3 microlitres Gene scan 500

Liz size standard.

Mixture was denatured at 95°C for three minutes followed by lowering the temperature to 4°C for three minutes. The denatured samples were loaded onto tray & transferred to auto sampler. Allelic ladder was run as a part of batch of samples.

Data Analysis : Gene Mapper[®]IDx software v 1.4 was used for analysis of samples. The samples were sized accordingly to internal size standard to assign fragment size & a sizing quality value to each peak.^[6] Comparison between the provided allelic ladders & amplified samples at the same locus were used to designate the alleles.

RESULTS & DISCUSSION :

This study screened a total of 1160 random individual samples. A total of 10 variant alleles were identified at 2 out of 15 STR loci tested. This study identified one variant allele at locus D13S317 (allele 7) and 9 at FGA locus (Allele 18.2, 19.2, 20.2, 21.2, 22.2, 23.2, 24.2, 25.2 & 27.2). Each variant allele was confirmed by re-extraction & amplification of each locus. Allelic ladder from kit contain alleles 8-15 at locus D13S317 and 17-26, 26.2, 27-30, 30.2, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 47.2, 48.2, 50.2, 51.2. The variant comprising the largest fraction of alleles is 22.2 & 21.2 at FGA and 7 at D13S317. However most alleles were quite rare with many occurring only a single profile reviewed in this study. (18.2, 19.2, 25.2 & 27.2 at FGA) The number of variant allele observations and corresponding allele frequencies examined in this study are shown in **Table 1**.

CONCLUSION : Many a times case work performed in our laboratory, involves one of the variant described in this study. Use of these variant eases the analysis & interpretation of results in troublesome cases. In addition to their benefit to paternity analysis, the variant alleles described herein act as a valuable tool for forensic & other human identification

laboratories that use CODIS STR's. (7) Care should be taken in kinship matching or paternity cases as incorrect designation of any of these deviation from allelic ladders could lead to misinterpretation of results or false exclusion. (8)

ACKNOWLEDGMENTS :

The authors wish to thank Director, FSL Delhi and Delhi Govt. for providing support and facilities to carry out this study.

REFERENCES :

- Butler JM. Fundamentals of Forensic DNA typing, Academic Press 2005
- Griffith RA, Barber MD, Johnson PE, Gillbard SM, Haywood MD, et al. (1998) New reference allelic ladders to improve allelic designation in a multiplex STR system. *Int J Legal Med* 111(5): 267-272.
- Chauhan T. Identification of Variant alleles at CODIS STR loci in Different Populations of India. *Int J Forensic Sci Pathol*. 2015;3(9):172-175.
- Quantifiler Duo Kits Product Bulletin, ThermoFisher Scientific.
- AmpFI STR Identifiler plus PCR amplification kit, User's Manual, ThermoFisher Scientific.
- Genemapper IDx 1.4 manual.
- Catherine Allor, M.S; David D. Einum and Marco Scarpetta Sept (2005) Identification and Characterization of Variant Alleles at CODIS STR Loci*. *J Forensics Sci*, Vol. 50, No. 5
- Huel RL, Basic L, Todorovic M, Smajlovic L, Eminovic I, et al. Variant alleles, Tri-allelic patterns and Point mutations observed in nuclear Short tandem repeat typing of populations in Bosnia and Serbia. *Croat Med J* 2007;48(4): 494-502.

Table '1'- Variant Alleles in random Indian population along with their frequencies

Locus Designation	Chromosome location	Alleles included in Identifiler Plus Allelic ladder	Variant allele	No. of observation	Allele Frequency
D13S317	13q22-21	8,9,10,11,12,13,14,15	7	(10)	0.0043
FGA	4q28	17,18,19,20,21,22,23,24,25,26,26.2, 27,28,29,30,30.2,31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 47.2, 48.2, 50.2, 51.2	18.2 19.2 20.2 21.2 22.2 23.2 24.2 25.2 27.2	(1) (1) (3) (10) (12) (6) (2) (1) (1)	0.00043 0.00043 0.00129 0.0043 0.00517 0.00258 0.00086 0.00043 0.00043

Original Research Paper

Gender and Age Differences in Eruption Age of Third Molar Teeth in Iranian Children and Young Adults

1. **SeyedJavad Mirhadi**, Forensic Medicine Research Center, Forensic Center of Hormozgan Province, Bandar Abbas, Iran.
2. **Saeed Mohammadi**, Forensic Medicine Specialist, Forensic Center of Hormozgan Province, Bandar Abbas, Iran
3. **Azadeh Memarian**, Assistant Professor of Forensic Medicine, Iran University of Medical Science, Tehran, Iran
4. **Samad Moini**, DDS, Forensic Center of Hormozgan Province, Bandar Abbas, Iran
5. **Katayoun Mottaghi**, DDS, Forensic Center of Hormozgan Province, Bandar Abbas, Iran
6. **HoseinJavadi Vasigh**, MD, Forensic Center of Hormozgan Province, Bandar Abbas, Iran
7. **Khatere Asadi**, MD, Forensic Center of Hormozgan Province, Bandar Abbas, Iran

ABSTRACT :

Background : No literature is available on the relationship between third molar developmental stages and age estimation among Iranian population, thus we aimed to measure the time course of third molar eruption and stages in Iranian children and young adults. Because this issue can be very useful in legal medicine field, we examined the people who referred to legal medicine centers because of the various injuries.

Methods : A total of 243 dental victims aged 14 to 24 years who consecutively referred to examination department of the General Directorate of Forensic Medicine in Bandar-e-Abbas city in Iran between March 2014 and March 2016 were assessed. All subjects were examined regarding third molar eruption and stages based on orthopantomogram and dental age were assessed based on the method proposed by Demirjian.

Results : Men reported lower mean age in most of developmental stages as compared to women (F and G stage in superior side and F stage in inferior side). Comparing the mean values the age in obese and non-obese individuals at different developmental stages of third molar teeth showed no difference. There were significant positive correlation coefficients between different variables particularly between contralateral molars than antimeres. The highest correlation coefficient was found between lower right and lower left third molars ($r=0.918$). The strongest correlation coefficient was found between upper right and upper left third molars ($r=0.949$) in men and between lower right and lower left third molars ($r=0.939$) in women. Considering only the fully developed third molar teeth status (stage H) showed higher number of fully developed third molar in men than in women as well as in obese than in non-obese subjects in all sides.

Conclusion : Our study indicates a significant earlier third molar development in men than in women only in relation to developmental F and G stages of maxillary molars and F stage of mandibular molar, but this difference is not shown in fully (H) developmental stage. The association between third molar developmental stage and chronological age may be independent to body mass index and obesity status.

Corresponding Author :

Dr. Saeed Mohammadi,

Forensic Medicine Specialist, Forensic Center of Hormozgan Province, Bandar Abbas, Iran

Contact : 0098-076322200337

E-mail : drsm1980@gmail.com

Article History:

Received: 4 November, 2018

Received in revised form: 4 December, 2018

Accepted on: 4 December 2018

Available online: 1 June 2019

KEYWORDS : 3rd Molar teeth, Demirjian, Forensic, Odontology

INTRODUCTION :

Age assessment in living individuals has become increasingly as an important issue in forensic medicine field over the recent years^[1-4]. In other word, chronological age is of relevance in legal proceedings in all populations that the age thresholds of legal importance frequently lie from 14 to 21 years. Nowadays, some comprehensive forensic medicine guidelines

recommend the age estimation based on some major physical and radiographic anthropometrics including head and neck, hands and feet, as well as dental dimensions^[5]. In this regard, dental age estimation has a special importance particularly among the relevant age subgroups. It is now agreed that the evaluation of third molar eruption is most important for age assessment based on dental characteristics. In addition,

because of ethnical and genetic variations in dental development, the study of the validity of age assessment by evaluating third molar eruption and stages is necessity for accuracy and precision in the determination of age in such populations^[6]. In fact, age assessment in young children is more precise as multiple teeth in their various stages of development can be utilized, while in adolescents and young adults, third molars can be the only teeth developing parameter that should be considered in age assessment. Thus, examination of third molars status can be very useful in estimating chronological age in children and young adults^[7,8].

MATERIAL AND METHODS :

A total of 243 dental victims of age 14 to 24 years who were referred to examination department of the General Directorate of Forensic Medicine in Bandar-e-Abbas city in Iran between March 2014 and March 2016 were assessed. All subjects were examined for third molar eruption and stages based on orthopantomogram and dental age were assessed based on the method proposed by Demirjian *et al*^[9]. Demirjian *et al.* proposed 8 stages of maturity, from stage A (single occlusal points calcification, without fusion) to stage H (apical end completed). To increase the accuracy of the estimation, one code for "follicle with no calcification" was added and named stage 0. Demirjian classification stages were scored from "1" to "8" and "follicles with no calcification" was scored "0"^[9]. The identification number, gender, date of birth, body mass index (BMI) and X-ray examination date of each subject were documented, and the eruption stages of the third molars were finally determined. Chronological age of each subject was confirmed by birth certificates and determining the difference between the age of birth and the date of referring.

RESULTS :

Table 1 shows the mean values and standard deviations of male and female ages at different developmental stages of third molar teeth. It was shown lower mean age in men compared to women for developmental stages F and G on superior side and F stage on inferior side. In the stage of full development (H stage), no difference was found between the two genders in the mean age of development of the molar teeth. Comparing the mean values and standard deviations of the age in obese and non-obese individuals at different developmental stages of third molar teeth (**Table 2**) showed no difference. **Table 3** displays the results of correlation between developmental stages of different molar teeth. There were significant positive correlation coefficients between different variables particularly between contralateral molars than antimeres. The highest correlation coefficient was found between lower right and lower left third molars ($r=0.918$). The strongest correlation coefficient was found between upper right and upper left third

molars ($r=0.949$) in men and between lower right and lower left third molars ($r=0.939$) in women. Considering only the fully developed third molar teeth status (stage H) showed higher number of fully developed third molar in men than in women as well as in obese than in non-obese subjects in all sides (**Table 4**).

Table 1 : Mean age (in years) and standard deviations of different stages of third molar teeth development in both genders

Stage	Male	Female	p-value
Right Superior			
D	16.00 ± 1.00	14.86 ± 1.22	0.413
E	15.83 ± 0.98	16.65 ± 2.09	0.374
F	15.75 ± 1.42	18.39 ± 2.38	0.002
G	18.04 ± 2.20	19.74 ± 2.34	0.010
H	21.57 ± 2.09	21.85 ± 1.62	0.504
Left superior			
D	16.00 ± 1.00	15.71 ± 2.63	0.922
E	15.83 ± 0.98	16.75 ± 2.11	0.324
F	16.53 ± 2.07	18.30 ± 2.41	0.029
G	18.04 ± 2.31	19.75 ± 2.29	0.011
H	21.57 ± 2.09	21.81 ± 1.59	0.560
Right inferior			
E	15.50 ± 1.23	16.25 ± 2.30	0.470
F	16.25 ± 1.65	18.36 ± 2.34	0.009
G	18.04 ± 2.20	19.16 ± 2.34	0.101
H	21.41 ± 2.22	21.64 ± 1.68	0.558
Left inferior			
D	15.50 ± 0.70	15.88 ± 2.36	0.836
E	16.00 ± 1.26	15.50 ± 1.65	0.535
F	16.42 ± 1.93	18.39 ± 2.29	0.016
G	18.33 ± 2.51	18.88 ± 2.26	0.460
H	21.48 ± 2.21	21.77 ± 1.59	0.472

DISCUSSION :

Estimation of the chronological age by assessing tooth development has been agreed as an accurate method and is independent to confounding factors like underlying disorders, malnutrition and level of physical activity. However, it seems that the age estimation based on development of third molar teeth can be strongly affected by the adolescent period when the third molar is the only variable dental indicator left. Because of ethnical differences and variant methodologies, the assessment of the relation between the chronological age and the developmental stage of the third molar teeth is essential. The present study tried to first assess the relation between

Table 2: Mean age (in years) and standard deviations of different stages of third molar teeth development according to BMI value

Stage	Overweight/ Obese	Non-obese	p-value
Right Superior			
D	15.00 ± 1.19	----	----
E	16.45 ± 1.92	16.00 ± 1.00	0.819
F	17.25 ± 2.47	18.50 ± 0.71	0.489
G	18.98 ± 2.32	17.50 ± 1.14	0.159
H	21.51 ± 1.96	22.22 ± 1.85	0.123
Left superior			
D	15.75 ± 2.43	----	----
E	16.52 ± 1.94	16.00 ± 1.00	0.795
F	17.48 ± 2.46	18.50 ± 0.71	0.570
G	18.95 ± 2.34	18.14 ± 3.07	0.418
H	21.49 ± 1.94	22.23 ± 1.90	0.115
Right inferior			
D	15.88 ± 2.36	----	----
E	16.00 ± 2.00	----	----
F	17.67 ± 2.35	16.00 ± 1.00	0.491
G	18.64 ± 2.28	17.75 ± 2.43	0.324
H	21.32 ± 2.06	22.17 ± 1.78	0.069
Left inferior			
D	15.80 ± 2.09	----	----
E	15.69 ± 1.49	----	----
F	17.73 ± 2.39	17.50 ± 2.12	0.897
G	18.46 ± 2.19	18.88 ± 3.48	0.664
H	21.47 ± 2.03	22.05 ± 1.88	0.240

chronological age and the developmental stage of the third molar and then the effect of the variables of gender and BMI (Body Mass Index). Significant earlier development in men was indicated than women in relation to developmental F and G stages of maxillary molars and F stage of mandibular molar.

However this difference was not shown in H developmental stage. Except for insignificant result between the two genders in fully developmental stage, our

Table 3 : Correlation between developmental stages of upper and lower third molars in males and females

	Right Superior	Left Superior	Right Inferior	Left Inferior
Total				
Right Superior	1	.901**	.691**	.691**
Left Superior	.901**	1	.681**	.676**
Right Inferior	.691**	.681**	1	.918**
Left Inferior	.691**	.676**	.918**	1
Men				
Right Superior	1	.949**	.546**	.580**
Left Superior	.949**	1	.508**	.556**
Right Inferior	.546**	.508**	1	.869**
Left Inferior	.580**	.556**	.869**	1
Women				
Right Superior	1	.837**	.718**	.708**
Left Superior	.837**	1	.730**	.698**
Right Inferior	.718**	.730**	1	.939**
Left Inferior	.708**	.698**	.939**	1

study results are in agreement with that of Thorson and Hagg^[10], Mincer et al^[11], Kullman^[12] and Gunst et al^[13] who had studied the third molar root development in relation to chronological age in Japanese, German and Caucasian populations respectively. In other study, significant earlier eruption of all molars was found in females than males^[14] that might be related to different population and genetic factors.

In our study, early or late developing third molars according to the chronological age was independent to individuals' body composition such as the obesity status. Some studies could demonstrate the correlation between developmental stage of the teeth and BMI in one gender but not in another. As shown by Anbiaee et al^[15], a significant correlation between BMI and dental development in the girls but the correlation coefficient was not high. To make the clinical prediction that increased pure BMI (regardless of age and sex which are required to CDC grouping) is related to accelerated dental development, the correlation coefficient needs to be at least 0.8.

Table 4 : The fully developed third molar teeth status (stage H) according to gender and BMI

	Gender		p-value	BMI status		p-value
	Men	Women		Non-obese	Obese	
Right Superior	75 (55.6%)	33 (32.7%)	< 0.001	85 (41.7%)	23 (71.9%)	0.031
Left Superior	72 (53.3%)	32 (31.7%)	< 0.001	2 (40.2%)	22 (68.8%)	0.032
Right Inferior	73 (54.1%)	42 (40.4%)	< 0.001	91 (44.4%)	24 (70.6)	0.038
Left Inferior	69 (51.9)	39 (38.6)	< 0.001	87 (43.3%)	21 (63.6%)	0.151

The correlation coefficient was not significant in the boys. There was a significant acceleration in dental development in the overweight and obese boys, whereas no significant differences were found in dental development of different BMI groups in the girls. Hilgers et al^[16] also studied the effect of children obesity on dental development and concluded that children with increase in weight and/or increase in BMI have increased dental development. In total, it seems that the development of molar teeth may be independent to body weight and body composition at similar chronological ages in both genders and thus considering the stage of teeth development could not be predicted by individuals. However, as indicated by our study and considering only fully developmental stage (H), higher frequency of fully developed molar teeth is expected in obese people.

Left-right symmetry in development age of the third molar expressed by the correlation coefficient was found in both males and females, but more interestingly, the pattern of this asymmetry was different in upper and lower sides in men and women so that the strongest correlation coefficient was found between upper right and upper left third molars, in men but between lower right and lower left third molars in women (may be referred to the difference in total body developmental patterns in men and women and probably in obese and non-obese subjects).

CONCLUSION :

Because of the difference in developmental stages of the third molar teeth between the genders and also between obese and non-obese subjects, designing a formula (including the variable of the fully development stage of third molar teeth as independent variables and gender, chronological age, and BMI status as the dependent variables) will be helpful in forensic investigations regarding the determination of age of either living subjects or dead ones whose heads or skulls are the only remains available for identification procedures.

REFERENCES :

1. Willems G. A review of the most commonly used dental age estimation techniques. *J Forensic Odontostomatol* 2001;19:9-17.
2. Teivens A, Mörnstad H. A modification of the Demirjian method for age estimation in children. *J Forensic Odontostomatol* 2001;19:26-30.
3. Teivens A, Mörnstad H. Comparison between dental maturity rate in the Swedish and Korean populations using a modified Demirjian method. *J Forensic Odontostomatol* 2001;19:31-35.
4. Rai B, Krishan K, Kaur J, Anand SC. Age Estimation from Mandible by Lateral Cephalogram: A Preliminary Study. *J Forensic Odontostomatol* 2008;27:24-28.
5. Schmeling A, Grundmann C, Fuhrmann A, Kaatsch H-J, Knell B, Ramsthaler F et al. Criteria for age estimation in living individuals. *Int J Legal Med* 2008;122:457-460.
6. Cunha E, Baccino E, Martrille L, Ramsthaler F, Prieto J, Schuliar Y et al. The problem of aging human remains and living individuals: a review. *Forensic Sci Int* 2009;193:1-13.
7. Narnbiar P. Age estimation using third molar development. *Malaysian J Pathol*. 1995;17:31-4.
8. Bhat VJ, Kamath GP. Age estimation from root development of mandibular third molars in comparison with skeletal age of wrist joint. *Am J Forensic Med Pathol*. 2007;28:238-41.
9. Demirjian A, Goldstein H, Tanner JM. A new system of dental age assessment. *Hum Biol*. 1973;45:211-27.
10. Thorson, J. and Hagg, U. (1991) : "The accuracy and precision of the third mandibular molar as an indicator of chronological age". *Swed. Dent. J.*, 15:18-22.
11. Mincer, H. H.; Harris, E. F. and Berryman, H. E. (1993) : "ABFO study of third molar development and its use as an estimator of chronological age". *J. Forensic. Sci.*, 38 : 379-390.
12. Kullman, L. (1995) : "Accuracy of two dental and one skeletal age estimation method in Swedish adolescents". *Forensic Sci. Int.*, 75: 225-236.
13. Gunst, K.; Mesotten, K.; Carbonez, A. and Willems, G. (2003) : "Third molar root development in relation to chronological age: a large sample sized retrospective study". *Forensic Sci. Int.*, 136:52-57.
14. Ahmed Hassan N. Orthopantomography And Age Determination Using Third Molar Mineralization In A Sample Of Egyptians. *Mansoura J. Forensic Med. Clin. Toxicol*. 2007;15(1):45-58.
15. Anbiaee N, Rashed Mohassel A, Bagherpour A. The Relationship between Body Mass Index and Dental Development by Demirjian's Method in 4- to 15-Year-Old Children in Mashhad. *J Dent Mater Tech* 2013; 2(3): 82-5.
16. Hilgers, K.K., Axridge, M., Scheetz, J.P. and Kinane, D.E. (2006) Childhood obesity and dental development. *Pediatric Dentistry*, 18, 16-22.

Original Research Paper

Effect of Room Temperature and Length of Exposure to recover quality DNA from Earphone Swab-Derived 126bp and 143bp Mitochondrial DNA at D-loop region

1. **Ahmad Yudianto**^{a,b,d}, Lecturer

2. **Simon Martin Manyanza Nzilibili**^{a,c}, MSc. Forensic Science Student

a. Forensic Science Program, Post Graduate School, Airlangga University, Surabaya – Indonesia.

b. Department of Forensic and Medico-legal, Faculty of Medicine, Airlangga University, Surabaya – Indonesia

c. Ministry of Health, Community Development, Gender Elderly and Children, Dodoma – Tanzania.

d. Human Genetic Laboratory, Institute of Tropical Disease, Airlangga University, Surabaya – Indonesia.

ABSTRACT :

Introduction: Exploration of crime scene devices left over and its surrounding is of significance in forensic identification. The significance is due to its association and/or potential harbor of biological evidence; ie., blood, blood spots, semen patches, vaginal swabs, buccal swabs, bones, or trace evidence. Such harboring of evidence from humankind proposes traceability under Locard's principle of contact.

Materials and Methods : Processing of cerumen expelled or traces logged from the 30 used earphones were subjected to 0, 1, 7, 14 and 20 days room temperature exposure.

Results : Length of exposure has a significant effect on decreasing the level of DNA steadily. Visualization of the results of PCR on 143bp (HVS I, nt 16268-16410) mtDNA D-loop region showed a positive (+) detection only for day 1 (4 [66.67%] samples) and day 7 (3[50%] samples). Visualization of the results of PCR on the 126bp (HVS II, nt 34-159) mtDNA D-loop region showed a positive (+) detection only for day 1 (2 [33.37%] of samples) and day 7 (6 [100%] of samples).

Conclusion: In conclusion, the length of exposure to room temperature has an effect on the quality of earphone swab-derived DNA. Decreased levels of earphone swab-derived DNA showed a significance value ($p < 0.005$) with regard to the effect of room temperature exposure

Corresponding Author

Ahmad Yudianto

Lecturer

Forensic Science Program, Postgraduate School, Airlangga University, 4-6 Airlangga Rd., 60286 Surabaya, Indonesia

Contact : +6281330198281

E-mail : yudiyn6sby@yahoo.co.id

Article History:

Received: 2 December 2018

Received in revised form: 2 January 2019

Accepted on: 2 January 2019

Available online: 1 June 2019

KEYWORDS : DNA quality, Ear phone swabs, Forensic Evidence, Mitochondrial DNA, Room temperature

INTRODUCTION :

Identification by means of Deoxyribonucleic Acid (DNA) analysis is modern and referred as primary independent preferred technical method^[1,2]. The profile accuracy is by its stability inside biological cells of samples like blood, semen, hairs and even organ hence making it suitable for isolation of the inquired^[3]. Traces of this biological evidence is either free disperse of the criminal environment or found logged and harbored in objects/devices lastly used and left at the scene by the perpetrator or victim^[4-6]. Such objects and devices include headsets or earphones. The association to the identification of crime perpetrator or victim and exoneration of innocent through these audio electronic devices is due to a user-contact theory supported by Locard's principle of material exchange^[7]. During its use in mobile phones, player voices or as hearing aid implants, the devices are attached to the outer ear skin, leading

to contact with expelled cerumen or degrading body cells which may yield DNA profile^[8-9]. The devices have gained innovative progress in forensic to yield biological sample and/or traces for generation of genetic profiles during investigative tasks of the reality. However, the results of DNA analysis quality on the generated profile are interfered by a number of environmental factors including room temperature^[10-11]. One alternative taken by forensic DNA experts to deal with this condition is the use of a mini- primer set^[12-13]. The technique is carried out by using a method of reducing the size of Short Tandem Repeat (STR) assays in the examination of the locus of the nuclear DNA^[14] and reducing the size of hypervariable segment [HVS] 1 or 2 of mitochondrial DNA (mtDNA), which is used as amplification products^[15]. The use and sequencing of mtDNA in forensic identification are based on ability to determine the genetic relationship, thus

expediting the process of individualization especially in cases involving a large number of individuals such as mass disaster^[16]. Specificity of mtDNA in genetic relation is by a contained non-coding region, the displacement loop (D-Loop) at positions 16024-576 of nucleotides (nt). This region is also called the control region since it is a segment that contains the elements of the replication origin, transcription initiation, and regulators^[17]. While acknowledging the techniques to handle samples impacted by environment, there remains a need to understand such effect on samples logged to earphone. This study, therefore, experimented association of room temperature on the effect of room temperature on the quality of earphones swab-derived mitochondrial DNA.

MATERIALS AND METHODS :

A total of 30 samples of earphone swab derived DNA was taken from respondents who agreed and signed a written informed consent. This consent was of a voluntary participation in the scholarly study aimed at knowledge exploration and contribution to the scientific community through findings sharing and publication. This scholarly aim with the purpose in understanding the effect of room temperature on samples logged to earphones as evidence that would be found at crime scene. As also, the study didn't fragment harmful content rather than the quality and trend of mtDNA on D-loop from the earphone swab derived sample. Hence, therefore, the intent preserved and refrained from profiling for personal and individualized identification.

Experimentation protocol :

The materials for DNA extraction were DNAzol reagent (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) and 70% ethanol (EMSURE®, Merck KGaA 64271 Darmstadt, Germany). The material for Polymerase Chain Reactions (PCR) were PCR mix -12.5µl (Promega Corporation, Madison, USA) comprising of dNTPs (ATP, CTP, TTP GTP), MgCl₂, Taq polymerase and Nuclease-free water; mtDNA primers - 143bp (HVS I, nt 16268-16410) AFDIL 5'CACTAGGATACCAACAAACC3' and 5'GAGGATGGTCAAGGGAC3'^[18], 126bp (HVS II, nt 34-159) AFDIL 5'TCTCCAAGCGGGTGGTGCATTTA3' and 5'AAATAATAGGCAGGAGAGGATATC3'^[15].

Then extraction and isolation of DNA were performed. Laboratory processing was conducted at 26.5°C. Positive control (K+) was used to control findings. Other measures included the use of sterile supply, material, and equipment, assigning a code to every sample, use of protective gears to prevent contamination, calibrated of machines together laboratory room sterilization and disinfection by Ultraviolet lamps. Room Temperature exposure on the level of earphone swab derived DNA Exposition of earphone samples was

conducted at 28.5°C to 30°C of room temperature from 0, 1, 7, 14 to 20 days.

Polymerase Chain Reaction amplification and electrophoresis visualization

Cell lysed and isolated during extraction produced DNA template for PCR processing. The template under this study was polymerized and amplified using two hypervariable segments: (HVS) II (126bp, nt 34-159) and HVS I (143bp, nt 16268-16410) of D-Loop region of the mtDNA as shown in **Figure 2 to Figure 5**.

Results of the DNA sample extracted were measured for DNA levels and purity by using Ultraviolet- visible Spectrophotometer (UV-1601, PC, Shimadzu, Japan).

RESULTS :

The measured average concentration levels of DNA are presented in **Table 1**.

Table 1: Amount of mtDNA isolated from earphone samples at varied exposure interval.

Earphone (samples)	Time of exposure to room temperature	Mean of DNA quantity (µg/ml) ± SD	Temperature range (°C)
30	Day 0	152.89 ± 85.71	28.5 - 30
30	Day 1	11.19 ± 5.58	28.5 - 30
30	Day 7	2.15 ± 0.81	28.5 - 30
30	Day 14	0.93 ± 0.79	28.5 - 30
30	Day 20	0.16 ± 0.42	28.5 - 30

Table 1 measurements portray a decreasing effect of DNA concentration from day 1 to the twentieth day as referred to a non- exposed sample of day zero. A reference at day zero measured levels of DNA at 152.89 then decreased to 11.19 after a twenty-four hours room temperature exposure, on seventh-day measurement dropped to 2.15, then to 0.93 and lastly to 0.16 on fourteenth and twentieth respectively. Decreasing trend of DNA levels is appreciated and visualized in **Figure 1**, in which a sharp drop is experienced on the first day then a gradual decrease is observed steadily to twentieth.

143bp and 126bp mtDNA amplicon profiling at day 1 and 7

Profile analysis using 143bp and 126bp resulted in a band contrast formed and presented in **Figure 2**. Amplification and visualization of samples exposed by day one and seven became positively detected under the two primers (143bp and 126bp). Though both positively detected, band of two samples appeared with faded contrast under 126bp after seven days of exposure as observed in **Figure 2**.

143bp mtDNA amplicon electrophoresis of day 1, 7, 14 and 20

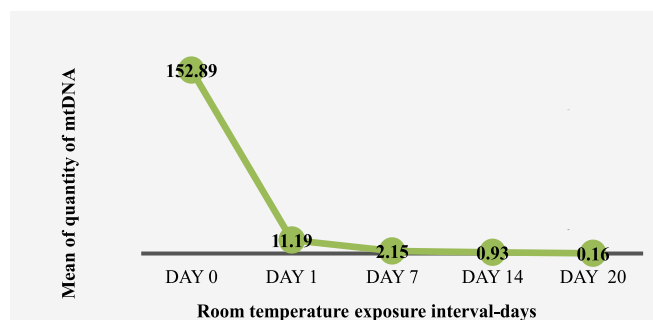
Under 2% agarose gel electrophoresis 143bp DNA amplicon, day 1 present positive [+] detection of two samples with faded bands and other 2 samples demonstrated a negative [-] detection while in day 7 samples there were 3 with negative [-]

detection (no band displayed) and there was only one sample that showed a positive [+] detection with a faint band too. Amplification and electrophoresis of samples by 143bp (HVS I, nt 16268-16410) D-loop mitochondrial DNA amplicon on day 14 and 20 using 2% agarose gel found that all samples displayed no band or negative detection as shown in **Figure 4**.

126bp mtDNA amplicon electrophoresis of day 7, 14 and 20

Amplification and electrophoretic visualization of 126bp mitochondrial DNA amplicon using 2% agarose gel on PCR swabbed earphone products exposed at 7, 14, and 20 intervals displayed variably. Day 7 exposed sample amplification gave a positive [+] contrast detection, bands were visible for the mtDNA as shown is presented in **Figure 5**. Contrary to day seven, amplification and electrophoresis of samples exposed for 14 and 20 days resulted into a no display of band contrast hence a negative [-] detection.

Figure 1: The trend of effects of the length of exposure on the mean of earphone swab derived DNA.



The measured mean levels of DNA as presented in Table 1 portray a decreasing effect of DNA. Extracted genetic material from less exposed earphone swabbed samples present before day seven evidence an elevated tabulation while a lowered graphical presentation and even gradual to those with maximized exposition. The graph confirms a sharp drop as sample get exposed to interfering factor (room temperature) – suggested by immediate cell lysis while in-vitro then the immediate destruction of primary DNA molecules hence a gradual damage continue steadily as interval persist. This trend supports the finding that the ability to profile DNA from environmental subjected sample gets diminishing with times, and in this case, the chance for identification of an individual by mtDNA D-loop profile get narrowed.

Relative extent effect of exposition on day 1, 7, 14 and 20

A representation of six samples for general percentage effect according to analysis is summarized in Table 2 and presented in Figure 6. The results show that, detection of the effects of

room temperature exposure on earphone swab-derived 143bp (HVS I, nt 16268-16410) and 126bp (HVS II, nt 34-159) mtDNA D-loop region depend also on the exposure interval and the D-loop specific region between the two (143bp and 126bp). Day one exposition analysis, 66.67% (4) of samples were positively detected at 143bp (HVS I, nt 16268-16410) D-loop region while 33.37% (2) were negatively detected similarly to those positively detected at 126bp (HVS II, nt 34-159) D-Loop region hence a v-like tabulation (day one graph) in Figure 6. On day 7; 50% (3) of samples were detected at 143bp (HVS I, nt 16268-16410) D-loop region and 100% (6) of samples detected at 126bp (HVS II, nt 34-159) D-loop region. Day 14 and 20, visualized a zero percentage of DNA positive detection due to exposure effect to room temperature on all earphone swab samples (143-bp (HVS I, nt 16268-16410) and 126bp (HVS II, nt 34-159) D-loop region). Relative tabulation and data presentation are referred to in **Figure 6** and **Table 2** respectively.

Figure 2: Electrophoretic visualization of 143-bp and 126-bp PCR products of earphone swab samples (M = marker, H1&H7=samples at day 1 and 7)

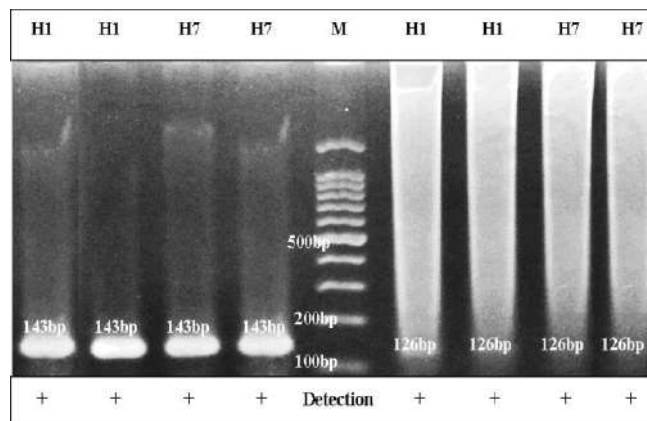


Figure 3: Electrophoretic visualization of 143-bp PCR products of arphone swab samples (M = marker, H1&H7 = samples at day 1 and 7)

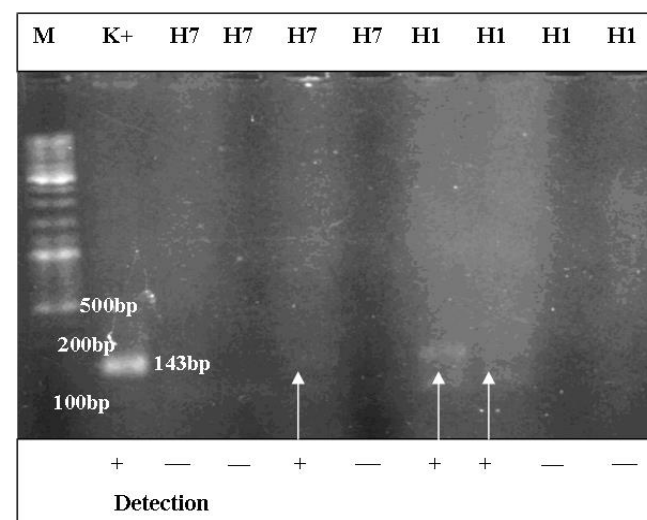


Figure 4: Electrophoretic visualization of 143-bp PCR products of earphone swab samples (M = marker, H14 & H20 = samples at day 14 and 20)

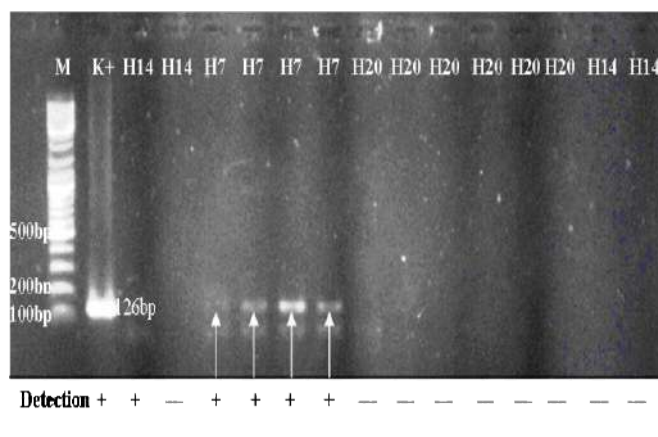
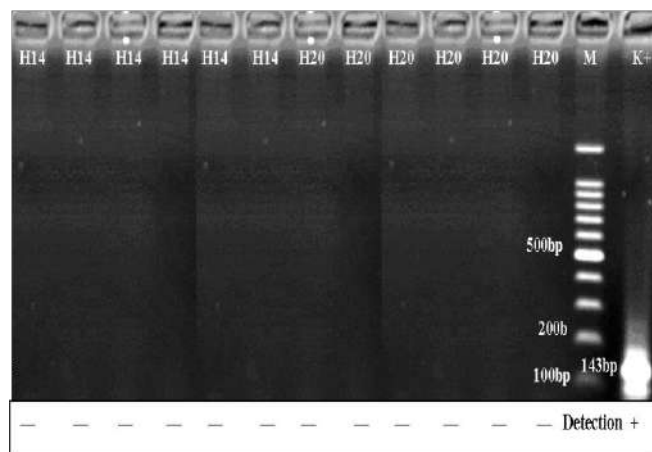


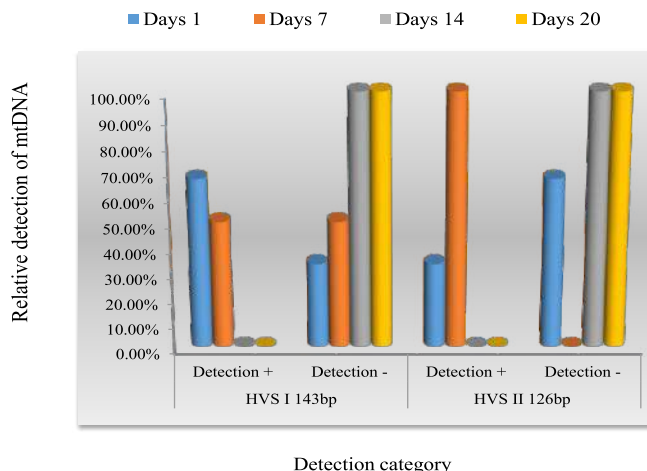
Figure 5: Visualization of 126-bp PCR products of earphone swab samples (M = marker, H7, H14 & H20 = samples at day 7, 14 and 20)



This relative presentation of six samples for general effect according to analysis is a summary of Table 2. The results show that, detection of the effects of room temperature exposure on earphone swab-derived 143bp (HVS I, nt 16268-16410) and 126bp (HVS II, nt 34-159) mtDNA D-loop region depend also on the exposure interval and the D-loop specific region between the two (143bp and 126bp). Day one detection relativity, 66.67% (4) of samples were positively detected at 143bp (HVS I, nt 16268-16410) D-loop region while 33.37% (2) were negatively detected similarly to those positively detected at 126bp (HVS II, nt 34-159) D-Loop region hence a v-like tabulation (day one graphs). On day 7; 50% (3) of samples were detected at 143bp (HVS I, nt 16268-16410) D-loop region and 100% (6) of samples detected at 126bp (HVS II, nt 34-159) D-loop region. Day 14 and 20, visualized a null percentage of DNA detection from all earphone samples at

143-bp (HVS I, nt 16268-16410) and 126bp (HVS II, nt 34-159) D-loop region).

Figure 6: Relative comparison of detection of earphone swab-derived 143-bp and 126-bp mtDNA D-loop region under room temperature



This relative presentation of six samples for general effect according to analysis is a summary of Table 2. The results show that, detection of the effects of room temperature exposure on earphone swab-derived 143bp (HVS I, nt 16268-16410) and 126bp (HVS II, nt 34-159) mtDNA D-loop region depend also on the exposure interval and the D-loop specific region between the two (143bp and 126bp). Day one detection relativity, 66.67% (4) of samples were positively detected at 143bp (HVS I, nt 16268-16410) D-loop region while 33.37% (2) were negatively detected similarly to those positively detected at 126bp (HVS II, nt 34-159) D-Loop region hence a v-like tabulation (day one graphs). On day 7; 50% (3) of samples were detected at 143bp (HVS I, nt 16268-16410) D-loop region and 100% (6) of samples detected at 126bp (HVS II, nt 34-159) D-loop region. Day 14 and 20, visualized a null percentage of DNA detection from all earphone samples at 143-bp (HVS I, nt 16268-16410) and 126bp (HVS II, nt 34-159) D-loop region).

Table 2: Results of detection of the effect of room temperature exposure on earphone swab-derived 143-bp (HVS I, nt 16268-16410) and 126-bp (HVS II, nt 34-159) mtDNA D-loop region.

Exposure Interval	HVS I 143bp nt: 16268 – 16410		HVS II 126bp nt: 34 – 159	
	Detection (+)	Detection (-)	Detection (+)	Detection (-)
Days 1	4(66.7%)	2(33.3%)	2(33.3%)	4(66.7%)
Days 7	3(50%)	3(50%)	6(100%)	0(0%)
Days 14	0(0%)	6(100%)	0(0%)	6(100%)
Days 20	0(0%)	6(100%)	0(0%)	6(100%)

RESULTS OF SAMPLE SEQUENCING :

Samples that remained to show a positive [+] detection were subjected to an analysis of sequencing for effects of exposure to room temperature. 143bp (HVS I, nt 16268-16410) and 126bp (HVS II, nt34□159) mitochondrial DNA D-loop fragments derived from earphone swab samples were sequenced using the Sanger dideoxy method by means of an Automatic DNA Sequencer based on the Dye Terminator Labeling method, protocol^[19]. The PCR product fragments sequenced were those amplified directly without cloning, a process called direct sequencing. This method was used since the process is fast and the products are the dominant nucleotide sequence of PCR-amplified DNA. Primer reaction (M1 - forward) was used to determine the nucleotide sequence of 126bp and 143bp in size in the sequencing reaction. The study was capable of determining the nucleotide sequence of the mtDNA D-loop region of the samples as shown in the below;

```
1st_BASE_2345711_20_143_143.ab1 for day 1
NNNNNNNNGGNNNNNNNNNNNNNNNNNNNANNNNGCA
TNAACCGNCAGAGCACATTA
```

```
GGGGCAGACCANNCTCGCCGCNGNANACGGGNC
NCCCGGGGAGACCAAAGNA
```

```
NCCCGNCGAGCCGGCANCTGNTGAGTCACGACC
CGAGAGGNGTGACGNCAGCCTGGGN
```

```
1st_BASE_397247_mtDNA_126bp1.ab1 for day 1
```

```
GTNGNNNTNNNTNCTCCTTNGNNGTCCCCGGC
GCGCCCCCTCAAGNNNNNNCTACTNGNCCTGAA
TTNATTACNGCCNCCTNNTCNCNTGANTNCTGCT
NCNNNANGNNNNNGCANNNNNNNNNNACGNNNN
NNGNNNNNGTNNANNNNNNNNNNNNNNNNNNN
CNTAGTCANNNGATNGTNTCTACNNNATNNNN.
```

DISCUSSION :**Room Temperature exposure on the level of Earphone swab derived DNA :**

DNA purity values obtained in this study were of 0.00 to 1.80 whereby the average level of DNA was 23.18 ± 57.58 ng/μl in which the purity value of 0.00 indicates no DNA in the sample. These parameters of DNA are an important factor in DNA analysis, especially with regard to the successful amplification of DNA samples. DNA integrity is central to DNA forensic investigation. This implies that, despite the relatively high levels of obtained DNA as observed in findings, fragmentation or degradation may reduce its significance. Though the level of DNA required in DNA analysis varies depending on the need and type of investigation still adequate quality of DNA is also required, in which DNA should be in a minimally degraded condition as also elaborated by (1). Measurement of DNA in this study has shown a tendency of decreased levels of

earphone swab- derived DNA in association with the length of exposure to room temperature (**Table 1**). Statistical tests were performed to determine the extent to which the length of exposure to room temperature had an effect on DNA levels. Kruskal-Wallis test and t-test of the levels of earphone swab-derived DNA on average showed a significant effect of the length of exposure to room temperature (significance value of 0000, significance limit of $p < 0.05$). The t-test of earphone swab-derived DNA levels showed a significance value of 0.002 and a significance limit of $p < 0.05$. Thus, there is a significant difference in the levels of DNA for all of the length of exposure.

In addition to the levels of DNA sample, PCR based investigation also requires an adequate quality of DNA. In this case, degraded DNA inhibits analysis of information. Severely degraded DNA prevents the primer from annealing the target DNA replication (20,21). According to (22), adequate visualization requires adequate DNA purity and DNA levels for successful investigation including identification and paternity test. Exogenous factors of DNA degradation are in several types either, (23,24). Type I degradation is in which DNA is usually degraded slowly by chemical processes for a relatively long period of time. Type II degradation is in which DNA is rapidly degraded by moisture, sunlight and even extremely high temperatures. Results of the study complement with the impact suggested in type II, that the environmental effects of temperature and length of exposure had an effect on the levels of DNA. Results of DNA measurement by spectrophotometer showed a significant decrease in the levels of DNA samples. The dependence of PCR-based DNA investigation on adequate quality of DNA is explained in a number of parameters. As discussed and established in literature the success of PCR highly depends on several factors, such as deoxyribonucleotide triphosphates (dNTPs), oligonucleotide primers, DNA template, buffer solution, reaction cycle, and enzyme. Additionally, it depends on the technical and non-technical factors, such as contamination. A sufficient quality of DNA means that DNA should be in good condition and, if it is degraded, the degradation should be as minimum as possible. Severely degraded DNA prevents the primer from annealing the target replicated DNA. Thus, a good quality of DNA constitutes a fundamental prerequisite for the overall PCR success. This is according to review collected by Cavanaugh & Bathrick (2018), PCR sensitivity is a function of the number of cycles and the levels and integrity of DNA.

Polymerase Chain Reaction amplification and Electrophoresis visualization

Visualization of PCR products was analyzed descriptively by

looking at the appearance of bands according to PCR product size. Positive detection [+] effects of room temperature exposure and the non-detection in the visualization of PCR products for these samples was related to DNA quality contained. DNA quality includes DNA levels, DNA purity and DNA condition state (degradation). In the present study, the average level of DNA was $0.93 \pm 0.79 \mu\text{g/ml}$ on day 14 and $0.16 \pm 0.42 \mu\text{g/ml}$ on day 20; the trend explains the degrading process. DNA degradation was among the causes of DNA detection failure in PCR-based DNA analysis in our study as well portrayed by Figure 4 and some bands in Figure 3 and 5. This is consistent with (26) with regard to several possible causes for failure detection of DNA, including a minimal amount of target DNA, DNA degradation that prevents the primer from annealing, lack of DNA polymerase and PCR cycle also presence of PCR inhibitors. The failure of amplification is characterized by the absence of bands on the electrophoresis results due to lack of DNA polymerase and PCR cycle. This can be controlled by the use of PCR master mix that contains Mg^{2+} and Taq DNA polymerase, dNTPs in quantities tailored to the needs of optimal PCR reaction and having a proven reliability in the current PCR reaction (27). From the article lack of cycle in a PCR reaction is controlled by optimizing the primers used for PCR while adhering to Mg^{2+} recommended concentration (should be 1.5 to 4 mM). An excessive concentration of Mg^{2+} causes a non-specific reaction with the presence of smear on electrophoresis. In contrary, the inadequacy of Mg^{2+} causes no amplified product or no band on the electrophoresis results as also guided in protocols (28). An excessive amount of Taq polymerase causes a smear formation (non-specific reaction) on electrophoresis, whereas inadequacy of it causes a reduced efficiency of amplification and non-appearance of bands. Tris-borate-EDTA (TBE) used in decalcification of samples may serve as a PCR inhibitor. This can be avoided by using ethanol to precipitate DNA extracted/isolated. Ethanol is a potential material to separate DNA from other components during DNA extraction (29). Some findings in this study show a failure of detection in the form of failed PCR amplification of DNA. This failure can be caused by degradation or damage to DNA from the effects of the length of exposure to room temperature. Cerumen attached to earphones contains detached epithelial cells of the outer ear canal. Those epithelial cells are not protected instead of directly exposed to external factors that cause degradation to contained genetic materials, DNA. According (30,31), DNA damage caused by external factors such as exposure to X-rays, chemical agents, spontaneous instability and extreme temperature will result in many types of damage, such as damage to DNA strands (both double and single strand), base damage (damage to DNA bases), sugar

damage and even presence of DNA crosslink and DNA-protein crosslink. Those environmental factors lead to degraded DNA. This degradation can be fast or slow, depending on the affecting factors and length of exposure. Findings of this our study shows that earphone swabs can still be used as a material for DNA analysis of mtDNA with regard to the effects of exposure to room temperature (28.5°C - 30°C) up to day 7. This is in potential consideration when applied to expedite forensic identification, especially in a mass disaster or criminal cases on the crime scene with little trace evidence.

Sample sequencing information derived

The positively [+] detected samples and analyzed for nitrogenous bases forming nucleotides sequence was in response of appreciating the damaging effect extent. Room temperature for samples exposed for day-1 and day-7 by 143bp and 126bp mitochondrial DNA D-loop fragments derived from earphone swab under Sanger dideoxy method portrayed potential effect impacted even to those still detected positive band contrast. The derived sequence of 126bp and 143bp mtDNA amplicon from earphone swab samples showed degraded nucleotides on day 1, as marked with 'N' in generated two sequences. This symbol 'N' according to the International Union of Pure and Applied Chemistry (IUPAC) is suggested to indicate the presence of ambiguities (32). Therefore a suggested room temperature effect per lapsed time interval.

CONCLUSION :

This study concludes that :

- The length of exposure to room temperature has an effect on the quality of earphone swab-derived DNA.
- Decreased levels of earphone swab-derived DNA showed a significance value ($p < 0.005$) with regard to the effect of exposure to room temperature.
- Visualization of the results of PCR on 143bp (HVS I, nt 16268-16410) mtDNA D-loop region showed a positive (+) detection only for day 1 (4 [66.67%] samples) and day 7 (3 [50%] samples) of room temperature exposure. Meanwhile, visualization of the results of PCR on 126bp (HVS II, nt 34-159) mtDNA D-loop region showed a positive (+) detection on similar exposure interval - day 1 (2 [33.37%] samples) and day 7 (6 [100%] samples) of room temperature.

ABBREVIATIONS :

bp: base pair(s); D-loop: Displacement loop; DNA: Deoxyribonucleic Acid; nt: nucleotides; HVS: Hyper Variable Segments; mtDNA: mitochondrial DNA; PCR: Polymerase Chain Reactions; STR: Short-Tandem Repeat; TBE: Tris-borate-EDTA.

ACKNOWLEDGEMENTS :

We appreciate and acknowledge the participation of volunteers who spent time and got involved in provide usage to earphones as evidence. Much gratitude to Postgraduate School, Forensic and Legal medicine program and the RSDS, Human Genetic Laboratory staff and all those who aided completion of this study.

Funding : None

Conflict of Interest : No conflicting interest

REFERENCES :

1. Dumache R, Ciocan V, Muresan C, Enache A. Molecular DNA analysis in forensic identification. Clin Lab 2016 ;62(1-2):245-8.
2. Ruitberg CM, Reeder DJ, Butler JM. STRBase: a short tandem repeat DNA database for the human identity testing community. Nucleic Acids Res 2001;29(1):320-2.
3. International Committee of the Red Cross. Missing People, DNA Analysis and Identification of Human Remains [Internet]. 2009. 52 p. Available from: www.icrc.org/eng/resources/documents/publication/p4010.htm
4. Ah Van Oorschot R, Ballantyne KN, Mitchell RJ. Forensic trace DNA: a review. Investig Genet. 2010;1:14.
5. Song GH, Huang FB, Gao JP, Liu ML, Pang WB, Li W Bin, et al. Effects of fluoride on DNA damage and caspase-mediated apoptosis in the liver of rats. Biol Trace Elem Res. 2015;166(2):173-82.
6. Shaler RC. Chapter 13: The Biological Crime Scene. In: Crime Scene Forensics: A Scientific Method Approach. 2011. p. 338-73.
7. Byard RW, James H, Berketa J, Heath K. Locard's Principle of Exchange, Dental Examination and Fragments of Skin. J Forensic Sci. 2016;61(2):545-7.
8. Shokry E, Filho NRA. Insights into cerumen and application in diagnostics: Past, present and future prospective. Vol. 27, Biochemia Medica. 2017.
9. Seo Y, Uchiyama T, Matsuda H, Shimizu K, Takami Y, Nakayama T, et al. Mitochondrial DNA and STR typing of matter adhering to an earphone. J Forensic Sci 2002 ;47(3):605-8.
10. Caputo M, Bosio LA, Corach D. Long-term room temperature preservation of corpse soft tissue: an approach for tissue sample storage. Investig Genet 2011;2(1):17.
11. Toothman MH, Kester KM, Champagne J, Cruz TD, Street WS, Brown BL, et al. Characterization of human DNA in environmental samples. Forensic Sci Int 2008;178(1):7-15.
12. Butler JM. Short tandem repeat typing technologies used in human identity testing. Biotechniques. 2007;43(4).
13. Hwan YL, Na YK, Myung JP, Woo IY, Shin KJ. A modified mini-primer set for analyzing mitochondrial DNA control region sequences from highly degraded forensic samples. Bio Techniques. 2008;44:555-8.
14. Coble MD, Butler JM. Characterization of New MiniSTR Loci to Aid Analysis of Degraded DNA. J Forensic Sci 2005;50(1):1-11.
15. Gabriel MN, Huffine EF, Ryan JH, Holland MM, Parsons TJ. Improved MtDNA Sequence Analysis of Forensic Remains Using a "Mini-Primer Set" Amplification Strategy. Vol. 46, Journal of forensic sciences. 2001. 247-253 p.
16. Alvarez-Cubero MJ, Saiz M, Martinez-Gonzalez LJ, Alvarez JC, Eisenberg AJ, Budowle B, et al. Genetic identification of missing persons: DNA analysis of human remains and compromised samples. Pathobiology. 2012;79(5):228-38.
17. Randi E, Lucchini V. Organization and evolution of the mitochondrial DNA control region in the Avian genus *lectoris*. J Mol Evol 1998;47(4):449-62.
18. Edson SM, Ross JP, Coble MD, Parsons T, Barritt SM. Naming the Dead -Confronting the Realities of Rapid Identification of Degraded Skeletal Remains. Forensic science review. 2004;16:63-90.
19. Slatko BE, Albright LM, Tabor S, Ju J. DNA Sequencing by the Dideoxy Method. Curr Protoc Mol Biol 2001;1-39.
20. Golenberg EM, Bickel A, Weihs P. Effect of highly fragmented DNA on PCR. Nucleic Acids Res. 1996;24(24):5026-33.
21. Mautner ME, Caputo M, Corach D. Degraded DNA samples made informative by using superprimers. Forensic Sci Int Genet Suppl Ser 2017;6(September):e448-50.
22. Psifidi A, Dovas CI, Bramis G, Lazou T, Russel CL, Arsenos G, et al. Comparison of eleven methods for genomic DNA extraction suitable for large-scale whole-genome genotyping and long-term DNA banking using blood samples. PLoS One. 2015;10(1):1-18.
23. Kawane K, Motani K, Nagata S. DNA degradation and its defects. Cold Spring Harb Perspect Biol. 2014;6(6):1-14.
24. Ghosal G, Chen J. DNA damage tolerance: a double-edged sword guarding the genome. Transl Cancer Res 2013;2(3):107-29.

25. Cavanaugh SE, Bathrick AS. Direct PCR amplification of forensic touch and other challenging DNA samples: A review. *Forensic Sci Int Genet.* 2018;32(October 2017):40–9.
26. Bartlett JMS, Stirling D. A short history of the Polymerase Chain Reaction. Humana Press [Internet]. 2003;226(PCR Protocols Volumen 226 de Methods in molecular biology):545. Available from:
<http://www.springerprotocols.com/Abstract/doi/10.1385/1-59259-384-4:3#>
27. Lorenz TC. Polymerase Chain Reaction: Basic Protocol Plus Troubleshooting and Optimization Strategies. *J Vis Exp.* 2012;(63):1–15.
28. PCR Troubleshooting | LSR | Bio-Rad [Internet]. [cited 2018 May 14]. Available from: <https://www.bio-rad.com/en-id/applications-technologies/pcr-troubleshooting?ID=LUSO3HC4S>
29. Schiffner L, Bajda EJ, Prinz M, Sebestyen J, Shaler R, Caragine T. Optimization of a simple, automatable extraction method to recover sufficient DNA from low copy number DNA samples for generation of short tandem repeat profiles. *Croat Med J.* 2005;46(1):578–86.
30. Hall A, Sims LM, Ballantyne J. Assessment of DNA damage induced by terrestrial UV irradiation of dried bloodstains: Forensic implications. *Forensic Sci Int Genet.* 2014;8(1).
31. Schuch AP, Garcia CCM, Makita K, Menck CFM. DNA damage as a biological sensor for environmental sunlight. *Photochem Photobiol Sci.* 2013;12(8):1259.
32. Swgdam. Guidelines for Mitochondrial DNA (mtDNA) Nucleotide Sequence Interpretation. *Forensic Sci Commun.* 200;5(2):1–5.

Original Research Paper

A Study on Snake Bite Poisoning in a Tertiary Care Hospital in Rural Odisha, India

1. **Ashim Mishra**, Associate Professor, Department of Forensic Medicine, SMIMS, Sikkim Manipal University, Gangtok
2. **S.N. Mohanty**, Medical Superintendent, AIIMS Bhubaneswar and Ex-Professor & HOD, Post Graduate Department of Forensic Medicine, M.K.C.G. Medical College, Berhampur, Odisha
3. **Prateek Rastogi**, Professor, Department of Forensic Medicine, KMC Mangalore, Manipal Academy of Higher Education.

ABSTRACT :

Introduction : The study was under taken to know the incidence of snake bite poisoning, the factors affecting survival and key findings during postmortem examination done at MKCG Medical college & Hospital.

Material and Methods : Clinico-epidemiological profile, analysis of postmortem findings and histopathologic examination was done to ascertain overall picture of snake bite.

Results : An overall incidence of 4.8 per 1000 admissions was found. 55 cases (61.8%) were due to poisonous variety and 34 cases (38.2%) were due to nonpoisonous snake bites. Majority of poisonous bites were neurotoxic (elapid) bites (70.9%). The overall mortality was 74.5%. Majority of the victims were males (61.8%) and between 21-30 years of age (34.8%). Most of the victims hailed from rural areas (77.5%) and reportedly worked as farmers (30.3%) and were bitten outside their homes (70.8%) during rainy season (57.3%). Diurnal predominance noticed in majority of the bites (67.4%), however on further categorization neurotoxic bites were well marked in morning hours (43.6%). Lower limbs were the most common site of bite (56.2%). Most of the non-poisonous cases did not reveal definite bite marks and grazing was seen in 73.5% cases. Two definite hemorrhagic puncture wounds corresponding to definite fang marks of being bitten by poisonous snake bite was found in 69.1% cases. The commonest symptom was local pain (41.6%), Ptosis being the most predominant sign of neurotoxic bite (23.5%) and oozing from bite site being the predominant sign in vasculotoxic variety (19.1%). The high rate of deaths during this study appears to be due to late presentation to tertiary care hospital attributed to prevailing traditional beliefs and customs and native treatment of snake bite by traditional healers causing delay in decision making.

Corresponding Author

Dr. Prateek Rastogi,

Professor,

Department of Forensic Medicine, KMC Mangalore,

Manipal Academy of Higher Education.

Contact : +91 94485-01376

E-mail : prateek.rastogi@manipal.edu

Article History:

Received: 4 December 2018

Received in revised form: 14 December 2018

Accepted on: 14 December 2018

Available online: 1 June 2019

KEYWORDS : Snakebite; Neurotoxic bite, Vasculotoxic bite, nonpoisonous bite

INTRODUCTION:

Snakebite is a serious but neglected problem in tropical and subtropical countries. However reliable data for morbidity and mortality are not available since there is no proper reporting system; the true worldwide burden of snake bite is not known. South Asia is the world's most heavily affected region, due to its high population density, widespread agricultural activities, numerous venomous snake species and lack of functional snake bite control programs. Moreover, many cases are not recorded in official statistics, as people seek traditional treatment methods. Earlier studies in Orissa state that around 1000 cases of snake bite deaths occur per annum⁽¹⁾ This prompted us to undertake the present study in rural Odisha

since there were numerous cases of snake bite reported every year with aim to find out the incidence, symptoms on arrival, different epidemiological profile and common postmortem findings in snake bite.

MATERIALS AND METHODS :

A total of 89 cases were assessed based on the inclusion and exclusion criteria in this prospective descriptive study, at MKCG Medical College & hospital, Berhampur, Odisha from 1.10.2011 to 30.9.2013. For convenience and accuracy, the casualty, the record section and the postmortem center were used for collecting the relevant information about the study subjects. 41 cases which succumbed were detailed for postmortem examination.

Exclusion criteria : History of unknown bites and LAMA (left the hospital against medical advice)

Data Collection: All the information regarding demographic and epidemiological parameters such as age, sex, residence, occupation, site of bite and place of bite, type of snake if identified etc. was obtained in the proforma. Pre referral treatment, time delay, symptoms on arrival and 20 minute clot retraction test was done and results were noted

Season : Classified as summer (March-June), rainy (July-October) and winter (November-February).

Time of bite: classified in 6-hour interval as 6Am to 12 Noon, 12Noon to 6Pm, 6Pm to 12 Midnight and 12 Midnight to 6Am.

Post mortem examination was carried out on 41 cases of snake bite victims out of total 89 cases of admission. Besides the usual parameters the other parameters of importance with special reference to snake bite like bite marks pattern and swelling were noted during external examination. In our study mild swelling was taken as minimal swelling of a less than 4 cm increase in limb circumference with no clinical evidence of local gangrene and gross swelling as resulting in local swelling of 4 cm or more^[2]. A histopathology of kidney tissue was done in all postmortem cases^[3]. The data was analyzed using SPSS Software version 20. 0.

RESULTS AND DISCUSSION:

Out of total 35,800 admissions in Medicine ward, in these two-year study period 1238 cases of poisoning were recorded with an incidence of 36.5 and 33.3 per 1000 admissions in 2011-12 and 2012-13 respectively. While only 173 cases of snake bite were admitted, the total study population was 89 owing patients who left against medical advice. The snake bite cases constitute about 13.9% of cases of total cases of poisoning cases. The incidence of cases with complaints of snake bite was 6.0 and 3.7 per 1000 admissions respectively in 2011-12 and 2012-13 with overall incidence of 4.8 per 1000 admissions in the total study period. [Table-1]

Our findings on incidence differed from studies done at Shongwe hospital where the incidence was 8.4/1,000 admissions but slightly higher than a study in India where the incidence was 3.8/1000 admissions^[4,5]. 55 cases (61.8%) were due to poisonous variety and 34 cases (38.2%) were nonpoisonous. Majority of poisonous bites were neurotoxic (elapid) bites (70.9%) and 29.1% were vasculotoxic bites. [Table-2] Our findings were similar to findings to a study at India where neuro paralytic features were seen in 40% and the haematotoxic manifestations were attributable to viper bites in 60%^[6].

The findings of researchers of Pakistan quite contrasted our study which showed that 3.1% of patients had neurotoxic

Table 1: Incidence of Snake Bite Cases

Study Period	Total number of hospital admission in medicine ward	Total number of poisoning cases	Incidence of poisoning cases per 1000 admission	Total number of snake bite cases	Percentage of snake bite cases to poisoning cases	Incidence of snake bite cases per 1000 admission
1 st Oct' 2011 to 30 th Sep' 2012	16828	606	36.5	101 (includes LAMA cases)	16.6%	6.0
1 st Oct' 2012 to 30 th Sep' 2013	18972	632	33.3	72 (includes LAMA cases)	11.4%	3.7

Table 2: Activity During Bite

Type	Sleeping	Working	Total
Non Poisonous	2 (5.9%)	32 (94.1%)	34
Poisonous			
Neurotoxic	14 (35.9%)	25 (64.1%)	39
Vasculotoxic	12 (75%)	4 (25%)	16
Total	28 (31.5%)	61 (68.5%)	89

snakebite, whereas rest of the cases were vasculotoxic (96.9%) in nature^[7]. It is obvious that relative incidence of toxic varieties would vary in different regions of the country and world according to the prevalent species of snakes. The higher incidence of neurotoxic bites in our study may be attributed to abundance of the Indian cobras and common krait in this area. The overall mortality in the study population was 74.5% was remarkably higher than several researchers^[6]. The reasons may be late reporting of cases, inadequacy of primary health centers in dealing with cases of snake bite, lack of proper road conditions in village hamlets, people seeking traditional treatment and ignorance of modern snake bite treatment in tribal dominated population. The victims often arrive in the casualty when conditions are critical where it is very difficult for any treatment approach. Poverty and ignorance forces the tribal population of this area comes to hospital only when situation gets out of hand. So only the poor serious patients depend on government machinery for treatment reflecting an abnormally higher mortality in my study. Although a large number of patients get cured since they had left against medical advice they could not be showed as study population, thus reflecting a higher mortality.

Out of total cases, 61.8% were males, the male–female ratio being 1.6:1 with highest preponderance in age group of 21-30 years of age (34.8%).

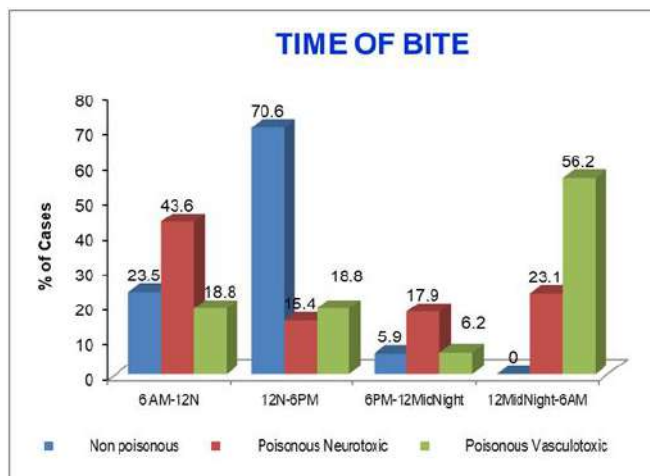
This finding was quite similar to findings of study at Pakistan where victims comprised of 61.55% males and 38.5% females but differed from study at Nepal where seventy-five per cent of the patients were in the age group 11-40 years^[7,10]. Most of the victims hailed from rural areas (77.5%) and reportedly working as farmers (30.3%). Other studies done at India indicating majority of the victims are from the rural areas (81.1%) clearly support our findings.^[3,9,11] Majority of the victims in our study were farmers (30.3%) similar but showing a lesser percentage than the study at Nepal where the common victims were farmers (44.2%)^[10]. Most of the victims were bitten outside (70.8%) and during rainy season (57.3%).

This is similar to other studies in studies at Pakistan and India^[7,8,12]. This study proved the fact that there is a definite seasonal pattern in cases of snake bites poisoning and rainfall forces snakes to venture out of their water-filled pits and there is increased human activity in fields during sowing season. Working bare-footed a normal practice in the people of these area could be one of the predisposing factors for coming in frequent contact with snakes.

In our study we get a slightly higher incidence than other studies of bites sustained during sleep due to the fact that this area is notorious for presence of 'koudia chiti' the common krait, which hides in crevices of wall and roof made of clay and tile. Moreover the cow dung is always present in courtyard used as a domestic fuel in this area which serves as an easy hiding place for this snakes.

A diurnal predominance was noticed in majority of the bites (67.4%), however on further categorization neurotoxic bites were well marked in morning hours (43.6%). (Figure 1)

Figure 1: Time of Bite

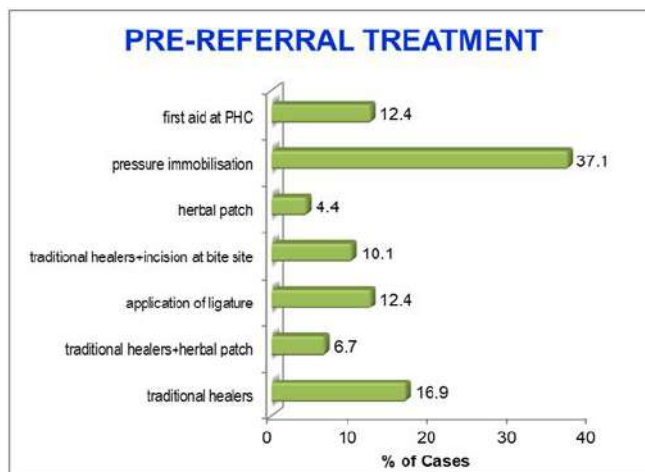


Our study contrasted with study at Malaysia which showed that most of the snake bites occurred during the 6-h evening period from 1800 to 2359 hours^[13]. The reason being all non-poisonous snakes bites occurred during day time and the cobra bites showed a higher incidence during daytime, proving that bites were chiefly an occupational hazard occurring when the snake gets startled or disturbed by human activity. Nearly all the bites of neurotoxic variety at night were due to kraits. The reason could be attributed to thatched houses, unclean environment providing shelters to snakes and unprovoked bites.

This finding suggest that the site of snakebite is predominantly determined by accidental or inadvertent contact of the reptile during the activities, the lower limb the first body part to be in contact with the creeping reptile. The higher percentage of upper limbs shows use of hand during rowing, clearing bushes and handling the sickle during farm related activities. The literacy status plays havoc in decision making behavior of the victims who are casual at the time of bite and rush at the last minute when conditions become too serious to handle.

Lower limbs were the most common site of bite (56.2%), similar to findings of studies done at India and Bangladesh where lower extremities were the commonest site. Bites at Uncommon areas like neck, groin and axilla occurred in 4.4% cases. The uncommon places like groin, neck and axilla were bitten during sleeping and all were due to common krait (koudia chiti) common in this area^[6]. Traditional methods of treatment like herbal patch, incision and witchcraft in combination were detected in 1/3rd of the cases. 16.9% went to traditional healers first before reaching hospital and majority were illiterate, followed by application of ligature in limbs in 11 cases. Pressure immobilization was followed in 37.8%. Only 12.4% went to nearby health facility to seek proper medical treatment from where referred to this tertiary care hospital. Incision at bite site was seen in 10.1%. (Figure 2)

Figure 2 : Pre referral treatment



Our Study clearly demonstrated a 100% fatality in cases who were subjected to traditional methods of treatment sighting zero added advantage.

Several researchers had shown local practices being still practiced having serious implication on survival^[8,10]. Our study also reflected the ignorance of the victims about modern snake bite management and beliefs on myths were responsible for their traditional treatment seeking behavior. Moreover, the pressure immobilization also shows a higher mortality and questions the importance of it in effective management.

Most of the cases do not reveal definite bite marks and grazing was seen in non-poisonous variety (73.5%). Two definite hemorrhagic puncture wounds corresponding to definite fang marks was found in 69.1% cases. Multiple teeth marks and grazing were observed in majority of non-poisonous snake bites. (Table 3)

Table 3 : Bite Mark Pattern

Bite Mark Pattern	Neurotoxic	Vasculo-toxic	Non-poisonous
Two definite hemorrhagic puncture wounds	28	10	1
Two hemorrhagic puncture wounds with blebs	6	4	0
Multiple Teeth Marks	2	0	8
Grazing	0	1	25
Four Hemorrhagic Puncture Wounds	3	1	0
Total	39	16	34

In neurotoxic (elapid) bite, normally in Indian cobra and common krait of our area, the site of bite contains fluid and hemolysed blood causing staining of vessels thus making the fang marks appear hemorrhagic.

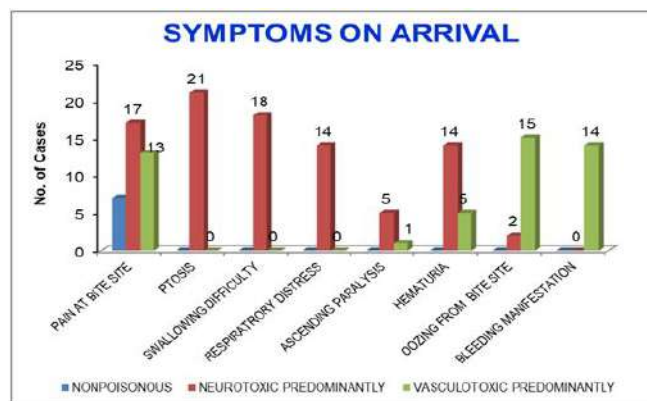
Our findings were similar to studies at Malaysia where fang marks were noted in 71.5% patients^[13]. A study in India pointed single puncture wound were found in 17.77% cases followed by double puncture wounds in 67.77% cases^[6].

The commonest symptom was local pain (41.6%), Ptosis was the most predominant sign of neurotoxic bite (23.5%) followed by oozing from bite site being the predominant sign in vasculotoxic variety (19.1%). (Figure 3)

Respiratory distress was found in 15.7% of victims on arrival while bleeding manifestation like bleeding from nose, gum bleeding, rectal bleeding occurred in 15.7% cases of vasculotoxic bites. Pain at bite site was found in 13 out of 16

cases of vasculotoxic bites. Hematuria was seen in 14 out of 39 cases of neurotoxic bites and only 5 out of 16 cases of vasculotoxic bites.

Figure 3: Symptoms on Arrival

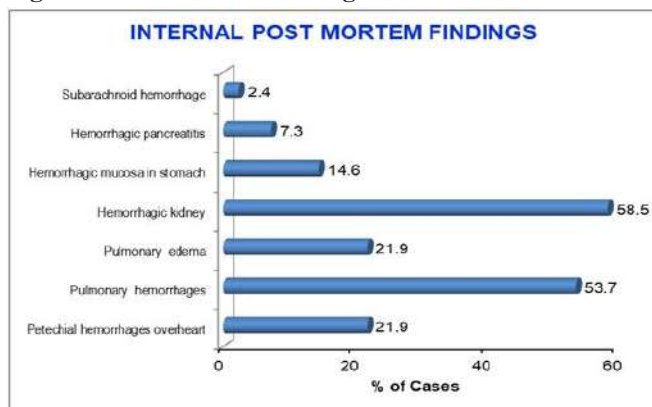


Ptosis was the most common symptom in elapidae bites followed by dysphagia. Our findings are different than the findings of another Indian study where 90.69% were having pain at the site of bite^[14]. Researchers at Malaysia observed 23.1% presented with 3.5% of cases presented with hemotoxicity and 13.5% with symptoms suggestive of neurotoxicity^[13].

In the cases taken in our study we observed that 38.3% patients reached hospital within 2-4 hours of snake bite. 21.4% seek medical treatment after 4 hours while 13.4% came after 6 hours of snake bite. Our findings differ from study done at Pakistan which found that 15% of victims reached hospital within 1 hour of the bite and 50% arrived and were treated within 1-3 hours; 25% within 3-6 hours and 10% arrived at hospital more than 6 hours after the bite while a study at Nepal found that 42% of the victims arrived at the treatment center within 2 hours of being bitten^[8,10]. The higher mortality in our present study could be attributed to decision delay, transportation delay and treatment delay which kills the valuable time making the victims more vulnerable. The commonest findings during postmortem were hemorrhagic kidney (58.5%) followed by pulmonary hemorrhages (53.7%). (Figure 4)

In elapid snake bites where the neurotoxic symptoms predominant the affection of vital centers of brain leads to respiratory paralysis and in such cases we have observed pulmonary hemorrhages and a local practice of giving small amount of water in drowsy patient lead to respiratory distress in snake bites victims could cause aspiration and pulmonary edema which was seen in 21.9% cases.

The older concept of the kidney being involved in most cases of vasculotoxic snake bites like the vipers is gradually yielding to recent research studies as undertaken by us where hemorrhagic kidney is observed in 19 cases out of 33 deaths due to neurotoxic bites (58.5% cases) and 5 cases out of 8

Figure 4: Internal PM Findings

deaths(62.5%) due to vasculotoxic bites.

Histo-pathological study of kidney reveals acute tubular necrosis in 39.02% cases, while combination of acute tubular necrosis and cortical necrosis was present in 14.63% cases. [Table-4] Earlier Indian studies states that bilateral renal cortical necrosis (BRCN) and less severe acute tubular lesions (ATL) occurs in kidney^[3,16].

Table 4 : Histopathology of Kidney Tissue

Type of bite by poisonous snake	Acute Tubular necrosis	Acute tubular necrosis+ cortical necrosis	Congested	Total
Neurotoxic	14	4	15	33
Vasculo-toxic	2	2	4	8
Total	16 (39.02%)	6 (14.63%)	19 (46.34%)	41

Our findings are similar to certain Indian studies where acute tubular necrosis and acute cortical necrosis were the most significant renal histopathological changes^[16]. This is a pointer to the fact that in spite of the neurotoxicity of the elapid group of snakes there is a significant renal involvement in such type of bites. It may be attributed to the renal tubules, the nephrons and renal cortex getting affected either by direct involvement of neurotoxins causing direct injuries to these tissues or hypotension induced shock resulting in injuries to renal tissue. There is evidence that renal blood flow diminishes substantially in toxic acute tubular necrosis, apparently as a result of arteriolar vasoconstriction.

CONCLUSION AND SUGGESTIONS:

Thus it is clear, the high rate of deaths during this study appeared to be due to non- recognition of the value of early presentation to tertiary care hospital attributed to prevailing traditional beliefs and customs and native treatment of snake bite by traditional healers thus causing the decision delay. Creating awareness in rural and tribal people, public education

proper training and skill in assessment of snake bite victims by peripheral doctors and health workers could play a pivotal role to decrease snake bite mortality.

REFERENCES:

- Jena I, Sarangi A. Snakes of Medical Importance and Snakebite Treatment, 1993. New Delhi: Ashish Publishing House,
- Bambery P. API Textbook of Medicine, 7th Edition, 2003. Mumbai: API.
- Pal M, Maiti AK, Roychowdhury UB, Basak S, Sukul B, Renal Pathological Changes in Poisonous Snake Bite. J Indian Acad Forensic Med, 32(1):19-21
- McNally SL, Reitz CJ. Victims of snake bite. A 5-year study at Shongwe Hospital, Kangwane, 1978-1982. S Afr Med J, 1987; 72(12):855-60.
- Lal P, Dutta S, Rotti SB, Danabalan M, Kumar A. Epidemiological Profile of Snakebite cases Admitted in JIPMER Hospital. Indian journal of community Medicine, 2001; 26(01): 1-3
- Halesha BR, Harshavardhan L, Lokesh A J, Channaveerappa PK, Venkatesh KB. A Study on the Clinico-Epidemiological Profile and the Outcome of Snake Bite Victims in a Tertiary Care Centre in Southern India. J Clin Diagn Res. 2013 Jan; 7(1): 122-126
- Nisar A, Rizvi F, Afzal M, Shafi MS. Presentation and complications of snakebite in a tertiary care hospital. J Coll Physicians Surg Pak, 2009; 19(5):304-7.
- Hayat AS, Khan AH, Shaikh TZ, Ghouri RA, Shaikh N. Study of snake bite cases at Liaquat University Hospital Hyderabad. Jamshoro J Ayub Med Coll Abbottabad, 2008; 20(3):125-7.
- Mohapatra B, Warrell DA, Suraweera W, Bhatia P, Dhingra N, Jotkar RM, Rodriguez PS, Mishra K, Whitaker R, Jha P; Million Death Study Collaborators.. Snakebite Mortality in India: A Nationally Representative Mortality Survey. PLoS Negl Trop Dis, 2011; 5(4): e1018.
- Sharma SK, Koirala S, Dahal G, Sah C. Clinico-epidemiological features of snakebite: a study from Eastern Nepal. Trop Doct, 2004; 34(1):20-2.
- Inamdar IF, Aswar NR, Ubaidulla M, Dalvi SD. Snakebite: Admissions at a tertiary health care centre in Maharashtra, India. S Afr Med J, 2010; 100: 456-458.
- Brunda G, Sashidhar RB. Epidemiological profile of snake-bite cases from Andhra Pradesh using immunoanalytical approach. Indian J Med Res, 2007; 125(5):661-8
- Chew KS, Khor HW, Ahmad R, Rahman NHK Abdul. A five-year retrospective review of snakebite patients admitted to a tertiary university hospital in Malaysia. International Journal of Emergency Medicine 2011, 4:41.
- Sudhir W, Rambhau G. Clinical Profile Of Snake Bite Cases in Marathwada, India. Indian Journal of Fundamental And Applied Life Sciences, 2011; 1 (4): 93-99.
- Nitesh P, Shantaram V. Clinical profile of snake envenomation – NIMS Experience. J. Association Physician India, 2001 ; 49: 57.
- Chugh KS, Pal Y, Chakravarty RN, Datta BN, Mehta R, Sakhuja V, Mandal AK, Sommers SC. Acute renal failure following poisonous snakebite. Am J Kidney Dis, 1984; 4(1):30-8.

Original Research Paper

Estimation of Stature and Gender from Percutaneous Tibial Measurements in population from North-West Uttar Pradesh.

1. **Somshekhar Sharma**, Assistant Professor, Department of F.M.T., Dr. D.Y. Patil Medical College, Navi Mumbai
2. **Rahul G Peddawad**, Associate Professor, Department of F.M.T., Dr. D.Y. Patil Medical College, Navi Mumbai
3. **Gurmanjit Maan**, Ex Professor and Head, Department of F.M.T., SRMS Institute of Medical Sciences, Bareilly, U.P.

ABSTRACT:

Stature estimation and gender determination is one of the prime tools for determining the identity in fragmented or mutilated remains. The present study aimed to assess the use of percutaneous tibial length in estimating stature and determining gender among a selected population in Bareilly, Uttar Pradesh. The study concluded that stature estimation is highly accurate with the measurement of percutaneous tibial length, but gender determination showed poor results in the selected population.

Corresponding Author

Dr. Rahul G Peddawad

Associate Professor,

Department of F.M.T., Dr. D.Y. Patil Medical College, Navi Mumbai Contact : +91

E-mail : rahul.peddawad@dypatil.edu

Article History:

Received: 10 November 2018

Received in revised form: 16 November 2018

Accepted on: 16 November 2018

Available online: 1 June 2019

KEYWORDS : Stature, Gender, Tibial Length

INTRODUCTION:

Anthropometry is a systematic study of measurements on man and it involves scientific techniques for taking various measurements and somatic observations on the living man. Identification of an unknown body and prediction of growth from specific body measurements are very important tasks in the fields of physical anthropology and forensic medicine. Height and weight are two factors among others required to establish individuality of an unidentified body^[1].

Identification of an unknown body of a person who died in mass disasters, wars, homicide, suicide or accidental deaths is a very important task in the fields of physical anthropology and forensic medicine. Estimation of the stature or the height is one of the steps for the identification^[2].

When any foot or arm or other body parts are recovered at scene of crime, their dimensions can provide valuable information about the height and sex of the body. There is a statistically significant correlation based on a relationship between body length and body segment which can be calculated using various statistical equations to estimate living height and sex. These dimensional relationships have been of interest to many artists, scientists, anatomists, anthropologists and in medico legal case work over many years^[3].

Gender determination from fragmented bodies is primarily based on pelvic morphology, or is determined by visual inspection of genitals, if present. In cases where partial skeletal remains are recovered, an accurate determination of gender may prove an uphill task due to features of sexual dimorphism

or due to absence of a community specific formula. Several studies have been conducted to provide for a specific method to determine gender from post cranial measurements. A region specific study is essential to determine if a particular ethnic group has been considered while formulating the equation for estimating gender or stature.

Several studies conducted in the past by a slew of western researchers have proved that different ethnic groups within the same geographical region have different anthropometric markers and the same can be said about differences among the two genders^[4,5,6].

The present study was done to determine the feasibility of applying regression formulae in estimating gender and stature from percutaneous tibial length in a specific population.

MATERIAL AND METHODS:

The present study was a cross sectional, observational study conducted among subjects from various institutions of Shri Ram Murti Smarak Trust. The study availed a sample size of 300 individuals with 149 males and 151 females. Sample size was calculated with SPSS software allowing a hypothesis with a confidence interval of 95 %. The resultant sample size was then taken in the study to provide an adequate and statistically significant data collection. An attempt was made to collect same number of samples from both genders. The study was conducted between December 2015 to December 2016, in a period of one year.

The tools used in the study were an anthropometric scale (Stadeometer) for determination of stature, blunt end

spreading calipers for measurements, Stationary and markers.

All the subjects were chosen as per the inclusion criteria as mentioned below and voluntary, informed, written consent was obtained prior to collection of anthropometric data.

Approval from Institutional Ethical committee was obtained prior to commencement of the study/

INCLUSION CRITERIA:

1. Adult males and females capable of giving informed consent.
2. Age between 20 years and completed 30 years only.
3. Residing in Bareilly.
4. Apparently healthy individuals were selected as subjects.

EXCLUSION CRITERIA:

1. Age below 20 years and above 30 years.
2. Non-residents of Bareilly.
3. Individuals with spinal deformities, H/O surgeries/deformities affecting stature or Lower limb measurements such as malformed fractures, kyphosis/scoliosis, any other congenital or acquired abnormalities.

OBSERVATIONS:

In the combined study of all subjects, it was noted that the total sample size of 300 exhibited no missing data entries. The maximum stature as recorded was 181 cms while the other end of the spectrum was 133 cms. Percutaneous tibial length was a maximum recorded value of 52.10 cms and minimum value as recorded was 37.70 cms respectively with a mean value of 43.85 cms.

Statistical analysis of the stature and percutaneous tibial length of the entire sample population including both genders and all age groups showed a Pearson co relation to be significant at 99 % confidence interval. (Table 1)

Table 1 : Pearson Correlation for PCTL in entire population.

Parameters		Stature	PCTL
Stature	Pearson Correlation	1	.832**
	Sig. (2-tailed)		.000
	N	300	300
PCTL	Pearson Correlation	.832**	1
	Sig. (2-tailed)	.000	
	N	300	300

Statistical analysis of the stature and Percutaneous Tibial Length (PCTL) of the sample population of male and female

population groups showed a Pearson co relation to be significant at 99 % confidence interval. This means that there was a statistically significant co relation between stature and the measurements of PCTL of male and female population as a whole without any segregation of age. (Table 2)

Table 2 : Gender Distribution and Co-relations with Percutaneous Tibial Length (PCTL).

Sex			Stature	PCTL
Male	Stature	Pearson Correlation	1	.816**
		Sig. (2-tailed)		.000
		N	149	149
	PCTL	Pearson Correlation	.816**	1
		Sig. (2-tailed)	.000	
		N	1	149
Female	Stature	Pearson Correlation	.491	.737**
		Sig. (2-tailed)		.000
		N	151	151
	PCTL	Pearson Correlation	.737**	1
		Sig. (2-tailed)	.000	
		N	151	151

** . Correlation is significant at the 0.01 level (2-tailed).

Linear regression analysis of data of percutaneous tibial length (PCTL) of females and stature revealed an equation as derived by simple linear regression analysis as follows:

$$Y = 0.2021(X) + 8.2859$$

Where Y= Stature of Individual.

X = Percutaneous Tibial Length

R² value of the equation was 0.35332 at 95 % CI.

Linear regression equation as depicted above has been formulated with a R² value of 0.35332. This denotes that the predictive value is significant but not highly significant in determining the variable component i.e. stature in this case from the definitive component i.e. Percutaneous tibial length in females of the study population.

Linear regression analysis of data of percutaneous tibial length (PCTL) of males and stature revealed an equation as derived by simple linear regression analysis as follows:

$$Y = 0.2897(X) - 3.0338$$

Where Y= Stature of Individual.

X = Percutaneous Tibial Length

R² value of the equation was 0.6384 at 95 % CI

Linear regression equation as depicted above has been formulated with a R² value of 0.6384. This denotes that the

predictive value is highly significant in determining the variable component i.e. stature in this case from the definitive component i.e. Percutaneous tibial length in males of the study population.

Based on the R^2 values of the two regression equations, it was determined that PCTL is a more effective marker for stature in males as compared to females, in the studied population group.

A binary simple logistic regression analysis was done for assessment of gender determination by Percutaneous Tibial Length (PCTL) for the data collected from the sample population, which revealed the following equation,

$$-47.575 + 2.026 \times (C) = D$$

where C = Percutaneous Tibial Length

D = Male Gender if Positive, Female gender if negative

The results of the equation for Percutaneous Tibial Length were that if the value is negative, it has a probability for being of female gender, while positive value has more probability for male gender of the population group under study.

- Efficacy of Percutaneous Tibial Length in prediction of Gender

		Predicted		
		Sex		Percentage Correct
		Male	Female	
Sex	Male	28	121	18.79%
	Female	42	109	27.81%
Overall Percentage				24.3%

R^2 value = 0.425 (Cox and Snell Method)

The prediction and evaluation of efficacy were calculated by comparison of recorded / observed values with values derived after formula substitution in SPSS software. The values upto second decimal point were included in the comparison. Based on the above results, it can be reported that Percutaneous tibial length is a poor predictor of gender in both gender subsets as well in the overall population group under study.

DISCUSSION:

The mean values of stature in our study were found to be 159.9 +/- 10.01 cms among both the sexes. In males it was found to be 163.46 +/- 6.354 cms while in females it was 153.27 +/- 4.232 cms. The above values are in concordance with the mean values by Twisha Shah, ArunAgnihotri and A Kaore, who reported similar ranges with different standard deviations among Indian subjects^[7,8,9].

In terms of gender distribution, a statistically significant correlation was found between stature and values of percutaneous tibial length among both males and females of

the study population. The correlation measured by means of P value was found to be highly significant in males, while correlation was significant among females. This indicates that the values of percutaneous tibial length among males are very good predictors of stature. This is in concurrence with the studies by ArunAgnihotri et al, Meligy et al, Mohanthi N K, Mysorekar and Nandedkar, Kaore A and Vijayamahantesh V also revealed statistically significant correlation among both genders, while stating that males showed better correlation compared to females^[8-13].

Our study is not in concurrence with studies by AbdiOzsalasn, AltayabAA, A Trivedi and Anitha M R, in the fact that the above authors reported highly significant P values among both the genders with stature and percutaneous tibial length. The probable reasons could be the differences in location, population sample size or socio-economic factors affecting the population^[14-17].

In terms of the regression equations, no equation holding similar value of Coefficients was found in studies by previously mentioned authors. This is in concordance with conclusions by Twisha Shah, Mohanthi N K and Anitha M R, who stated that population specific studies are needed as population groups may show different regression equations.^[7,13,16]

In estimating gender, the scenario was unencouraging as far as percutaneous tibial length was considered. In determining gender, percutaneous tibial length was a poor predictor with less than 60 % correct outcomes on using tibial length alone. Our study concurs with various studies by AltayebAbdalla Ahmed et al and Christian Armah et al in the fact that tibial length is a poor predictor of gender^[14,18].

CONCLUSION:

There are a variety of studies that enumerate relationships between stature and percutaneous measurements of the body. The studies were unanimous in stating that a relationship exists that may or may not conform to the devised regression equations as derived by our study, however every author(s) has mentioned an equation that aids in delineating the stature.

Authors from various regions of the country and abroad have attempted to discern an equation that may be applicable to the general population or even universally, but the equations cannot counter the demographic and racial diversity and provide for a singularity that may prove applicable in proper effect to the whole population. Thus it was decreed by a variety of review papers and original articles that it is indeed the need of the day to enhance studies at a community or region level and formulate equations that cater to the specified demography or population subgroup.

LIMITATIONS: The study is limited in its small sample size and lack of applicability in entire population subset of the region.

Source of Funding: Self

Conflict of Interest: Nil

REFERENCES:

1. Iscan MY. Anthropometry. In: J. Siegel, P. Saukko, G. Knupfer, editors. Encyclopedia of forensic sciences, London: Academic Press; 2000. p.284-290.
2. Golda, Stephanie (2010). "A Look at the History of Forensic Anthropology: Tracing My Academic Genealogy". Journal of Contemporary Anthropology. 1 (1).
3. Singh S, Potturi BR (1978). Greater Sciatic notch in sex determination. J Anat 125: 619-624.
4. Rogers TL (2005). Determining the sex of human remains through cranial morphology. J Forensic Sci 50: 493-500.
5. Hinkes, MJ (July 1989). "The role of forensic anthropology in mass disaster resolution". Aviat Space Environ Med. 60: 60-3
6. Spradley MK, Jantz RL (2011) Sex estimation in forensic anthropol- ogy: skull versus postcranial elements. J Forensic Sci 56:289-296
7. Twisha Shah, Patel MN, Nath S, Shobhana K Menon (2015) A Model for Construction of Height and Sex from Shoulder Width, Arm Length and Foot Length by Regression Method. J Forensic SciCriminol 3(1): 102. doi: 10.15744/2348-9804.2.402.
8. Arun Kumar Agnihotri, SmitaKachhwaha, VandnaJowaheer, Ashok Pratap Singh. Estimating stature from percutaneous length of tibia and ulna in Indo-Mauritian population. Forensic Science International 187 (2009) 109.e1-109.e3
9. Kaore A et al. Stature Estimation From Tibial Length NJIRM 2012; Vol. 3(2). April-June: 51-56.
10. Mysorekar, V.R., Nandedkar, A.N. and Sarma, T.C.S.R., 1984. Estimation of stature from parts of ulna and tibia. Medicine, Science and the Law, 24(2), pp.113-116.
11. Manal M.S. El-Meligy ,Randa H. Abdel-Hady, Ragaa M. Abdel-Maaboud , Zaghloul T. Mohamed. Estimation of human body built in Egyptians. Forensic Science International 159 (2006) 27-31.
12. Vijayamahantesh S N, Vijayanath V. Determination of Height from Percutaneous Tibial Length among Bagalkot Population. Indian Journal of Forensic Medicine& Toxicology. January-June., 2013, Vol.7, No. 1 . Pg. 221-222.
13. N. K. Mohanty, "Prediction of height from percutaneous tibial length amongst Oriya population," *Forensic Science International*, vol. 98, no. 3, pp. 137-141, 1998.
14. AbdiOzaslan, BeytullahKarada, MelekOzlemKolusayin, Ahsen Kaya. Stature estimation from bi-acromial and bi-iliocrisal measurements. Rom J Leg Med [19] 171-176 [2011].
15. AltayebAbdalla Ahmed. Estimation of sex from the lower limb measurements of Sudanese adults. Forensic Science International 229 (2013) 169.e1-169.e7
16. Anitha MR, Bharathi D, Rajitha V, Chaitra BR. Estimation of height from percutaneous tibial length among South Indian population. Ind J of ClinAnat and Physiol, October-December 2016;3(4);405-407.
17. TrivediA ,Saxena S ,Morya R , Jehan M , Bhadkaria V . Stature Estimation Using PerCutaneous Tibial Length in People of Gwalior Region. IOSR Journal of Dental and Medical Sciences, .Volume 13, Issue 5 Ver. I. (May. 2014), PP65-70.
18. Christian Armah, Chrissie StansieAbaidoo, Joshua Tetteh, Thomas Diby, ObedOhene-DjanAtuahene, Nancy Darko& Atta KusiAppiah (2017): A preliminary anthropometric study of height and sex determination using percutaneous humeral and tibial lengths, Australian Journal of Forensic Sciences, DOI: 10.1080/00450618.2016.1257065.

Original Research Paper

Clinico-Epidemiological Profile of Snake Bite Patients in a Tertiary Healthcare Centre in South India

1. **Vrinda Lath**, Undergraduate Student, Kasturba Medical College, Manipal, Manipal University, India
2. **Shankar M Bakkannavar**, Associate Professor, Department of Forensic Medicine and Toxicology, Kasturba Medical College, Manipal, Manipal Academy of Higher Education
3. **Ranjith Immanuel James**, Assistant Professor, Department of Forensic Medicine and Toxicology, Christian Medical College, Vellore, India
4. **Pradeep Kumar G**, Professor, Department of Forensic Medicine and Toxicology, Melaka Manipal Medical College, Manipal, Manipal University, India

ABSTRACT:

Introduction: Snake bite is a common medical emergency affecting the rural poor. India has the highest rates of envenomation with 81,000 envenomation and 11,000 deaths. Such high figures in spite of availability of antivenin is a cause for concern. The availability of medical records of the victims of snake bite enabled the analysis of the factors contributing to such high mortality and morbidity associated with snake bite.

Aims and Objectives: To study the prevalence of poisonous and non-poisonous snake bites in the part of Coastal Karnataka with reference to age, sex, occupation, part of body bitten, time of bite and seasonal variation. To know the systemic envenomation of various types of poisonous snakes and their effective management in reducing the mortality and morbidity rate. To have a common knowledge of the types of poisonous snakes common in this locality and their clinical manifestations. To study the socio-economic impact of snakebite cases in the region.

Results: A total of 454 case records were analyzed from 2010 to 2014. 9.69% of the victims died. Male to female ratio was 2.19:1. The highest incidence was between the ages 31 and 40 which was 19.82 % (n=90). Fang marks were recorded in 45.38% of the cases. 16.9% of them reported to a hospital within an hour of the bite. 33.03% reported within 24 hours of the bite. In 65.41% of the cases, the site of snake bite was found to be the lower limb. 23.8 % (n=50) of the identified snakes were cobras. 66.66% (n=140) were vipers. 28.64% of the bites occurred between 18:00 hours and 0:00 hours. 33.92% (n= 154) of the incidents occurred outdoors. Ayurvedic medication was given in 38.23% while a tourniquet was tied in 29.41% of cases. 15.19% of the cases had haemotoxic manifestations while 18.9% had neurological manifestations. 77.5% had local reactions.

Conclusion: Snake bite is an important occupational hazard that causes preventable death and disability. Delay in hospitalization, use of traditional medicine and infection are factors contributing to significant mortality and morbidity. Awareness regarding outdoor safety and early hospitalization can significantly reduce the incidence of snake bite.

Corresponding Author

Dr. Shankar M Bakkannavar,

Associate Professor,

Department of Forensic Medicine and Toxicology, Kasturba Medical College, Manipal, MAHE

Contact : +91 9110240992

E-mail : shankar.mb@manipal.edu

Article History:

Received: 22 December 2018

Received in revised form: 3 January 2019

Accepted on: 3 January 2019

Available online: 1 June 2019

KEYWORDS : Key words: Snake bite; medical emergency; envenomation; Ayurvedic medication; West Costal region

INTRODUCTION:

Snake bite is a cause of significant mortality and morbidity in tropical regions. A medical emergency, it often requires intensive care¹. Snake bite was added to the list of neglected tropical diseases by the WHO in 2007². More than 5 million people are bitten by snakes worldwide, per year³. Of these, around 4, 21,000 envenomings occur, resulting in 20,000 deaths⁴. It is estimated that the number of envenomings could be as high as 1.8 million and deaths 94,000⁴. About 4, 00,000

are left with permanent disabilities³. These figures were found to be conservative when compared to official records⁵. A major hurdle encountered in providing accurate estimates is the low hospital attendance. Not many victims seek treatment at hospitals and prefer traditional healers¹.

India has the highest annual number of envenomings, which is estimated to be 81,000 and 11,000 deaths⁴. Rural India, most of all, bears a high burden of snake bite⁶. People belonging to the lower socio-economic strata are most impacted, especially

those who are engaged in agriculture and forestry⁶. Bites due to unskilled handling of snakes have also been reported.

In India, four venomous snakes are most often encountered. Known as “the big four”, they are the Indian Cobra or Common Cobra (*Naja naja*), Russell's viper (*Daboia russelli*), Saw scaled viper (*Echiscarinatus*) and Common krait (*Bungarus caeruleus*)⁷. Polyvalent Anti-Snake Venom (ASV) is available for these four snakes. Besides being home to the “big four”, Karnataka is also home to some other highly venomous snakes, notably the King Cobra (*Ophiophagus hannah*), Malabar pit Viper (*Trimeresurus malabaricus*) and the Hump-nosed pit Viper (*Hypnale hypnale*)⁷. Mortality rates remain high in spite of availability of polyvalent Anti-snake venom (ASV). The lack of trained medical professionals and scarcity of health care facilities, delay in hospitalization and preference for traditional healers are some factors that contribute significantly to the disability and death associated with snake bite. A study revealed that less than 40% of the victims reported to public hospitals⁸. Traditional methods of treatment and popular first aid methods have been found to be ineffective and even dangerous¹.

Besides causing death, Snake bite also results in severe disability and sequelae such as infection, tetanus, scarring and contracture^{1,2}. Also to be considered with the high morbidity are the psychological sequelae associated with disability and the high financial burden. Often snake bite may require prolonged duration of hospitalization and intensive care¹.

Kasturba Hospital, Manipal, is a tertiary care center located in the town of Udupi in coastal Karnataka. Patients from the coastal region as well the Western Ghats present to this hospital. The location and the availability of the medical records makes this center ideal to study the incidence of snake bite and the factors that contribute to the high mortality and morbidity associated with the disease.

MATERIALS AND METHODS:

The 5 years (2010 – 2014) retrospective study was conducted at a tertiary care teaching hospital in west coast of South India. All the snake bite cases admitted during the study period were analyzed. The Institutional Ethical Committee approval was obtained to access the data from Medical Records Section of the Hospital. A detailed information regarding demographic and epidemiological parameters such as age, sex, residence, occupation, site of bite and place of bite, time and month of bite, type of snake (if identified), any first aid received outside, time to reach the hospital, etc., were obtained from the medical records. The clinical examination details like presenting symptoms, signs and complications arising out of snakebite, period of survival (if the patient had died) were noted. The details regarding the physicians' opinion on identification of

type of snake bite (Vasculotoxic, Neurotoxic, musculotoxic and Non-poisonous) were recorded. In case of death and subsequent post mortem (PM) examination, the details of PM examination were collected from the PM reports, Police Inquest and Police requisition letters.

The data collected was tabulated in Microsoft Excel and analyzed using descriptive statistics by Statistical Package for Social Sciences (SPSS) version `16 using percentage.

RESULTS:

A total of 454 cases of snake bite were analyzed from the year 2010 to 2014. Out of total cases, 9.69% of the victims did not survive. The male to female ratio was 2.19:1. The highest incidence in both groups was in the age group of 31-40 (n=90) while among the males it was between the ages of 21-30 which was 21.79% (n=68) followed by age 31-40 which was 19.87% (n=62). Among women the largest number of victims were found in 5th and 6th decades amounting to 22.53% each. 6.6% of the victims were below the age of 10. As shown in **Table no. 1**, 28.19% of the victims were engaged in agriculture. 18.28% were engaged in manual labour followed by students (13.43%), homemakers (23.12%) and others accounting to 14.78% were salaried or people engaged in business, unemployed or children. In 54.62% of the cases, fang marks were not visible while in 20.48% of the cases, a single fang mark and in 22.9% of cases 2 fang marks were found. 1.54% of the victims were found to have 3 fang marks and 0.4% were found to have multiple fang marks. Of the 454 victims who reported to the hospital, 16.9% of them reported to a hospital within an hour of the bite. 33.03% reported within 24 hours of the bite. 7.7% of the victims reported after 48 hours. In 65.41% of the cases, the site of snake bite was found to be the lower limb of which 58.24% were over the feet and 13.13% over the toes. The site was the upper limbs in 25.99% of the cases of which in 55.08% of the site was the fingers. The site of snake bite was over the head and face in 0.44% and the trunk in 0.66% of the cases. Among the males, 25.64% (n=80) of the bites occurred over the upper limb while among the females, 26.76% were over the upper limb. The site of bite was not recorded in 5.2% of the cases. **Table no. 2** shows the cases based on months of occurrence of the bite. 42.73% (n=194) of the bites occurred during the months of October- January followed by June-September (29.07%) and February-May (25.77%). Distribution of cases based on time of incident is shown in **Table no. 3**. The total of 28.64% of the bites occurred between 18:00 hours and 0:00 hours and 27.10% of the bites occurred between 12:00 hours and 18:00 hours. 6.38% of the bites occurred between 0:00 hours and 6:00 hours. Among the males, 30.14% of the bites occurred between 18:00 hours and 00:00 hours. Among females, 32.4% of the bites occurred

between 12:00 hours to 18:00 hours. In 56.17% of the cases the place of the incident was not recorded as shown in **Table no. 4**. 33.92% (n= 154) of the incidents occurred outdoors. 9.91% (n=45) of the bites occurred indoors. 34.30% of the male victims and 33.10% of the female victims were bitten outdoors. 8.01% of the male victims and 14.9% of female victims were bitten indoors. As shown in **Table No. 5**, the snakes were identified in (n=210) 46.25% of the cases. 23.8 % (n=50) of the identified snakes were cobras. 66.66% (n=140) were vipers. Two were identified as Malabar pit vipers and one as Russell's viper. 6.66% (n=14) were kraits of which one was identified as a Common Krait and one as a Banded Krait. Among the non-venomous snakes identified, one was a Python and the other was a Checkered Keelback. The details about first-aid given or not and its type has been shown in **Table no. 6**. Administration of first aid was documented in 68 cases. The type of first aid given was not documented in 2 cases. Ayurvedic medication was given in 38.23% while a tourniquet was tied in 29.41% of cases. 25% of the victims were treated by a native practitioner while 7.35% used traditional medicines. When the presenting symptom was considered as shown in **Table No. 7**, pain was present in 47.13%, and swelling in 64.97% of the cases. In 77.5 % (n=352) local reaction was present in the form of pain, swelling, ulceration etc. 15.19% of the cases had haemotoxic manifestations while 18.9% had neurological manifestations. 1 patient presented with photophobia. 4 patients presented with dysarthria. The system involved is shown in **Table No.8**. The complications secondary to snake bite are shown in Table No. 9. 30.39% of the victims had cellulitis. Acute Kidney Injury was recorded in 20.26% of the cases. Sepsis and septic shock occurred in 6.14% and 4.18% of the victims. Myocarditis and encephalopathy were noted in 1.10% of the patients. There were 2 case of rhabdomyolysis and purpura fulminans. 1 case of Hemolytic Uremic Syndrome was recorded.

Table 1 : Distribution of Cases Based on Occupation

Occupation	%
Agriculture	28.19
Manual labour	18.28
Student	13.43
Housewife	23.12
Others	14.78
Not recorded	2.2

Table 4: Distribution of Cases Based on Place of Incident

Place of bite	Male	Female	Total
Indoors	25 (08.01%)	20 (14.09%)	45 (09.91%)
Outdoors	107 (34.30%)	47 (33.10%)	154 (33.92%)
Unknown	180 (57.69%)	75 (52.81%)	255 (56.17%)
Total	312 (100%)	142 (100%)	454 (100%)

Table 2 : Distribution of Cases Based on Season

Months	Sex		Total
	Female	Male	
June - September	41	91	132
February - May	37	80	117
October - January	61	133	194
Month not recorded	3	8	11
Total	142	312	454

Table 3: Distribution of Cases Based on Time of Incident

Time	Male	Female	Total
12 am-6am	21 (06.73%)	08 (05.64%)	29 (06.38%)
6am-12 noon	61 (19.55%)	27 (19.01%)	88 (19.38%)
12 noon-6pm	77 (24.67%)	46 (32.40%)	123 (27.10%)
6pm-12am	94 (30.14%)	36 (25.35%)	130 (28.64%)
Not known	59 (18.91%)	25 (17.60%)	84 (18.50%)
Total	312 (100%)	142 (100%)	454 (100%)

Table 5 : Type of snake

Snakes	Sex		Total
	Female	Male	
Banded Krait	1	0	1
Common Krait	6	7	13
Cobra	21	29	50
Russell's viper	38	100	138
Malabar pit viper	0	2	2
Checkered keel back	0	1	1
Python	0	1	1
Unidentified	76	172	248
	142	312	454

Table 6: Details about First Aid Management

Type of First-aid	Sex		Total
	Female	Male	
Local Medication	0	1	1
Ayurvedic Medicine	10	14	24
Type of first aid not known	0	2	2
Local Medication	2	2	4
Native Practitioner	6	11	17
Tourniquet	6	12	18
Tourniquet + Ayurvedic medication	0	2	2
No details about first aid	118	268	386
	142	312	454

Table 7: Presenting Symptoms

Presenting Symptom	Number	%
abdominal pain	20	4.40%
chest pain	5	1.10%
Local pain	214	47.13%
swelling	295	64.97%
vomiting	54	11.89%
seizure	6	1.32%
hematemesis	17	3.74%
melena	6	1.32%
bleeding from oral cavity	15	3.30%
bleeding gums	7	1.54%
hemoptysis	4	0.88%
ptosis	14	3.08%
breathlessness	17	3.74%
Loss of consciousness	15	3.30%
hematuria	3	0.66%
ecchymosis	14	3.08%
Decreased urine output	34	7.48%
discoloration	46	10.13%
respiratory distress	2	0.44%
blurring of vision	9	1.98%
diarrhea	7	1.54%
ulcer	24	5.28%
necrotic patch	5	1.10%
fever	15	3.30%
paralysis, paresis	8	1.76%
altered sensorium	7	1.54%
rash	5	1.10%

Table 8: System Involved

System involved	Number	%
local reaction	352	77.53%
Haemotoxic manifestations	69	15.19%
Neurological manifestations	86	18.94%
Gastro-Intestinal disturbances	54	11.89%
Renal disturbances	34	7.48%
Cardiac manifestations	12	2.64%

DISCUSSION :

Snake bite, as stated by the WHO, is one of the most neglected diseases that affects mainly the rural poor⁶. In spite of the fact that it is preventable and treatable, it continues to have a very high burden owing to lack of timely intervention and management. The factors contributing to such a high burden have been analyzed. In a total of 454 cases, the clinical features and epidemiological factors including age wise distribution,

Table No. 9: Complications

Complication	Number	%
Gangrene	21	4.62%
Cellulitis	138	30.39%
Acute kidney injury	92	20.26%
Sepsis	28	6.16%
Septic shock	19	4.18%
Cardiac arrest	10	2.20%
Respiratory failure	4	0.88%
Paralysis	3	0.66%
Multiple organ dysfunction syndrome(MODS)	15	3.30%
Disseminated Intravascular Coagulation (DIC)	17	3.74%
Coagulopathy	13	2.86%
Compartment syndrome	22	4.84%
Encephalopathy	5	1.10%
Myocarditis	5	1.10%

location and time of incident, the presence of fang marks, time to present to the hospital, clinical features and complications were studied. 72.90% of the victims were male while 27.1% were females. The male to female ratio was 2.19:1. The ratio obtained in the present study was found to be higher than that obtained in the National Representative Mortality Survey (1.4:1)⁵ as well as a similar study conducted in Hassan, Karnataka (1.5:1)⁹. In a study conducted by Sharma et al¹⁰, the male to female ratio was 4.25:1. 9.69% of the victims died. The study conducted in Hassan, Karnataka⁹, found the mortality to be 3.8%. Of the patients studied in Ahmedabad, Gujarat, only three died¹¹ while in the study conducted in Jewahar Institute of Postgraduate Medical Education and Research (JIPMER), Pondicherry¹², the mortality was 13.5%. The higher mortality was attributed to delay in hospitalization due to distance and opting for traditional medicine before presenting to the hospital¹². This may be applicable to the present study as well. The mortality rate due to Snake bite in Karnataka is high⁵. The result of the present study may be influenced by the fact that, being a tertiary care center, already under treatment and complicated cases are referred here. The mortality may be higher as some of the patients were discharged against medical advice due to financial constraints and could not be followed up. The highest incidence was found to be in the age group of 31-40 while among males, the highest was in the age groups of 21-30 which was 21.79% (n=68). Among females, the highest number of victims was between the age groups of 41-60 which was 22.53% each. According to the study conducted in Hassan District, Karnataka, South India⁹, the males comprised 60.5% of the victims and were mostly between the ages of 20-40.

48.62% of the males were in the age group of 20-39. While 70.42% of the female victims were in the age group of 20-49, the largest number of female victims was in the age group of 20-29 years (25.35%). The age groups with the highest incidence belong to the working age group. This indicates a high social and economic burden on the victims' family as well as the society. In the present study, 28.19% of the victims were engaged in agriculture. 13.43% were students and 23.12% were housewives. The results of the present study correspond to the results of the national representative survey⁵. In the study conducted in Gujarat, 53.40% of the victims were farm laborers and 17.80% were farmers¹¹. Venomous snakes including the Cobra, Krait and Viper prefer to enter agricultural land where prey (frogs and rodents) is abundant. This increases the likelihood of farm workers encountering these snakes. The presence of fang marks was not detected or recorded in 54.62% of the cases. The fang marks, when detected, ranged from one (20.48%) to multiple (0.4%) though predominantly two fang marks were found (22.9%). In the study conducted in Ahmedabad, Gujarat, bite marks were seen in 70% of the cases^[11]. The reason for non-detection of fang marks may be due to local distortion of the skin as a result of inflammation or ulceration and in some cases, the fang marks may be concealed, as is known to occur in Krait bites¹. The site of snake bite was found to be the lower limb in 65.41% and upper limb in 25.99% of the cases. A very small number of patients were bitten over the face, neck and trunk. This is in concurrence with other studies where the site of bite was predominantly the lower limb^{9-11,13}. The largest percentage of bites have occurred over the lower limbs, mostly over the feet. These have occurred mostly when the victims were walking. Some bites occurred after the victim stepped on the snake. The bites over the upper limb mostly occurred while handling vegetation or during unskilled handling of snakes. A very small percentage of victims were bitten over the face, neck or trunk. 16.9% of the victims reported to a hospital within an hour of the bite. 33.03% reported within 24 hours of the bite. This corresponds to the results of the study conducted in Ahmedabad, Gujarat¹¹ where 14% of the victims received treatment within an hour of the incident and 64.84% of the victims, were treated within 6 hours of the bite. Similar results were found in the study conducted in Hassan, Karnataka, South India⁹. The higher rate of early reporting can be attributed to better awareness and education among the communities. 7.7% of the cases reported over 48 hours after the bite. The delay in hospitalization may be due to poor access to medical facilities, financial constraints, use of traditional medicines, native practitioners or lack of education. Ayurvedic medication was given in 38.23% of the cases in which first aid was documented. A tourniquet was tied in 29.41% of cases.

25% of the victims were treated by a native practitioner while 7.35% used herbal medicine in the form of roots and leaves. According to the study conducted in Ahmedabad, Gujarat, North India¹¹ First aid given was in the form of application of tourniquet (16.2%), local application of lime, chillies, herbal medicine, etc., (1%). The application of a tourniquet has been found to be a popular method of first aid¹¹. The use of a tourniquet is no longer recommended as it may result in limb ischemia and gangrene, as was found in one case. Other methods of first aid such as incision and application of herbal medication are also ill-advised¹. The snakes were identified in (n=210) 46.25% of the cases. 23.8% (n=50) of the identified snakes were cobras. 66.66% (n=140) were vipers. Two were identified as Malabar pit vipers and one as Russell's viper. 6.66% (n=14) were kraits of which one was identified as a common krait and one as a banded Krait. Among the non-venomous snakes identified, one was a python and the other was a checkered keel back. The results are consistent with other studies conducted in other regions in the vicinity. The percentage of viper bites was 60% in the study conducted in Hassan, Karnataka, South India⁹. A study conducted in Maharashtra, Central India found that 55.26% of the venomous snakes were vipers while 27.63% were neuroparalytic (elapid)¹⁴. In this hospital, the snake is identified based on the history given by the patient or the clinical manifestations. In some cases the snake is brought to the hospital. In this study, in many cases (53.5%), the species of snake could not be identified. Of all the viperine bites, one has been attributed to the Russell's viper and two to the Malabar Pit Viper. Of the Krait bites, one was reported as a common krait while one was reported as a Banded Krait. The type of snake is often not identified as the snake was either not seen or not identified by the victim. In dry bites or patients presenting with sepsis and shock, the clinical features cannot be used to identify the type of snake. Accurate identification of the snake where possible is important as it influences the administration of antivenin as well as for documentation. One of the snakes has been reported as a Banded Krait which is outside the geographic distribution of that snake which is normally found in the North Eastern States⁸. In this study, the time of bite was recorded in 81.50% of the cases and 55.74% of the bites occurred during the daytime, 28.64% occurring between 6pm and 12 am while 6.38% of the bites occurred between 12am and 6am which is in agreement with the study conducted by Anjum A, Husain M, Hanif SA, Ali SM, Beg M, et al.(30.2%)¹⁵. This may be due to non-usage of flashlights when outdoors. A similar result was obtained in the study conducted in Hassan, Karnataka, South India⁹ while in the study conducted in Ahmedabad, Gujarat, North India 61.2% of the bites occurred at night or early morning¹¹. This difference may be related to the time of outdoor activity.

Bites that occur late at night and early in the morning may be during open sanitation which is highly prevalent in rural India, especially in the north. Elapid bites have been reported to be more common in the North while viperine bites are common in the South¹⁶. Elapids (cobras and Kraits) actively seek out prey therefore may cross people while moving at night while Vipers are am bush predators. This behavior, associated with outdoor activity and changing forest cover may influence these patterns. The largest number of bites occurred in the months of October-January (42.73%), which corresponds to winter season. This finding differs from that of the study conducted in Hassan, where the peak incidence was found to be between July and September⁹. The National Representative Survey also reports peak incidence between July and September which corresponds to the monsoon⁶. Other studies have found the peak incidence to be corresponding to monsoon months^{10,13}. In the North, the incidence is low during the winters as snakes hibernate, whereas they are active throughout the year in the South. The peak incidence in this study corresponds to the autumn paddy harvesting period which falls between October and January¹⁷. A large part of agricultural land is used for cultivation of paddy¹⁷. There is also an abundance of prey (rodents) during the harvest season. The incidence may also be related to human outdoor activity. The list of explanations is not exhaustive and warrants further investigation. The place of bite and circumstances were recorded in 43.83% of the cases. A majority of cases occurred outdoors (33.92%) while only around 9.91% of the bites occurred indoors. A larger percentage of female victims (14.9%) were bitten indoors. This is in concordance with the study conducted in Hassan, South India⁹, where (n=149) 82.7% of the victims were bitten outdoors. Most of the bites have occurred outdoors while the victims were working in fields, walking or playing. Some of the indoor bites were Cobra bites which occurred in the bathroom. Sleeping on the floor puts people at risk of Elapid, especially Krait bites. The most common presenting symptom was a local reaction (77.53%) which includes signs of local inflammation such as pain, swelling, local rise of temperature or tenderness which is in agreement with the study conducted in Hassan, South India⁹ (58.89%). In a study conducted in Jharkhand, North India (49%) of the cases presented with local signs of inflammation¹³. This difference may arise due to the higher number of krait bites in the North as compared to the south. Krait bites are known to cause minimal local inflammation¹. 18.9% of the patients had some form of neurological manifestations such as ptosis, respiratory or cardiac manifestations, paresis or paralysis. This symptoms are seen in Elapid bites although they were noted in some viperine bites as well. The high percentage of neurological manifestations may

be due to anxiety which may result in alarming symptoms such as loss of consciousness or vasovagal syncope¹. Pre-paralytic symptoms such as vomiting, drowsiness and blurring of vision are typical of krait bites¹⁹. 15.19% had hematological abnormalities such as bleeding from gums, haematuria, bleeding from the oral cavity or ecchymotic patches. A case of subconjunctival haemorrhage was also recorded. Haemotoxic manifestations are typical of viperine bites¹. 7.14% developed symptoms suggestive of renal involvement such as decreased urine output. This was mainly recorded in case of viperine bites however, there are reports of renal symptoms in elapid bites. 11.8% of the patients had episodes of vomiting while 4.4% of the patients complained of abdominal pain. These symptoms were noted in both viperine and elapid bites. 1.3% (n=6) cases had both hematological and neurological manifestations. This is known to occur in severe envenomation, which is why the syndromic approach has not been recommended as an effective method of identification of the snake¹. The study conducted in Ahmedabad, Gujarat found 52% of the cases had neuromuscular manifestations and 9.6% hemorrhagic manifestations. 1.2% had both hemolytic and neurological deficits¹¹. In the study conducted in Hassan, Karnataka 72 cases (40%) had neurological manifestations and the haematotoxic manifestations were attributable to viper bites in 108 cases (60%)⁹. Sepsis and septic shock occurred in 6.14% and 4.18% of the victims. Most of the victims developed cellulitis (30.39%). Locally, necrotizing fasciitis (5.9%) and gangrene (4.62%) were recorded. 20.26% of the patients developed Acute Kidney Injury. This is a complication known to occur following Viperine bites¹. Elapid bites mostly resulted in cardiac and respiratory complications. In the study conducted by Sharma N¹ et al. in North India, 19.01% of the patients developed renal failure¹⁰. High mortality was associated with sepsis and Multi-organ dysfunction.

LIMITATIONS:

It is a retrospective study. The information obtained is indirect, i.e., it was obtained from medical records. As this was not a planned study, deficiencies have been found in the data.

CONCLUSION:

India has been reported to have the highest number of envenomations as compared to other regions⁴. The incidence of snake bite in West Coastal part of South India and the Western Ghats region is very high as people live in close proximity to snake habitat. This part is home to "the big four"; the Indian cobra, Russell's viper, Saw-scaled viper and the common krait⁷. Most of the bites were viperine followed by elapids (cobras and kraits). In many cases the snake has not been identified. Most of the bites have occurred during the daytime which corresponds to the time when people venture

into the forests or fields to carry out their work. While many victims presented to the hospital within 24 hours of the bite, a smaller number presented late, often resulting in adverse outcome. Both Viperine and Cobra bites result in local reactions. Some bites, Krait in particular, elicit a mild or no local reaction. Haemotoxic manifestations are seen in viperine bites while elapid bites result in neurological manifestations. In certain cases both neurological and hematological manifestations were noted. The unacceptably high death and disability associated with snake bite prompted the WHO to recommend that it be a notifiable disease². The incidence of snake bite is highest among the rural poor, especially those engaged in agriculture and forestry, making it an occupational hazard. It is now being recommended that Snake Bite be included as an occupational disease¹. Another group affected by this disease are children and the elderly. Although the number of victims is not as high as the middle aged and young adults, these groups also face severe consequences of the disease.

RECOMMENDATIONS :

The disease may be better studied at the primary level or with door-to-door questionnaires. Snake bite can, to an extent be avoided if proper care is taken. People engaging in any outdoor activity should be adequately covered. Wearing boots is highly recommended. Walking bare feet in the fields especially paddy fields, is discouraged. As far as possible, venturing into snake habitat should be avoided at night. If necessary, a flashlight should be carried. Any vegetation should be thoroughly checked before being handled. Open sanitation should be discouraged. In case of a bite, immediate medical attention should be sought and if possible, the snake be identified. The handling of snakes should be avoided as much as possible. Granaries should be kept away from houses. Plant material such as wood should not be stored indoors. Homes should be kept rodent-free. Termite mounds should be cleaned. Cyclists or motorists should avoid running over snakes. Medical personnel working in rural areas should be adequately trained. A list of regional snakes with identification (posters/flash cards) should be released to allow easier and more accurate identification of snake species. Importantly, education of the public should be actively carried out and information regarding the do's and don'ts of an encounter with snakes should be provided. The public should be educated regarding effective first-aid measures.

No Conflict of Interests

No funding from any source

Ethical Clearance: Obtained from Institutional Ethical Committee

REFERENCES :

1. Warrel David A (2016) Guidelines for the management of snake-bites World Health Organization. 2nd edition; <http://apps.who.int/iris/bitstream/10665/249547/1/9789290225300-eng.pdf> Accessed on 09.05.2017
2. World Health Organization (2007) Rabies and envenomings. A neglected public health issue. Geneva: WHO.
http://www.who.int/bloodproducts/animal_sera/Rabies.pdf Accessed on 09.08.2017
3. José Maria Gutiérrez, David A. Warrell, David J. Williams, Simon Jensen, Nicholas Brown, Juan J. Calvete, Robert A. Harrison (2002) The Need for Full Integration of Snakebite Envenoming within a Global Strategy to Combat the Neglected Tropical Diseases: The Way Forward. *PLoS Negl Trop Dis* 7(6): e2162.
4. Kasturiratne A, Wickremasinghe AR, de Silva N, Gunawardena NK, Pathmeswaran A, Premaratna R, Savioli L, Lallo DG, de Silva J (2008) Estimation of the global burden of snakebite. *PLoS Med* 5(11): e218.
5. Harrison RA, Hargreaves A, Wagstaff SC, Faragher B, Lalloo DG (2009) Snake Envenoming: A Disease of Poverty. *PLoS Negl Trop Dis* 3(12): e569
6. Mohapatra B, Warrell DA, Suraweera W, Bhatia P, Dhingra N, Jotkar RM, Peter S Rodriguez, Kaushik Mishra, Whitaker R, Prabhat Jha (2011) Snakebite mortality in India: A nationally representative mortality survey. *PLoS Negl Trop Dis* 5:e1018
7. Whitaker R. (2006) Common Indian Snakes, A Field Guide. 2nd edition. Macmillan, Chennai.
8. Chippaux JP. (1998) Snakebites: appraisal of the global situation. *Bull World Health Organ* 76: 515–524
9. Halesha BR, Harshavardhan L, Lokesh AJ, Channaveerappa PK, Venkatesh KB. (2013) A study on the clinico-epidemiological profile and the outcome of snake bite victims in a tertiary care centre in southern India. *J Clin Diagn Res* 7(1):122-6.
10. Sharma N, Chauhan S, Faruqi S, Bhat P, Varma S. (2005) Snake envenomation in a north Indian hospital. *Emerg Med J* 22(2):118-20.
11. Jarwani B, Jadav P, Madaiya M (2013) Demographic, epidemiologic and clinical profile of snake bite cases, presented to Emergency Medicine department, Ahmedabad, Gujarat. *J Emerg Trauma Shock* 6(3):199-202.
12. Lal P, Dutta S, Rotti SB, Danabalan M, Kumar A (2001) Epidemiological profile of snakebite cases admitted in

JIPMER Hospital. Indian J Community Med 26:36-8.

13. ShubhankerMitra, Abhinav Agarwal, BU Shubhankar, SahilMasih, ViswajitKrothapalli, Brian Mark Lee, JeevanKuruvilla, Reginald Alex(2015)Clinico-epidemiological Profile of Snake Bites over 6-year Period from a Rural Secondary Care Centre of Northern India: A Descriptive Study. ToxicolInt 22(1): 77–82.
14. Bhalla G, Mhaskar D, Agarwal A(2014) A study of clinical profile of snake bite at a tertiary care centre. ToxicolInt21(2):203-8.
15. Anjum A, Husain M, Hanif SA, Ali SM, Beg M, Sardha M (2012) Epidemiological Profile of Snake Bite at Tertiary Care Hospital, North India. J Forensic Res. 3:146.
16. Vivek Chauhan and Suman Thakur(2016)The North–South divide in snake bite envenomation in India J Emerg Trauma Shock. 9(4): 151–154.
17. Profile of Agriculture Statistics Karnataka State. Karnataka State Department of Agriculture, Bengaluru.<http://raitamitra.kar.nic.in/ENG/statistics.asp> Accessed on 10/09/2017
18. WhitakerR. Why Does India Have So Many Snakebites? Accessed on 15/7/2017
<http://indiansnakes.org/content/why-does-india-have-so-many-snakebites-rom-whitaker>
19. Francis N P Monteiro, Tanuj Kanchan, Prashantha Bhagavath, G Pradeep Kumar(2011) Krait bite poisoning in Manipal region of Southern India. J Indian Acad Forensic Med.; 33(1): 43.

Original Research Paper

Age Estimation of Individuals Beyond 45 Years of Age By CT Scan of Skull

1. **Kanika Kohli**, Assistant Professor*

2. **O.P. Aggarwal**, Professor*

3. **Amit Mittal**, Professor & Head**

*Department of Forensic Medicine, MMIMSR, Mullana, Ambala, Haryana, India

**Department of Radiodiagnosis, MMIMSR, Mullana, Ambala, Haryana, India

ABSTRACT :

The study was conducted as age estimation of living is most important issue to the court and to common citizen as well. Community relies on medico-legal expert for justice. The present study was conducted among 100 cases with an objective of estimating age by CT Scan of the skull radiologically and to see the closure of sutures in the Department of Forensic Medicine, MMIMSR, Mullana. So there are 100 patients and it's clearly seen that more predominant age group for CT- Scan is above the age of 60 years. As the age advances the closure of suture also increase its percentage. Males studied are 60 in number and the females studied are 40 in number. In 45-50 years of age Lambdoid, Parieto mastoid and Squamous sutures closes completely in 50 %, 22.2% , 44.4% of cases respectively. In 51-55 years of age Lambdoid, Parieto mastoid and Squamous sutures close completely in 70%, 65%, 45 % of cases respectively. In 56-60 years of age Lambdoid, Parieto mastoid and Squamous sutures closes completely in 72.2%, 61.1%, 66.7% of cases respectively. In 61-65 years of age Lambdoid, Parieto mastoid and Squamous sutures closes completely in 75%, 85%, 90% of cases respectively. In 66-70 years of age Lambdoid, Parieto mastoid and Squamous sutures closes completely in 75.8 % 87.5 %, 91.7 % of cases respectively. Arcus senilis is present between the age of 60-70 years. Wrinkles on the face starts appearing at the age above 50 years and is present between the age of 55-70 years. Hair in auditory meatus is applicable only on cases of male and is present maximum in 75 % of cases between the age of 65-70 years. Presbycusis is present between the age of 65-70 years. So all the above findings are necessary for the estimation of age. When the dispute is in the age of 60 years and 70 years the most reliable indicator is squamous suture closure and the least reliable is lambdoid suture.

Corresponding Author :

Dr. Kanika Kohli

Assistant Professor

Department of Forensic Medicine, MMIMSR, Mullana,
Ambala, Haryana, India

Contact : +91- 9728405109

E-mail : kanikaghotra86@gmail.com

Article History:

Received: 7 February 2019

Received in revised form: 20 February 2019

Accepted on: 20 February 2019

Available online: 1 June 2019

Keywords: CT Scan, skull, Lambdoid suture, Parieto-mastoid suture and Squamous sutures

INTRODUCTION :

Identification of an individual is very difficult, whether the individual is alive, dead or skeletal remains. The identification of human remains is a multifaceted one that encompasses religious, moral, social, investigative or technical and legal fields. The identification of a dead body is required in cases of sudden and unexpected deaths like fire, earthquakes, explosion, railway or aircraft accidents, mutilated or decomposed bodies. In India, identification of unknown unclaimed dead bodies, found at the outskirts of the village or lying in a ditch or nearby and also due to rapid decomposition in hot seasons or damaged by wild animals, is very difficult. In these cases, age estimation is very important factor for identification along with others like race, sex, colour etc.⁽¹⁾ Therefore, for age estimation, cases are being referred frequently to Forensic Medicine experts. Age estimation can

be a critical part of forensic investigations. Unfortunately, age determination is also one of the most difficult aspects of the investigation of death of a skeletonised individual. Community relies on medico-legal experts for justice on this issue. If proper age is not given, it is injustice to the public and profession. Different types of age groups (Gestational age, Infancy to puberty, Puberty to adulthood and Adulthood to old age) are generally considered for age estimation.⁽²⁾ Estimation of age for identification in unknown decomposed dead bodies or from skeletal remains or in living persons is very important for civil as well as medico-legal cases like criminal responsibility, judicial punishment, marriage, kidnapping, rape, infanticide, consent, age of retirement, income tax calculations etc. Since skeletal remains are often incomplete, the availability of a variety of methods that are applicable to different skeletal elements is essential.⁽³⁾ After 25 years,

determination of age is very difficult. Skeletal age determination was considered one of best method.⁽¹⁾ It includes age estimation from sutures of skull, changes in the vertebrae, changes in other bones and fusion of body of sternum with xiphisternum and manubrium.

The forensic community has in general been slow to implement the modern diagnostic imaging modalities, partly due to unawareness of its potentials and probably also for financial reasons. Now CT and other imaging techniques such as magnetic resonance imaging are however gaining access to forensic medicine, as an increasing number of forensic institutes are installing CT scanners.⁽⁴⁻⁹⁾

MATERIAL AND METHODS :

After taking voluntary consent from participants, present study was carried out on 100 cases between age groups of 45-70 years, who came to the department of radio diagnosis, MMIMSR Mullana for CT scan head. CT scans was evaluated for sutural fusion and then age estimation was done according to sutural fusion and also find out any co-relation between menopause in female, cataract, presbycusis, arcus senilis, wrinkles on face, hair in auditory meatus and closure of followings different sutures of skull.

1. CT Scan for lambdoid suture,
2. CT Scan for parieto-mastoid suture,
3. CT Scan for squamous suture.

Age of each individual studied is confirmed from birth certificate, service record, driving license, passport, ration card or voter's card. Persons without age proof were excluded.

RESULTS :

Maximum number of cases were from the age group of 66-70 years i.e. 24 (24%), lowest number of cases were from the age group of 45-50 and 56-60 years i.e. 18 (18%). Majority of the patients were males (60 cases; 60%). The numbers of female patients were forty (40%). Maximum number of cases i.e. 68 (68%) were of complete closure of lambdoid suture and lowest number of cases i.e. 5 (5%) had less than half closure of lambdoid suture.(Table 1)

Table1: Degree of Lambdoid Suture closure

	Frequency	Percent
Less than half	5	5.0
Half	9	9.0
More than half	18	18.0
Complete	68	68.0
Total	100	100.0

Maximum number of cases i.e. 66 (66%) were of Complete closure of Parieto Mastoid suture and lowest number of cases i.e. 3(3%) less than half closure of Parieto Mastoid suture.(Table 2)

Table2: Degree of Parieto-mastoid Suture closure

	Frequency	Percent
No closure of suture	3	3.0
Less than half closure of suture	9	9.0
Half Closure of suture	7	7.0
More than half closure of suture	15	15.0
Complete Closure of suture	66	66.0
Total	100	100.0

Maximum number of cases i.e. 68 (68%) were of Complete closure of Squamous suture and lowest number of cases i.e. 4 (4%) less than half closure of Squamous suture.(Table 3)

Table3: Degree of Squamous Suture closure

	Frequency	Percent
No closure of suture	4	4.0
Less than half closure of suture	7	7.0
Half Closure of suture	7	7.0
More than half closure of suture	14	14.0
Complete Closure of suture	68	68.0
Total	100	100.0

Arcus senilis was present in 51% of cases and absent in 49% of cases. Wrinkles on the skin of the face were present in 82% of cases and absent in 18% of cases. Hair in the auditory meatus was present in 71.7% of cases and was absent in 27.3% of cases. Cataract was present in 73% of cases and is absent in 27% of cases. Presbycusis was present in 33% of cases and absent in 67% of cases. Memory was intact in 67% of cases and not intact in 33% of cases. History of menopause was present in 76% of cases and absent in 23% of cases. (Table 4).

Table4: Distribution of different physical parameters

	Present		Absent	
	Case	%age	Case	%age
Arcus Senilis	51	51	49	49
Wrinkles on the skin of the face	82	82	18	18
Cataract	73	73	27	27
Presbycusis	33	33	67	67
Memory	67	67	33	33
Hair in auditory meatus (n=60)	43	71.7	17	28.3
History of menopause (n=40)	31	77.5	9	22.5

DISCUSSION :

In present study the lambdoid suture closure starts at 45 years of age in about 50% of cases and increase upto 76 % of cases upto the age of 70 years. **Krogman**⁽¹⁰⁾ concluded that Lambdoid suture closure started at age of 31 years from gross skeletal examination, while **Parikh**⁽¹⁾ gave 45-50 years as age for closure of Lambdoid suture. But Parikh and Krogman agree on one thing that the closure of Lambdoid suture is variable i.e. it may or may not close at all. **ParmarP**⁽¹¹⁾ concluded that by age of 55 years Lambdoid suture closure is evident on x-rays (**Table 5**).

Table 5. Comparison of time of closure off lambdoid suture (in years) with that observed by other authors.

Author	Year	Time of Closure	Method
Krogman ⁽¹⁰⁾	1962	31	Gross skeletal
Parikh ⁽¹⁾	1990	45-50	X-ray
Krishan ⁽¹⁴⁾	2001	About 55	X-ray
VijNandy A ⁽¹⁵⁾	2001	50-55	X-ray
Pradeep et al ⁽²⁾	2001-2004	45-55	CT Scan
Reddy K.S.N ⁽¹⁶⁾	2007	About 45	X-ray
Karmakar R.N ⁽¹⁷⁾	2010	45-50	X-ray
Pragnesh Parmar et al ⁽¹¹⁾	2012	45-55	X-ray
Present study	2014	45-70	CT Scan

Krogman's study was done from gross skeletal examination because during those days, CT scans were not available and his

study was only done on bones. So in his study, the skull sutures were seen with the naked eyes, so there is possibility of the observer variation as he noted that the earliest age for suture closure was 31 years. Moreover, he was of the opinion that suture closure of lambdoid, parieto-mastoid & Squamous suture is variable i.e. it may or may not close at all⁽¹⁰⁾.

In present study, the parieto-mastoid suture closure starts at the age of 45 years but only in 22 % of cases and increases upto 87 % of cases upto the age of 70 years. **Krogman**⁽¹⁰⁾ gave 50 years age for Parieto-mastoid suture closure from gross skeletal examination, while **Parikh**⁽¹⁾ gives 55 years age for closure of Parieto-mastoid suture from x-ray. **Pradeep**⁽²⁾ observed 55-60 years for closure of parieto mastoid suture from CT scan. **ParmarP**⁽¹¹⁾ concluded 70 years age for Parieto mastoid suture closure from x-ray (**Table 6**).

Table 6. Comparison of time of closure of Parieto-mastoid suture (in years) with that observed by other authors.

Author	Year	Time of Closure	Method
Krogman ⁽¹⁰⁾	1962	About 50	Gross skeletal
Parikh ⁽¹⁾	1990	55	X-ray
Pradeep et al ⁽²⁾	2001-2004	50-60	CT Scan
Reddy K.S.N ⁽¹⁶⁾	2007	40-50	X-ray
Karmakar R.N ⁽¹⁷⁾	2010	60-70	X-ray
Pragnesh Parmar et al ⁽¹¹⁾	2012	55-70	X-ray
Present study	2014	55-70	CT Scan

In present study, the squamous suture closure starts at the age of 45 years in about 44% of cases and increases upto 91.7 % of cases upto the age of 70 years. **Krogman**⁽¹⁰⁾ gave 50 years age for Squamous suture closure from gross skeletal examination, while **Parikh**⁽¹⁾ gave 60 years age for closure of Squamous suture. **Pradeep**⁽²⁾ observed 65 years age for squamous suture closure from CT scan. **ParmarP**⁽¹¹⁾ concluded 70 years age for Squamous suture closure from x-ray. **Pillay**⁽¹²⁾ concluded 65 years age for Squamous suture closure from x-ray (**Table 7**).

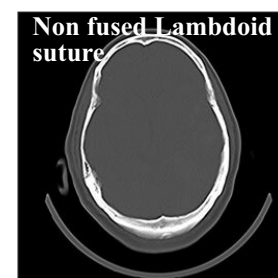


Table 7. Comparison of time of closure of Squamous suture (in years) with that observed by other authors.

Author	Year	Time of Closure	Method
Krogman ⁽¹⁰⁾	1962	50	Gross skeletal
Parikh ⁽¹⁾	1990	60	X-ray
Nandy A ⁽¹⁵⁾	2001	70	X-ray
Pradeep et al ⁽²⁾	2001-2004	60-65	CT Scan
Reddy K.S.N ⁽¹⁶⁾	2007	60	X-ray
Pillay ⁽¹²⁾	2008	60-65	X-ray
Pragnesh Parmar et al ⁽¹¹⁾	2012	60-70	X-ray
Present study	2014	55-70	CT Scan

Arcus senilis is present between the age of 60 – 70 years. Wrinkles on the skin of the face appear from the age of 50 years and keeps on increasing with the age. Hair in auditory meatus is applicable only in case of male which starts appearing at the age of 55 years and extends upto the age of 70 years. Memory is intact in almost 100% of cases upto the age of 60 years and reduces by 50 % of cases in the upto 65 years but as the age advances the memory i.e. the retaining power of the person keeps on reducing.

In the present study, the lambdoid suture was not fused in 7 cases in the age group of 66-70 years which is supporting the earliest studies by **Murli Manju et al⁽¹³⁾** that the prolonged patency of the lambdoid suture is due to external forces acting on it because of the greater number of muscles are attached to the occipital bone. Hence the lambdoid suture closure is least reliable.

CONCLUSION :

Maximum number of patients in the study group were aged above the age of 60 years. As the age advances the closure of suture also increase its percentage. The squamous suture is the best suture to determine age between the age of 65-70 years as in the present study it was completely closed in 91.7% cases. When the dispute is between the age of 60 years and 70 years, the most reliable indicator is squamous suture closure and the least reliable is closure of lambdoid suture.

REFERENCES :

1. Parikh CK. Parikh's text book of medical jurisprudence, forensic medicine and toxicology. 6th ed. CBS Publisher and Distributors, New Delhi, India.2004: 2-10.
2. Singh P, Oberoi SS, Gorea RK and Kapila AK. Age

estimation in old individuals by CT scan of skull. JIAFM. 2004;26(1):10-3.

3. Stout SD, Dietze WH, Iscan MY, Loth SR. Estimation of age at death using cortical histomorphometry of the sternal end of the fourth rib. J Forensic Sci.1994;39: 778-84.
4. Leth PM. Computerized tomography used as a routine procedure at postmortem investigations. Am J Forensic Med Pathol. 2009; 30(3): 219-22.
5. Poulsen K, Simonsen J. Computed tomography as routine in connection with medicolegal autopsies. Forensic Sci Int. 2007;171(2-3):190-7.
6. Rutty GN, Morgan B, O'Donnell C, Leth PM, Thali M. Forensic institutes across the world place CT or MRI scanners or both into their mortuaries. J Trauma. 2008;65(2):493-4
7. O'Donnell C, Rotman A, Collett S, Woodford N. Current status of routine post mortem CT in Melbourne, Australia. Forensic Sci Med Pathol. 2007; 3:226-32.
8. Yamazaki K, Shiotani S, Ohashi N, Doi M, Kikuchi K, Nagata C. Comparison between computed tomography (CT) and autopsy findings in cases of abdominal injury and disease. Forensic Sci Int. 2006; 162(1-3):163-6.
9. Thomsen AH, Jurik AG, Uhrenholt L, Vesterby A. An alternative approach to Computerized Tomography (CT) in forensic pathology. Forensic Sci Int. 2009;183:87-90.
10. Krogman WM. The human skeleton in forensic medicine. Springfield Illinois, USA. Charles C Thomas. 1962: 215-8.
11. Parmar P, Rathod GB. Determination of age by study of skull suture. Int J Cur Res Rev. 2012; 4(20):127-33.
12. Pillay VV. Textbook of forensic medicine and toxicology: Identification. 14th ed. Paras publishing, Hyderabad. 2004: 62.
13. Murlimanju BV, Gupta C, Samiullah D, Prabhu LV, Kumar CG and Somesh MS. Morphological investigation of cranial sutures in Indian Human adult skulls. Rom J MorpholEmbryo.2011; 52(3): 1097-1100.
14. Vij K. Identification. In: Textbook of Forensic Medicine and Toxicology Principles and Practises.4th ed. Elsevier.2008:50-8.
15. Nandy A. Identification of individual In: Principles of Forensic Medicine including Toxicology .3rd ed. New central book agency (p) ltd, Kolkata.2010:132-8.
16. Reddy KSN. Identification. In: The Essentials of Forensic Medicine and Toxicology.29th ed. Devi KS. 2010:72.
17. Karmakar RN. Forensic Medicine and Toxicology: oral, practical and MCQ. 3rd Ed. Academic Publisher. 2010:13.

Original Research Paper

A study of deaths due to burn injuries brought for medico legal autopsies at a tertiary care hospital in northern region of Himachal Pradesh.

1. **Susheel Sharma, Associate Professor***
2. **Vijay Arora, Professor and Head***
3. **Vipul Parmar, Junior Resident***
4. **Saurbh Sharma, Junior Resident***
5. **Mukul Sharma, Demonstrator***

*Deptt. of Forensic Medicine, Dr. R. P. Govt. Medical College, Kangra at Tanda, HP-176001

ABSTRACT :

Introduction : Invention of fire creates development as well as devastating impact on human. India has an estimated burn incidence of 6-7 million and about 1.4 lakh burn deaths in a year.

Materials and Methods : In present study five year data of burn deaths autopsied in our institute was scrutinized.

Results : Burn deaths comprised of 190 (15.62%) cases out of 1216 of the total medicolegal autopsies, Maximum cases were reported in the winter season. The age group of 19-30 years accounted for the maximum number of cases i.e 78 (41.05%). Females 121 (63.68%) outnumbered the males 69 (36.31%) in burn deaths. Maximum 67 (35.26%) cases showed burn injuries on 51-75% total body surface area. The average extent of injury was 61.21% total body surface area. Accidents 170 (89.47%) were the most common manner. Burn shock 94 (49.47%) was the leading cause of death followed by Septicaemic shock in 76 (40.00%) cases. Kerosene smell was appreciated in 140 (73.68%) cases.

Conclusions : Present study will provide analysis on how burn mortality is distributed across different seasons, age groups, sex and manner of its occurrence.

Corresponding Author :

Dr. Vijay Arora

Professor and Head

Dep. of Forensic Medicine, Dr. R. P. Govt. Medical College, Kangra at Tanda, HP-176001

Contact : +91-9418089939

E-mail : drvjarora@gmail.com

Article History:

Received: 19 January 2019

Received in revised form: 25 January 2019

Accepted on: 25 January 2019

Available online: 1 June 2019

Keywords: Medico legal, Burn death, Burn shock.

INTRODUCTION :

Invention of fire was a revolution in the history of mankind. Every invention has its own dark side for which mankind has to face its repercussion. Fire aided in the development of human and its culture, but also gives the most devastating form of trauma known as burn.

A Burn is defined as an injury to the skin or other organic tissue primarily caused by heat or due to radiation, radioactivity, electricity, friction or contact with chemicals.

According to world health organisation (WHO) about 2,65,000 deaths occurs each year from fires alone globally, with more deaths from scalds, electrical burns, and other forms of burns for which data are not available. The majority of these deaths occur in low and middle-income countries, with almost half occur in the WHO South-East Asia Region.¹

India, the second most populous country in the world with over a billion people has an estimated annual burn incidence of 6-7 million, based on data from major hospitals when extrapolated to whole of the country, which is the second largest group of injuries after road accidents² and about 1.4 lakh burn deaths.³

In hospitals, burn victims treated as per standard protocols, but medicolegal aspect usually ignored resulting in delayed or denial of justice. In this study we are analysing the various medico-legal aspects related with the burn deaths which will help in dealing with the consequences of the burn death.

OBJECTIVES:

The aim of this study is to analyze burn deaths across all ages in dead bodies brought in the mortuary of DRPGMC, Tanda. The study will provide analysis on how burn mortality is distributed across different seasons, age groups, sex and manner of its occurrence.

METHODOLOGY:

The study is retrospective document based study conducted in the Department of Forensic Medicine and Toxicology, DRPGMC, Tanda, HP. In present study, five year (1 Jan 2011-31 Dec 2015) medico-legal documents scrutinized for the objective of the study.

OBSERVATIONS:

Burn deaths comprised of 190 (15.62%) cases out of 1216 of the total postmortem examination conducted during the period 2011-2015 in the Department of Forensic Medicine, Dr. R. P. Govt. Medical College Kangra at Tanda. **(Table-1)**

Table 1: Year wise incidences of death due to burn injuries.

Total No.	2011	2012	2013	2014	2015	Total
Post Mortems	203	243	227	267	276	1216
Burn Deaths	36 (17.73%)	47 (19.34%)	37 (16.29%)	47 (17.60%)	23 (08.33%)	190 (15.62%)

Maximum 78 (41.05%) cases were conducted in winter i.e Oct-Feb months and least 46 (24.21%) in rainy i.e July-Sep months. **(Table-2)**

Table 2: Seasonal variation in burn deaths

Season	2011	2012	2013	2014	2015	Total
Winter (oct-Feb)	17	12	18	21	10	78 (41.05%)
Summer (March-June)	8	23	10	19	6	66 (34.73%)
Rainy (July- Sep)	11	12	9	7	7	46 (24.21%)
Total	36	47	37	47	23	190

The age group of 19-30 years accounted for the maximum number of cases i. e 78 (41.05%). Females 121 (63.68%) outnumbered the males 69 (36.31%) in burn deaths. **(Table-3)**

Table 3: Age and gender wise distribution of burn deaths

Age wise distribution	Male	Female	Total
0-18 years	6	14	20 (10.52%)
19-30 years	14	64	78 (41.05%)
31-40 years	17	21	38 (20.00%)
41-50 years	16	10	26 (13.68%)
51-60 years	9	6	15 (07.89%)
61-80 years	5	6	11 (05.78%)
More than 80 years	2	0	2 (01.05%)
Total	69 (36.31%)	121 (63.68%)	190

Maximum 67 (35.26%) cases showed burn injuries on 51-75% total body surface area. The average extent of injury was 61.21% total body surface area (TBSA). **(Table-4)**

Table 4: Shows total body surface area involved with burn injuries

% of body surface area	Male	Female	Total
0-25%	3	5	8 (04.21%)
26-50%	22	32	54 (28.42%)
51-75%	27	40	67 (35.26%)
76-100%	17	44	61 (32.10%)
Total	69	121	190

Accidental 170 (89.47%) was the most common manner, followed by suicidal 14 (07.36%) and homicidal 6 (03.15 %) **(Table-5)** Burn shock 94 (49.47%) was the leading cause of death followed by Septicaemic shock in 76 (40.00%) cases. **(Table-6)** Kerosene smell was appreciated in 140 (73.68%) cases. **(Table-7)**

Table 5: Shows manner of death due to burn injuries.

Manner of death	2011	2012	2013	2014	2015	Total
Accidental	33	45	33	40	19	170 (89.47%)
Suicidal	3	1	2	4	4	14 (07.36%)
Homicidal	0	1	2	3	0	6 (03.15%)
Total	36	47	37	47	23	190

Table 6 : Shows cause of death in burn injuries.

Cause of death	2011	2012	2013	2014	2015	Total
Septicaemic shock	24	23	4	15	10	76 (40.00%)
Hypovolumic shock	0	6	3	0	0	09 (04.74%)
Neurogenic shock	0	1	0	0	0	01 (00.53%)
Burn shock	11	15	28	27	13	94 (49.47%)
Electrocution	1	2	1	5	0	09 (04.47%)
Laryngeal edema	1	0	1	0	0	01 (00.53%)
Total	36	47	37	47	23	190

Table 7: Shows inflammable material used in deaths due to burn injuries.

Inflammable material	2011	2012	2013	2014	2015	Total
Kerosene oil	34	30	28	30	18	140 (73.68%)
Other	2	17	0	17	5	50 (26.32%)
Total	36	47	37	47	47	190

DISCUSSION:

During the period of study, the fatal burn deaths comprised of 190 (15.62%) cases out of 1216 of the total medicolegal autopsies which means about every 6-7th autopsy conducted in the department was a burn death. Our findings are comparable with the study of Batra AK (23.3)⁴, Ambade VN et al (21.6%)⁵ and Gupta RK et al (10.79%)⁶. The difference in the percentage is due to differences in the region from where study was carried out. In Himachal Pradesh about 65% population resides in northern six districts, which are covered medico-legally by the Department of Forensic Medicine, DRPGMC Tanda. The Department is authorized to conduct medico-legal autopsies in cases of deaths in jurisdictions of Police station Nagrota Bagwan, deaths in DRPGMC Hospital, Tanda and cases referred for autopsies from peripheral hospitals of Kangra and adjoining districts.⁷

Maximum cases were reported in the winter season (Oct-Feb) followed by summer (Mar-Jun) and rainy season (July-Sep) which can be explained as Northern region of Himachal Pradesh is a hilly and rural area. In winter season, the people usually burn wood for keeping themselves warm and for cooking etc. In the present study, out of 190 burns deaths, 121(63.68%) were females and 69 (36.31%) were males. This finding is consistent with the study of Batra A K (80.8%)⁴, Ambade V N et al (74.2%).⁵ In this part of the country, females mostly work in the kitchen as well as help in taking care of the cattle and this makes them susceptible to burn accidents. Moreover, in Indian society cruelty to females in demand of dowry also leads to suicidal and homicidal burn deaths.

In the present study the most common age group involved was 19-30 years accounting for 78 (41.05%) cases. In females the most common age group involved was 19-30 years accounting for maximum 64 cases while in males the most common age group involved was 31-40 years accounting for maximum 17 cases. This is consistent with observations of Singh et al⁸ and Gupta M et al⁶ who reported two thirds of fatal burn cases in the young age group (21-40 years). Lack of awareness and adequate safety measures leads to maximum burn deaths in this age group.

In the present study, maximum 67(35.26%) cases, had burn injuries on 51-75% total body surface area followed by 76-100% in 61(32.10%) cases, 26-50% in 54 (28.42%) cases and 0-25% in 8(4.21%) cases. The present study also revealed that in 67.4% cases, burns extend more than 50% of total body surface area. The average extent of injury was found to be 61.21% of total body surface area. This finding is consistent with the study of Singh D et al⁸, Bang RL et al⁹ and Song C et al.¹⁰ It indicates that mortality is higher in cases where burns extend in more than 60% of TBSA in spite of providing better treatment and care.

The present study shows that 170 (89.47%) cases were due to accidental burns, 14 (07.36%) were suicidal and 6 (03.15 %) were homicidal. Thus accidental burns were more common as compared to suicidal & homicidal burns. This finding is consistent with other studies conducted by Batra et al, Ambade et al, Gupta et al and Singh et al.^{4,5,6,8}

The cause of death in maximum 94 (49.47%) cases was found to be burn shock followed by Septicaemic shock in 76 (40.00%) cases. Our findings are consistent with Nath et al¹¹ who observed shock as the cause of death in 65.74% and septicemia in 28.7% cases. In study done by Tasgaonkar et al¹² 3.38% deaths were due to neurogenic shock, 36.38% cases due to oligemic shock, 29.54% cases due to septicemic shock and 30.46% cases due to acute tubular necrosis and complications of septicemia. Dasari et al¹³ had observed shock in 8% case, toxemia and shock in 18% cases and septicemia in 71% cases of their study. Instantaneous death in case of burns is due to neurogenic shock.

The inflammable material used in maximum 140 (73.68%) cases was found to be Kerosene oil. Our finding is consistent with other Indian studies conducted by Ambade et al and Singh et al.^{5,8} In study done by Chawla et al¹⁴ smell of kerosene was perceived in 4% cases. As the duration of hospital stay increases, the chances of perceiving smell of inflammable substance decreases due to their volatile nature and cleansing by the hospital staff.

CONCLUSION:

The study reveals that in the northern region of Himachal Pradesh burn injuries involving the 61.21% of total body surface area usually prove fatal however in India about 30-50% of total body surface area, if burnt is usually considered fatal.¹⁵ Smell of kerosene perceived on autopsy should be carefully noted as it serves important evidence in court-of-law. Shock and septicemia are the causes of death following burn injuries. Proper rehydration and prevention of hospital acquired infection is the key to prevent deaths in burn cases.

ETHICAL ASPECT - In the present study the findings

obtained will not be used for any legal or court purpose. Since all the cases are medico-legal in nature, the identity of the deceased not disclosed at any stage.

Financial support and sponsorship - Nil.

Conflicts of interest - There are no conflicts of interest.

REFERENCE:

1. Burns - World Health Organization (Mar 6, 2018) retrieved from <https://www.who.int/news-room/fact-sheets/detail/burns>.
2. Gupta J L, Makhija L K, Bajaj S P. National programme for prevention of burn injuries. Indian J Plast Surg 2010;43, Suppl S1:6-10
3. Burns | National Health Portal Of India (Sep 6, 2016) retrieved from <https://www.nhp.gov.in/disease/skin/burns>
4. Batra AK, Burn mortality: Recent trends and socio-cultural determinants in rural India. Burns 2003; 29(3): 270-5.
5. Ambade VN, Godbole HV. Study of burn deaths in Nagpur, Central India. Burns 2006; 32(7): 902-8.
6. Gupta RK, Srivastava AK. Study of fatal burns cases in Kanpur (India). Foren Sci Int 1988; 38(2): 81-9.
7. Notification: Home-C-(B) 15-4/2013, Govt of Himachal Pradesh, Deptt. of Home, Dated: 04.08.2015.
8. Singh D, Singh A, Sharma AK, Sodhi L. Burn mortality in Chandigarh zone: 25 years autopsy experience from a tertiary care hospital of India. Burns 1998; 24(2):150-56.
9. Bang R et al. Burn mortality during 1982 to 1997 in Kuwait. Eur J Epidemiol 2000; 16(8): 731-9.
10. Song C, Chua A. Epidemiology of burn injuries in Singapore from 1997 to 2003. Burns Jan 2005; 31 Suppl 1:518-26.
11. Nath A, Das P, Chakraborty PN. Burnt wives of Agartala: a retrospective study from Medicolegal autopsies of a Tertiary Hospital of Tripura, Northeast India. Int J Emerg Trends Sci Technol 2015; 2(7):2842-46.
12. Tasgaonkar GV, Zine KU, Meshram VP, Parchake MB, Sukhadeve RB, Hosmani AH. Study of trends of burn deaths at Aurnagabad region in India. Sch J App Med Sci 2015; 3(5C):2013-8
13. Harish D, Kaur C, Singh A, Kumar A. A comprehensive analysis of deaths due to burns in a tertiary care centre. J Punjab Acad Foren Med Toxicol 2013; 13(2):68-73
14. Chawla R, Chanana A, Rai H, Aggrawal AD, Singh H, Sharma G. A two years burns fatality study. JIAFM 2010; 32(4):292-7
15. Rao NG. Textbook of Forensic Medicine and Toxicology ,2ND edition, Jaypee, New Delhi; 2010, P 314.

A Case Report

Role of Vaginal Swab Examination to Apprehend the Culprit in Sexual Assault & Murder Case

1. **Naresh Kumar**, Biology Division, Forensic Science Laboratory, Home Department, GNCT of Delhi, Rohini, Delhi.
2. **Amit Chauhan**, Amity Institute of Forensic Sciences, Amity University, Uttar Pradesh, Noida
3. **Ritika Gupta**, Biology Division, Forensic Science Laboratory, Home Department, GNCT of Delhi, Rohini, Delhi.
4. **Deepa Verma**, Director, FSL Rohini
5. **S.K. Shukla**, Amity Institute of Forensic Sciences, Amity University, Uttar Pradesh, Noida

ABSTRACT :

In the era of advancement and modern technology, PCR technology has substantiated its valuable portrayal and become the witness of tremendous changes. This technology is significant to observe the changes and its accuracy in DNA profiling of forensic cases. DNA fingerprinting is considered perpetual, ubiquitous and exclusive for the establishment of individuality. It is based on the polymorphism in DNA sequence. Careful examination of trace evidences along with STR technology can be a milestone, which can change the favour of any cases. In this present case, DNA technology helped to whack the perpetrator of the murder of a young girl. The physical exploration had no sign of any fresh sexual assault, yet the vaginal swab was recovered. In any sexual assault, vaginal swab epitomizes the distinct ratio of male and female DNA because of limited number of sperms and high concentration of female cell. A forensic scientist always uses this ratio to determine the identity of culprit by differential extraction. Due to the limited amount of vaginal swab, scientists prefer the direct examination rather than differential extraction. In this case, direct examination helped to extract out enough information/ profile of culprit, that was sufficient to nab him and put behind the bar.

Corresponding Author :

Naresh Kumar,

Biology Division, Forensic Science Laboratory, Home Department, GNCT of Delhi, Rohini, Delhi.

Contact : +91 99719-18173

E-mail : nareshkumar2982@yahoo.com

Article History:

Received: 29 October 2018

Received in revised form: 16 November 2018

Accepted on: 16 November 2018

Available online: 1 June 2019

KEYWORDS : Polymorphism, Male Fraction, Vaginal Swabs, Mixed Profile etc.

INTRODUCTION :

Sexual assault has become the major issue of concern in the society/ Nation. Sexual assault holds a hard step to curb crime against the females. The government and judicial system are framing stringent laws for such crimes¹. In this type of crime, no eye witness exists except the evidences i.e. biological fluids, physical evidences which are considered as a mute witness with certainty and often recovered from the isolated location of crime. The accurate scientific collection and preservation process of these evidences establish a link between the victim and the perpetrator and the scene of occurrence². In such crime, vaginal swab mixed with semen, sweat, saliva, pubic hairs or several types of physical evidences carry the information about an individual involved in that crime³. Vaginal swab in sexual assault contains different ratio of male and female DNA due to limited number of sperms and high quantity of female cells. Advance technologies i.e. quantitative PCR and conventional PCR are very helpful to convince the sexual contact of victim and accused⁴. The presence of any other substance like colour of cloth, nature of swab also affects the quality and quantity of DNA and its

profile. The latency period for examination also affect the quantity of DNA of male fraction in the vaginal swabs⁵.

In this case, the samples were collected from the crime scene, body of the victim at the time of autopsy which were transported to the accredited laboratory later. The semen sample was identified based on the direct acid phosphatase test⁶. In addition of this confirmation, the presence of spermatozoa was also confirmed from the shape, size and staining of sperms. The assertive regions of DNA are replicated through the process of amplification to generate detectable amount of DNA⁷. During the analysis of juridical samples, a problem was encountered when inhibition was found in the collected samples. These samples were collected from the crime scene, under garments, adhere clothing's of the victim. It is possible that the samples collected from the scene of crime may contain the humic acid and the adhere clothing of victim may contain the inhibitor dye specially denim clothes⁸. It can be removed by re-purification or block the activity of inhibitors. Number of methods has been developed to improve the amplification in presence of inhibitors by use of sodium hydroxide or other method of purification. The failure of PCR

amplification can occur due two reasons either due to degradation or inhibition. Real time PCR can be accessing to determine the presence of inhibition in the samples. The duo kit used in quantification of DNA will help to determine the inhibition of the sample. If inhibition is found, it can be removed by re-purification or washing of DNA.

BACKGROUND :

A 14 years old girl was found unconscious in her house by a neighbour. Immediately shifted to the hospital and declared brought dead. Matter was reported to the police station because the body was having injury marks on upper lips and neck. Contusions and lacerations were found on upper lips. Four major reddish bruises of dimension 1 cm, 0.8cm, and 2cm were found on front of the neck while multiple reddish abrasions were found in crescentic shape on front and side of neck. On dissection of the neck extra vasations of blood were detected. Bruises were present on the neck muscles and Cricoid cartilages were fractured. Mucosa of tracheal lumen was congested and tracheal lumen was found having froth. Both lungs were congested and oedematous while Heart and pericardial sac were also congested. Smothering and throttling was given as cause of death.

All injuries were anti-mortem and had approximate time duration of 16 hours to one day prior to post-mortem. As the Nothing adverse was detected in her vaginal inspection but swab was taken for precautionary measure and sent to the laboratory for detection of semen. Lab reported semen on vaginal swabs. DNA profiling confirmed a mixed profile of deceased and a unknown male.

Lab reports changed the whole investigation. Earlier it was a case of murder but now it was converted to the Protection of Children from Sexual Offences (POCSO) Act, 2012 (This act deals with sexual offences against persons below 18 years of age, who are deemed as children). During the investigation police collected the blood samples of 10 suspects and 3 were from the same family.

MATERIALS AND METHODS :

The received samples were preserved at 4°C by the time examination started. After comparing the seals on the parcel in correspondence with the sample seal provided by the investigation officer, the samples were opened. The primarily examination of the sample was conducted for the detection of semen by acid phosphates test which was found positive and it was followed by the confirmation of spermatozoa by microscopic examination. The vaginal swabs were further analysed for DNA isolation along with blood samples of accused (**Figure 1-2**). A 3/4 portion of vaginal swab was cut into microcentrifuge tube of 1.5 µl. The samples were added

with Forensic buffer, Proteinase K, SDS and dithiothreitol as per the recommendation of protocol. Then kept for overnight for the digestion of cells. The sample was not proceeded for differential extraction as it might cause loss of male fraction during extraction of DNA. In case of sexual assault, the separation of female fraction is not full proof technique, but it helps to reduce the female portion present in the samples. The samples were further centrifuged the very next day and supernatant was discarded. As per the protocol of organic extraction, the phenol chloroform extraction process was performed to extract the DNA from the samples.

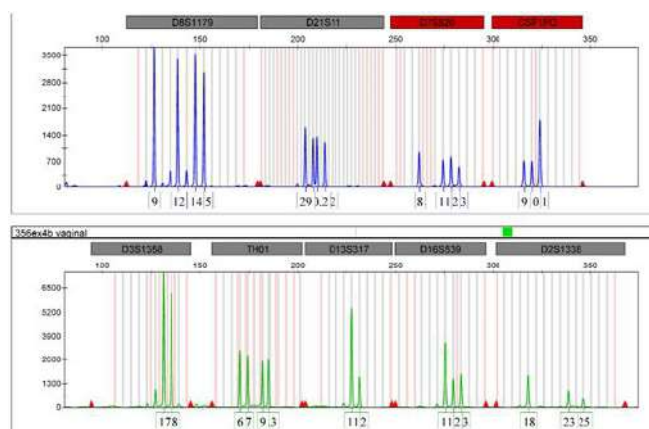


Figure 1 : DNA profile at 9 markers showing the mix profile in autosomal STR

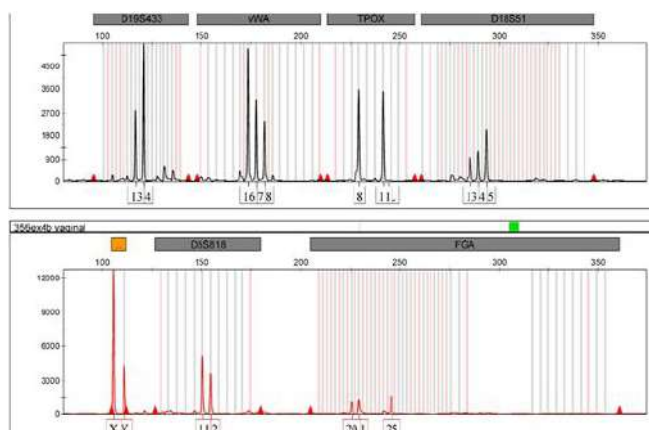


Figure 2 : DNA profile at 7 markers showing the mix profile in autosomal STR

Real Time PCR :

PCR was performed by using Quantifiler Duo kit of applied Bio system as per manufacture protocol. Total volume of sample was 25µl consisting the reaction mix, primer and DNA sample. Standards were used with the samples to compare the accuracy of results. These results helped us to determine the availability of human and male DNA in the samples. This is

reliable technique to arbitrate the gender from forensic sample while amelogenin based technology is not fully reliable. Samples were diluted as per value given by RT-PCR and approximately 1ng/ μ l DNA was used for sequencing the samples. AmpF1 STR Identifiler plus kit was used for PCR amplification (Applied Bio system). This kit contains 16 markers and out of them, one marker for sex determination namely amelogenin. PCR product 1 μ l was mixed Hi-Diformamide 24.5 μ l and .5 μ l 500 LIZ™ was used. Electrophoresis was done on the instrument Genetic Analyser (Applied Bio system 3500XL). Samples run in the instrument and detail alleles determined using Gene Mapper ID-X software 1.4.

RESULT& DISCUSSION :

The sample taken for isolation were quantified using duo kit and found to be having the human male DNA in the vaginal swabs while the rest garments gave the female profile of deceased itself. Quantity of human DNA was 1.24ng/ μ l while male DNA was .87ng/ μ l. As the quantity of male was less than 1ng/ μ l, the other samples were not taken for differential extraction method.

At the time of examination of the vaginal swabs there was no theory of fresh sexual assault. Hence a mixed DNA profile was prepared and sent to investigation officer to provide the suspect blood to match with the male profile found in the swabs (Figure 3 and 4).

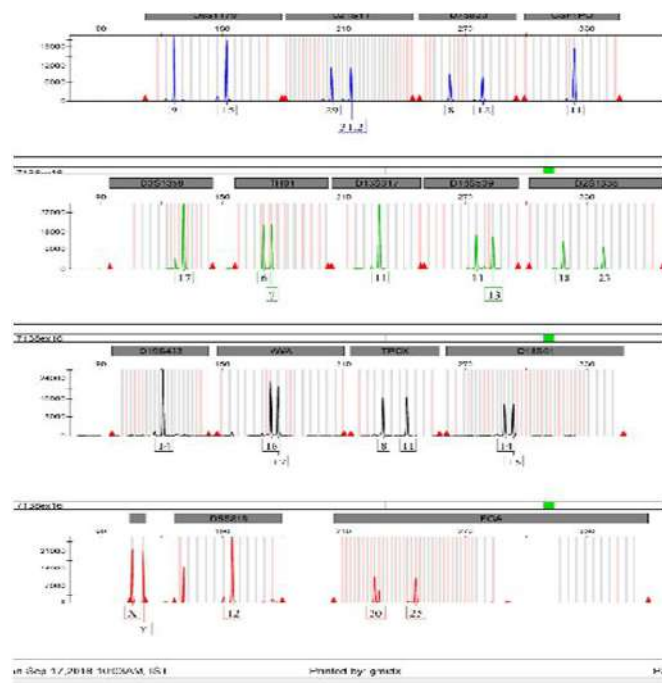


Figure 3 : DNA profile of accused (autosomal STR) from reference sample

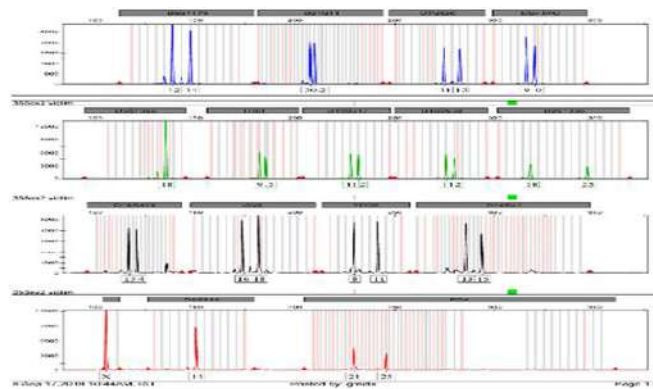


Figure 4 : DNA profile of deceased (autosomal STR) from reference sample

CONCLUSION :

The observation of this case indicates that, in cases of minor victim vaginal swabs collection plays an important role in detection of semen. Quantity of male and female fraction in any swabs should be taken directly without any differential extraction. If the ratio is not less than 20 time of male to the female fraction, direct DNA profiling may be done. If the result of sample gives the mix profile of deceased and perpetrator the Y-STR may also be used for such profiling of the case. This case was concluded based on autosomal STR because of the involvement of three suspects including accused from same family.

SOURCE OF FUNDING: Forensic Science Laboratory, Delhi

REFERENCES :

- Lewis J, Baird A, McAlister C, Siemieniuk A, Blackmore L, McCabe B et al. Improved detection of semen by use of direct acid phosphatase testing. *Sci Justice*. 2013 Dec;53(4):385-94.
- Davies A Wilson E. The persistence of seminal constituents in the human vagina. *J Forensic Sci*. 1974; 3:45-55.
- Kumar N, Ritika G, Aanchal M, Dhruv S, Shukla SK. Role of vaginal washing in semen detection and DNA profiling in delayed medical examination of sexual assault cases. *Journal of forensic Science & Criminology*. 2017; 5(5):1-5.
- Ley BL, Jankowski N, Brewer PR. Investigating CSI:Portrayals of DNA testing on a forensic crime showand their potential effects. *Public understanding of Science*. ; 21(1):51-67.
- Magalhães T, Dinis-Oliveira R J , Silva B, Corte-Real F, Nuno Vieira D. Biological Evidence Management for DNA Analysis in Cases of Sexual Assault. *The Scientific World Journal*.2015:1-11
- Bourke MT, Scherzinger CA, Ladd C, Lee HC. NaOH treatment to neutralize inhibitors of Taq polymerase. *J Forensic Sci*. 1999 Sep;44(5):1046-50.
- Kumar N, Guar V, Sharma D, Shukhla SK. Comparative evaluation of bone, teeth & Blood gauze in DNA analysis obtained from unidentified dead bodies. *Journal of Punjab academy of Forensic Medicine and toxicology*. 2016; 16(2):117-120.
- Thomson WC. Subjective interpretation laboratory error and the value of forensic DNA evidence;Three case studies. *Genetica*. 1995;96:(153-68).

A Case Report

Acrylamide Versus Agarose Efficiency to recover Trace DNA (without PCR) on 70% Ethylated Swab Versus Distilled Water Swab from Spectacles

1. **Simon Martin Manyanza Nzilibili**^{ac}, M Sc. Forensic Science Student

2. **Muh. Abduh Dwi Putra**^a, M Sc. Forensic Science Student

3. **Ahmad Yudianto**^{abd}, Lecturer

a Forensic Science Program, Post Graduate School, Airlangga University, Surabaya – Indonesia.

b Department of Forensic and Medico-legal, Faculty of Medicine, Airlangga University, Surabaya – Indonesia

c Ministry of Health, Community Development, Gender Elderly and Children, Dodoma – Tanzania.

d Human Genetic Laboratory, Institute of Tropical Disease, Airlangga University, Surabaya – Indonesia.

ABSTRACT :

Introduction : Swabbing method is one of the established methods in recovering forensic traces. The used swab is suggested to be effective while in a moist state. Different fluids have been proposed then with minimal highlight to ethylated swabbing (prepared from ethanol) which is also found usable and friendly during extraction and storage of sample among other efficacy.

Material and Methods : This study analyzes the significant use of 70% ethylated swab run simultaneously with distilled water swab to potentiate the findings under 6% acrylamide and 2% agarose electrophoresis without PCR. Besides assessing the use of these swabs (70% ethyl and distilled water) and its electrophoretic reaction on the two gel, this study also studied the usefulness on spectacle as a potential source of traces.

Results : 70% ethylated swab is found to concentrate DNA sample (1421 µg/ml) two times more compared to distilled water swab (654.5 µg/ml). In terms of purity of recovered DNA, 70% ethylated swab presented nearly similar measurement to distilled water swab (1.219 to 1.176 purity ratio respectively). Agarose electrophoresis was not able to detect DNA molecule hence the absence of visualized DNA fragments as band contrast from both swabs. On acrylamide gel electrophoresis, DNA molecule migration displayed multiple bands contrast with 70% ethylated swab; deep and concentrated towards positive electrode while lighter bands lagged near the negative end. Distilled water swab contrasted one deep band near the negative terminal electrode.

Conclusion : 6% acrylamide gel electrophoresis in absence of PCR is effective to profile trace DNA compared to agarose. In addition, 70% ethylated swab is a useful method in recovering trace DNA to successful DNA analysis. Also, the spectacle is a potential evidence for trace DNA either found at the crime scene for criminal linkage or purposed forensic investigation.

Corresponding Author

Simon Martin Manyanza Nzilibili

Forensic Science Program, Postgraduate School, Airlangga University, 4-6 Airlangga Rd., 60286 Surabaya, Indonesia

Contact : +255 7654 34320

E-mail : nzilibili@gmail.com

Article History:

Received: 31 October 2018

Received in revised form: 30 November 2018

Accepted on: 30 November 2018

Available online: 1 June 2019

KEYWORDS : 6% Acrylamide, 2% Agarose, PC, 70% Ethyl Swab, DNA, Spectacle , Trace evidence

INTRODUCTION :

Recovery of micro to macro Deoxyribonucleic acid (DNA) trace evidence is studied to involve adhesive tape, forceps and vacuum methods^[1,2]. Swab method besides is also discussed and presented to work properly and far better while moist in recovering such trace evidence^[2-7]. Opted and used moisturizing fluid, in that case, remains of high and efficacy determinant. To explore the effectiveness, experimental studies done have explored the success of ethyl moist swabbing though most still recommend water moistening option^[3,8]. Thus, in the DNA retrieval study; 95% ethanol swab^[9] was found suitable to recover DNA at high yield than distilled water similar to^[10], in which a 25% ethylated alcohol

swab yielded the most compared to 50% and distilled water swab. Emphasis is on maximized retention of minute natured sample residing on swab after recovery against loss or blow-away^[1] Contrary to a noted good use as swab fluid, ethyl on the other side is presented as decontaminant and lyses catalyst^[11] which means it has a destructive effect in opposition to recovery usefulness. However such encounters that interfere with sample evidence are well interpreted during DNA profile from extraction to electrophoretic visualization. Furthermore, depending on sample state (DNA molecule size) agarose and acrylamide reaction are also studied to respond differently^[12-14] Despite substantial trials on alcohol fluid, assessment of specified ethyl (70%) against distilled water swab for effective

swabbing and its comparative reaction on agarose and acrylamide electrophoresis is yet established. This study, therefore, dedicates investigation on the efficient use of a 70% ethylated swab supply by using spectacle evidence convinced by reasons discussed in next section.

Why 70% Ethylated Alcohol swab?

The percentage of alcohol on the treatment of biological sample has a direct effect relation. On DNA extraction and preservation usage, 70% ethyl has evidenced a flexible treatment of biological samples which allow morphological exploration afterward^[15]. This is in contrast to high or low percentage in respect to volatile, drying blow-away, non-flexibility performance, and degradation prone. Also; compared to other alcohol groups such as isopropanol, ethanol is highly precipitous that resuspend DNA pellet easily. Catalyse insolubility of DNA molecules by forming H-bonds with water during isolation (decrease hydration ability of water to DNA) reduced decaying. Lower dielectric lead DNA to aggregate and concentrate with cat-ions below lighter molecules under phenol-chloroform extraction^[16,17]. Referring to the percentage used in the reference above, ethylated alcohol swab envisions a special use in recovering DNA trace evidence, 70% ethyl alcohol swab is optimized for consideration as explained earlier. This ground prompted on the exploration of the usefulness of 70% ethylated swab through the admission of spectacle as useful and reliable as a suggested source of trace biological evidence based on Locard's and Kick's contact and silent witness respective principles. Appreciation of 70% ethyl swab and admission of spectacle in addition to normal referred evidence such as clothes, knife, vehicles, firearms, bedding, food, condoms, lip cosmetics, wallets, jewellery, glass, skin, bullet, paper, cables, windows and door lockers/handle, stones and watch^[2] broaden exhibits. Either in the recovery of such micro to macro exhibits like hair, dust, soil, glass particles, fluids, touched surfaces, clothes^[1] as forensic evidence, limited information is on spectacles as a potential source of biological trace evidence. Apart from compilation through literature reviews of the established useful properties of the ethyl alcohol in forensic evidence; this study complement finding used to recover trace DNA from spectacles especially through the use of 70% saturation. Aggregation of this information potentiates establishing special 70% ethyl swab for trace evidence swabbing recovery as reported in this study that compared to most recommended water swab.

MATERIALS AND METHODS :

Present Study was conducted at Human Genetic laboratory of Airlangga University to recover trace DNA from spectacles of two voluntary participants. Two sterilized cotton swabs were

taken for sample collection. One swab was a readymade 70% ethylated which swabbed one spectacle and other was dry swab moistened by 1cc of distilled water and swabbed the second spectacle. Swabbing researchers used sterilized gloves and squeezed extra fluid from swabs to avoid sample loss. Swabs were immediately soaked in 2 different tubes, filled with 4cc distilled water for overnight to allow down settling of DNA biological traces. For DNA extraction uppermost fluids was removed while down settled sample solution retained. Then 0.5cc of each sample was isolated in a sterile centrifuge tube; pipetted with 1cc of DNAzol (Invitrogen, ThermoFisher Scientific, Waltham, MA, USA), vortexed and incubated for 15 minutes. Then vortexed with 0.2cc of Chloroform (Merck KGaA, 64271 Darmstadt, Germany) followed by a centrifuge of 8,000rpm for 10 minutes. The separated supernatant was obtained into Eppendorf with isopropanol 1cc (EMSURE®, Merck KGaA, 64271 Darmstadt, Germany) followed by 15 minutes incubation and centrifuge at 12,000 rpm for 10 minutes then with care followed by discard of supernatant fluid again leaving settled and concentrated pellet. The pellet was washed with 0.5cc of 70% ethanol (EMSURE®, Merck KGaA 64271 Darmstadt, Germany) followed by 15 minutes incubation and then centrifuge at 12,000 rpm for 5 minutes which again followed^{[18][19]} by removal of supernatant. Finally, 50µl of distilled water resuspend formed DNA pellet for spectrophotometer and electrophoresis.





Table 1 : Concentration and Purity of Spectacle DNA evidence on the ethylated and non-ethylated swab

Sample Code	Absorbance 260 nm	Absorbance 280 nm	DNA Concentration (ng/ul)	DNA Purity
Distilled Water Swab-A	0.187	0.159	654.5	1.176
70% Ethylated Swab-B	0.406	0.333	1421	1.219



Electrophoresis

Electrophoresis involved both acrylamide and agarose gel without Polymerase Chain Reaction (PCR) (purposely to find out precise and accurate of 70% ethylated swab method and spectacle referenced to distilled water swab for recovery and profile of trace DNA). Percentage preparation and concentration decision of these two gels is by the size of fragments measured and needed to be resolved for optimal separation for visualization. The measured DNA fragments concentration (**Table 1**), decided acrylamide gel to be prepared at 6% concentration then stained with silver nitrate while agarose was at 2% concentration with ethidium bromide stain then visualized in Ultraviolet transilluminator with reference to^[13]. As to the reference made, 6% acrylamide concentration is determined between 80-800bp of native to 50-500 of denatured DNA while 2% agarose concentration is determined between 200-1500bp. Charged molecules then responded in accordance with visualization of **Figure 3**.

Figure 1 : Concentration of DNA extracted from 70% ethyl and distilled water swabbed spectacle.



Spectrophotometer Measurements

Quantification spectrophotometry measurement read in order to establish concentration and quality parameters of 70% ethylated swab in reference to distilled water swab but also the potential extent of spectacle evidence in yielding significant biological sample for DNA. Using Ultraviolet-visible Spectrophotometer (UV-1601, PC, Shimadzu, Japan) DNA Concentration was given by absorbance reading at 260nm and 280 in UV-1601 and DNA Purity was given by Optical Density (OD) OD260/OD280 ratio, refer **Table 1**

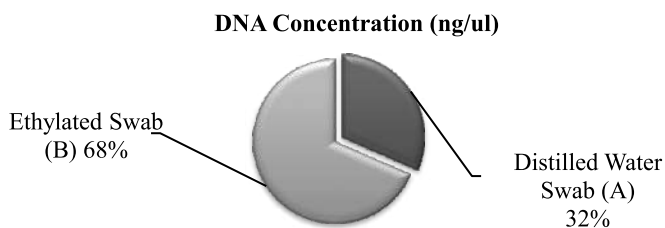


Figure 2 : Purity of DNA Extracted from 70% Ethyl and Distilled Water Swabbed Spectacle.

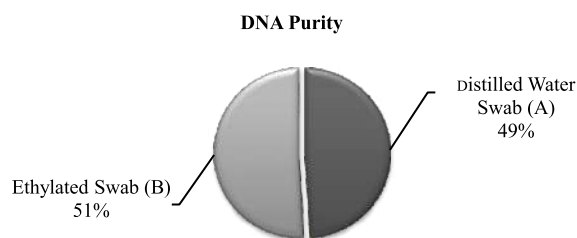
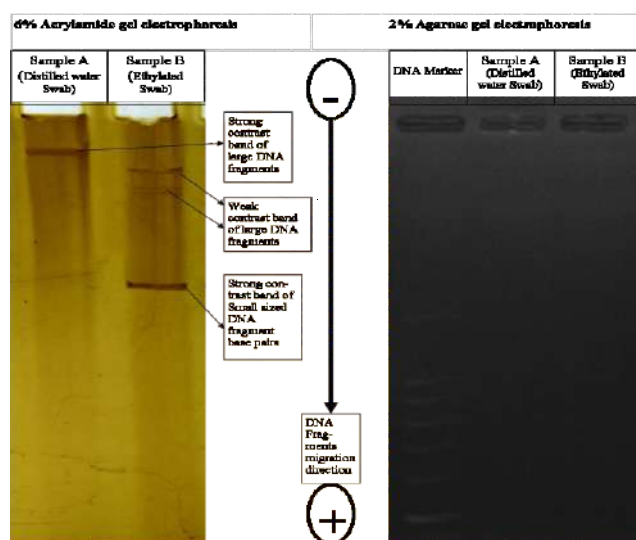


Figure 3 : Electrophoretic reaction between 6% acrylamide and 2% agarose gel from a DNA sample recovered through 70% ethyl and distilled water swabbed spectacle evidence.



Measures to control the study

Contamination and control measures of analyzing foreign and un-targeted genetic adhered. The adhered measure included the use of sterile supply and equipment, one-sided and direction swabbing, constant laboratory temperature at 26.5 oC, use of PPE gears, use of maintained and calibrated of machines together with twice a week sterilization and disinfection of the laboratory room by Ultraviolet lamps.

RESULTS AND DISCUSSION :

70% Ethyl swab recovery method

According to literature, alcoholic swab has exemplified usefulness in varied percent. As from this study, a 70% is reasonably examined and presented to substitute and establish a useful ethylated swab potential to maximizing recovery of trace biological evidence. Compared the two used swab: 70% ethyl alcoholic swab and distilled water swab, finding presented a more closely results in spectrophotometer measurement (**Table 1**) but quite different in electrophoresis band contrast (**Figure 3**) between the swabs at different gel electrophoresis. Concentration reading measured almost three times in 70% ethylated swab compared to distilled water swab as per **Figure 1** presentation. This concentration gives an interpretation that, 70% ethylated swab recover more DNA sample compared to possible amount able to be recovered by distilled water swab. In forensic profiling analysis, this interpretation gives a meaning to the usefulness of increased probability and assured recovering the adequate amount of sample from the targeted evidence of traces that is potential to enable successful profiling results during experimentation.

Deducing purity measurement; spectrophotometry purity increases confidence in the usage of the ethylated swab. Estimated chances of increased degradation and destruction of genetic materials as anticipated through previous few reported application in disinfection and decontamination is a contrary scenario. According to the studies; used ethyl as destructive agent forecasted expectation that this study also might generate a lowered and minimized purity by the fact of its destructing ability (micro-organisms discussed similar to the structure of traces). The purity of the 70% ethylated swab recovered sample is nearly similar above distilled water swab. Despite the fact that both 70% ethylated swab and distilled water swab are below the recommended limits of purity (1.6-2.0) (**Table 1**), the findings are promising that use of modern extraction and isolation techniques would purify to acceptable limits. The nature of results measured project useful pellet (of acceptable limit) upon with agreed contribution of effective recovery of ethyl swab. Generation of these findings proposes a step forward in utilizing the significance and modernized extraction and amplification reaction that would segregate Ribonucleic acid (RNA) and protein ingredients to attain worthy and sufficient DNA decisive profile from 70% ethylated swab.

Electrophoretic reaction on 6% Acrylamide and 2% Agarose Gel

DNA fragments resulted from isolation and electrophoresis under both acrylamide and agarose gels without PCR are presented as seen in **Figure 3**. Despite the fact that PCR and sequencing technique amount to accuracy and discriminatory efficiency of genetic profile the cost consequences and technology always hinder accessibility to struggling community, this study explored the option of effective and affordable electrophoresis only techniques. Aiming to profile establishment, the study used an isolated DNA pellet from two different swabs (70% ethylated and distilled water) into the two electrophoretic gel with the standard similar experimental condition. From the two gels type of electrophoresis, 2% agarose electrophoretic gel didn't respond to visualization of bands resulted from DNA fragments migration irresponsiveness, **Figure 3**. The failure is established to be due to the ability to work better and resolving many large molecules compared to minute fragments, hence suitable to high sized DNA. This characteristic property is also associated with low-resolution ability – meaning to challenge reaction when amplification of fragmented, or minute molecules is skipped. These findings complement findings established and discussed by different contributors^[12,13].

Compared to acrylamide gel electrophoresis, DNA fragments mobility is observed by bands contrast stain. The success of

6% acrylamide gel is supported by its resolution and a higher ability to analyses of small DNA molecules. In such case, acrylamide gets preference to this traced DNA by being more sensitive to the ability to resolve small molecules. Successfulness of acrylamide gel is well appreciated on both ethylated and distilled swab, variation and responding differences are certainly by swabbing technique. This acrylamide electrophoresis of DNA band contrast appearance to both swabs sample is by fragments migration effect of polarization and sizing. Migration of DNA fragments from negatively charged electrodes to positive is established in different numerous band level especially to sample of the 70% ethyl swab as presented in **Figure 3**. These band contrasts portray the size, length and strength of the DNA extracted from these two kinds of swabs (ethylated and distilled water swabs). As discussed in concentration and purity above (**Table 1**),

DNA obtained through 70% ethylated swab is with nearly similar purity but higher amount as portrayed in Figure 1&2, this information gives the connotation that both swabs yielded pure DNA capable to be analyzed in electrophoresis, **Figure 2**. The meaning is that through this electrophoresis without PCR amplification of DNA targeted locus, DNA has successfully been electrophorized as full charged fragments and migrated to appropriate contrast level. However, from the displayed bands, ethylated swab contrast more bands compared to water swab; this implies that 70% ethyl swab recover more amount of DNA sample with various strength and size leading to a differed migration of which – small sized fragments are lighter and migrate faster with contrast level closer to Anode electrode refer **Figure 3**. The longer and larger sized DNA fragments recovered in the ethylated swab and water swab appear to contrast closer to the cathode electrode due to the slower migration of charged fragments. The findings interpret varied implication of argument; first, both swabs (70% ethylated and distilled water) recover significant amount but ethylated swab recover more to significant minute traces evidenced by different band contrast and even concentrated more at a lower level (closer to positive end). Second, the purity of the DNA sample recovered by ethylated swab is higher with excess concentration compared to water swab. Third, minute and increased extraction of the sample are of useful quality as being able to be profiled on electrophoresis even without PCR primer amplification.

Due to this fact establishment, both 2% agarose and acrylamide gel electrophoresis are challenged by stature and nature of profiled DNA molecules due to the absence of PCR amplification. Though the two gel present with varied quality and profile response, acrylamide stand out compared to agarose electrophoresis. By this result, 6% acrylamide is

recommended to work far better and remain a suggested option even in absence of PCR and amplification techniques for genetic profile and DNA discrimination among individuals.

Spectacle

As other evidence found at crime scene, through this study; spectacle is evaluated to useful and potential evidence able to be used as a source of trace DNA for profiling as result of contact from a human that used before. Through a well-established recovery method, spectacle exhibit can significantly contribute the logged and harbored amount of DNA in a contacted sample from specific individual used before with basic observation established from non-PCR'd electrophoresis.

CONCLUSION :

DNA spectrophotometer and band visualization contrast under 6% acrylamide gel depict and suggest; 70% ethylated swab to be a useful and strong method that recover large and enough sample for DNA profile establishment. As for these reasons stated above in signifying conduction of this study, 70% of ethyl is in that manner recommended due to being a flexible percent that tolerates further morphological treatment of DNA sample from recovery to temporal storage before processing in the laboratory. The study also brings attention to successful electrophoresis in consideration of profiling DNA even in the absence of or limited PCR technique by using 6% acrylamide gel. Finally, usage of spectacle as a potential source of DNA sample either found at the crime scene for criminal linkage or to purposed forensic inquiry investigative profiling.

Measures to Control the Study

Contamination and control measures of analyzing foreign and un-targeted genetic adhered. Then adhered measure included the use of sterile supply and equipment, one-sided and direction swabbing, constant laboratory temperature at 26.5 o C, use of PPE gears, use of maintained and calibrated of machines together with twice a week sterilization and disinfection of the laboratory room by Ultraviolet lamps.

Abbreviations

DNA: Deoxyribonucleic Acid; OD: Optical Density; PCR: Polymerase Chain Reactions; RNA:

Ribonucleic Acid; TBE: Tris-borate-EDTA

Acknowledgements

Authors convey gratitude to volunteers who provided spectacles for study execution, Human Genetic laboratory staff for technical assistance and Mr. Stanley Elias for English and language improvement.

Funding : None

Conflict of Interest : No conflicting interest

REFERENCE :

1. Fisher BAJ, Fisher DR, Kolowski J. Forensics demystified. McGraw-Hill; 2007. 272 p.
2. Ah Van Oorschot R, Ballantyne KN, Mitchell RJ. Forensic trace DNA: a review. *Investig Genet.* 2010;1:14.
3. Van Oorschot R, Phelan DG, Furlong S, Scarfo GM, Holding NL, Cummins MJ. Are you collecting all the available DNA from touched objects? *Int Congr Ser.* 2003;1239(C):803–7.
4. Jack Dillon, Debra Figarelli, David Sylvester WT. Collecting DNA Evidence at Property Crime Scenes. DNA Initiative. 2009;1–30.
5. Oregon State Police. Physical Evidence Manual. 2015.
6. Puritan. Swabbing for Trace Evidence vs. Swabbing for Blood and Other Fluids. 2016. Available from: <https://blog.puritanmedproducts.com/swabbing-for-trace-evidence-vs-swabbing-for-fluids> Accessed on Jan 11, 2018
7. Adamowicz MS, Stasulli DM, Sobestanovich EM, Bille TW. Evaluation of methods to improve the extraction and recovery of DNA from cotton swabs for forensic analysis. *PLoS One.* 2014;9(12):1–18.
8. Raymond JJ, van Oorschot RAH, Walsh SJ, Roux C. Trace DNA analysis: Do you know what your neighbour is doing?. A multi-jurisdictional survey. *Forensic Sci Int Genet.* 2008;2(1):19–28.
9. Slrichantrawonq K, Rerkamnuaychoke B, Ramathibodi M. DNA retrieval on flip-flops. :30–6.
10. Hildebrand DP, Shamji F, Yamashita B, Wilkinson D. DNA Sampling from the Trigger and Handgrip of Discharged Firearms. 2004;1-10
11. Gršković B, Zrnec D, Popović M, Petek MJ, Primorac D, Mršić G. Effect of ultraviolet C radiation on biological samples. *Croat Med J.* 2013;54(3):263–71.
12. Taylor CY, Guillén IA, Nazabal M, Fernández JR, Silva JA. Electrophoretic techniques applied to the detection and analysis of the human microsatellite DG10s478. *J Biomol Tech.* 2007;18(5):298–305.
13. Barril P, Nates S. Introduction to Agarose and Polyacrylamide Gel Electrophoresis Matrices with Respect to Their Detection Sensitivities. In: *Gel Electrophoresis - Principles and Basics.* Magdeldin S(editor)
14. Stellwagen NC. Electrophoresis of DNA in agarose gels, polyacrylamide gels and in free solution. *Electrophoresis.* 2010;30(Suppl 1):1–14.
15. Oswald N. The Basics: How Ethanol Precipitation of DNA and RNA Works. *Bitesize Bio.* 2007;1–15. Available from: <https://bitesizebio.com/253/the-basics-how-ethanol-precipitation-of-dna-and-rna-works>. Accessed on Jan. 15, 2018
16. Brennan J. What Does Ethanol Do in a DNA Extraction?. *Sciencing.com.* 2017. Available from: <https://sciencing.com/ethanol-do-DNA-extraction-8336005.html>. Accessed on Jan. 15, 2018
17. Zumbo P. Ethanol Precipitation. *Weill Cornell Med Coll.* 2013;1932(Pauling):1–12.
18. Chen H, Rangasamy M, Tan SY, Wang H, Siegfried BD. Evaluation of five methods for total DNA extraction from western corn rootworm beetles. *PLoS One.* 2010;5(8).
19. Chomczynski P, Mackey K, Drews R, Wilfinger W. DNAzol: A reagent for the rapid isolation of genomic DNA. *Biotechniques.* 1997;22(3):550–3.

A Case Report

Writing On Unconventional Surface

1. **Sarita Sharma**, Senior Scientific Assistant (Documents), Forensic Science Laboratory, Delhi
2. **Jyoti Singh**, Assistant Professor, Amity University , Noida, UP
3. **Anurag Sharma**, Assistant Director, Forensic Science Laboratory, Delhi
4. **Deepa Verma**, Director, Forensic Science Laboratory, Delhi
5. **Madhulika Sharma**, Ex- Director, Forensic Science Laboratory, Delhi
6. **S.K Shukla**, Director, Amity Institute of Forensic Science, Amity University , Noida, UP

ABSTRACT :

Examination of writings on unconventional or unusual surfaces by unusual writing instruments is one of the challenging areas of document examination. It has its limitations due to non availability of suitable comparable materials in similar terms & conditions. The present case deals with the writing executed on wall by a person in which he mentioned the name of the people responsible for mental agony and stress which led him to commit suicide. Thorough scientific examinations were conducted which started with the processing of crime scene and furnishing a definite conclusion.

Corresponding Author

Sarita Sharma

Senior Scientific Assistant (Documents),
Forensic Science Laboratory, Delhi
Contact : +91 8860413004
E-mail : drsarita0707@gmail.com

Article History:

Received: 12 December 2018
Received in revised form: 15 January 2019
Accepted on: 15 January 2019
Available online: 1 June 2019

KEYWORDS : Unconventional Surface, limitation, Suicide Crime Scene, Writings

INTRODUCTION :

Examination of two cases cannot be carried out in a similar manner. Every case is unique in its examination and conclusion. Examination of Writings executed on unconventional surfaces using unconventional writing instruments is one such area where limitations are much more as compared to examination of writings executed on conventional surfaces using conventional writing instruments. Forensic Document Examination is the study based on the basic principle of comparison of 'like with like'. Forensic Document Examiner has to face many challenges while examination of different types of cases, as every case is unique in itself. In case of examination of unconventional writing surface, the basic principle of comparison of 'like with like' may not be perfectly followed as the standard materials for comparison of such writing surfaces are on conventional or usual surfaces. The abnormal substrates affect the overall presence and line quality of a writer's writing including abrupt interruptions and unevenness¹. The specimen writings on unconventional surface such as wall can be taken as it is not impossible thing to do. But, in case of suicide and murder where executor of writings is dead, specimen writings cannot be taken and in such cases document examiner has to depend on the old admitted writings. Examination of writings on unconventional surfaces depend on the type of unconventional surface, type of unconventional writing instrument, amount of matter or material written, mental conditions of the writer, posture of the writer.

Unconventional surfaces are unusual surfaces. These are of many types such as wall, wooden log, body surface, stones, metals, mirror, soil etc. Writers may use almost any surface or

substance to leave their mark. Text may be painted, carved, or cut. With latest & advanced technology available such as laser cutter, 3D printer can also be used. Digitally produced text can also be seen. Unconventional or unusual writing instrument may also be used such as blood and other body fluids, spray paints, lipstick, liquid sindoor, coal, brick, soap, kajal, nail polish, scratching with pointed tool etc. Totty² reported a case of handwriting on an unusual surface – stomach. Writing on unconventional surfaces is basically circumstantial depending upon the availability of writing surface & writing instrument. Apart from this, the mood or mental condition at the time of writing also plays the role like joy, sorrow, depression, excitement, happiness, confusion, etc . Huber and Headrick³ described various external and internal factors, conditions and circumstances that can have an influence on the appearance of handwriting, bearing in mind that we usually do not have any influence over factors of an external nature. Unconventional surface or unconventional writing instruments are also used in various cases such as murder, suicide, robbery, threat, to defame, trickery, to reveal/hide the identity etc.

Brief Case History:

Information was received by police about a man found murdered by hanging from the ceiling fan and jewellery along with other valuable items found stolen. During interrogation with other family members it was revealed that it might be the case of suicide. Crime scene investigation was done by a forensic team, where walls were found to be scribbled in Hindi (**Figure 1-4**).

One lipstick was also found at the spot which was probably used to write on the wall. Wife of deceased claimed that her husband was murdered by her in laws. After comparison of

Figure 1



Figure 2



Figure 3

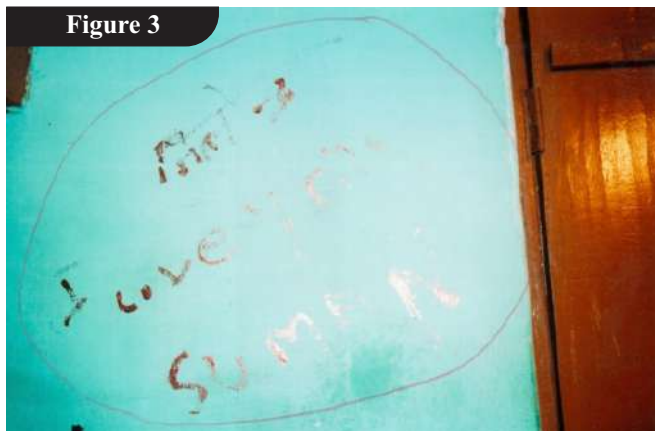


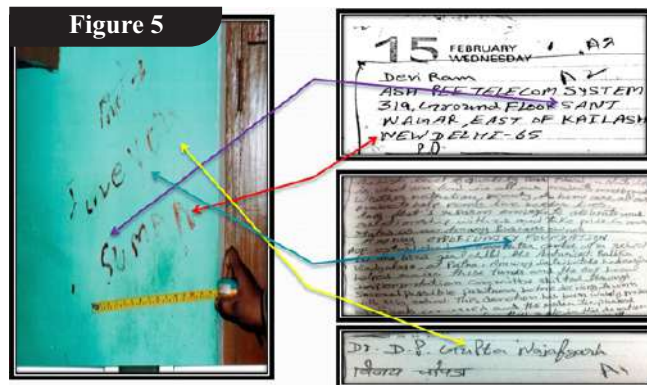
Figure 4



writing written on wall with the admitted writing of a deceased, investigating agency could solve the mystery of suicide or murder.

Examination and Analysis : Beside the original document itself, a photograph permits the most extensive technical study⁴. Irregular interruptions appear in writing executed on unusual surfaces⁵. Photographs are the most important tools in the examination of such cases. The scientific examination of questioned writings present on the walls and comparison with the standard writings as supplied by the family of deceased which were considered as admitted genuine writings, revealed similarities in both general and individual writing characteristics. The commonly occurring letters could be found in both the sets of writings (Figure 5).

Figure 5



Result and Discussion : Writing as a whole process is one of the most permanent and unconscious habits⁶. It is observed that when a person writes on a wall with his standing posture, the distance between the words and letters & interlineal distance vary when compared to the writings executed in normal conditions. Wall being the unconventional surface, has major irregularities on surface which caused writing instrument to change the direction & follow the irregularity to some distance. Purpose of executing the document may have an effect on writing. In this case, the deceased was in highly mentally/emotionally unstable condition which may have reflected in his writing on wall. He used lipstick to write on the wall. Deceased might have used some conventional writing instrument but due to his mental condition and in fit of anger and rage or might be due to unavailability of conventional surface, he wanted to leave the mark to hint his other family members for reason of committing suicide. He scribbled the name of the male friends of his wife. In this case, the successful effort have been done to evaluate the common, general & individual characteristics between questioned & standard writing eg- movement impulses, start, spacing, pen pauses, manner of formation of characters with respect to each other, commencing & terminating ends etc. After examining each & every minute & inconspicuous details of the writings it was concluded that the pictorial appearance had shown some differences in formation but substantial inherent characteristics had been found similar despite of irregular surface & unconventional writing instrument in case of questioned writings. It is needless to mention that the appropriate standard materials are the key of a successful examination.

REFERENCES :

1. De Angelis CM. Effects of writing surfaces and author's position on handwriting. *Journal of Questioned Documents Examination* 1997;6:10-70
2. Totty RN. A case of handwriting on an unusual surface. *Journal of Forensic Science Society* 1981;21:349-350.
3. Huber RA, Headrick AM. *Handwriting Identification: Facts & Fundamentals*: Boca Raton, New York; CRC Press.1999.
4. Hilton O. *Scientific examination of questioned documents*1982. New York : Elsevier Science Publishing Co.
5. Joshi MC, Garg Rakesh K. Examination of handwriting on an unusual surface in a suicide case: dead persons do tell tales- conduct a forensic investigation for the cause of humanity and justice, *Problems of Forensic sciences* 2015;101:56-65
6. Osborn AS. *Questioned Documents*. Albany : Boyd Printing Co 1928.

A Case Report

Scanning Electron Microscopic Finding of Heart in a Case of Celphos Poisoning

1. **Manoj Kumar Pathak**, Professor and Head*

2. **Rohini**, Research Scholar*

*Department of Forensic Medicine, Institute of Medical Sciences, BHU, Varanasi, Uttar Pradesh

Corresponding Author

Dr. Manoj Kumar Pathak, Professor and Head

Department of Forensic Medicine,

Institute of Medical Sciences, BHU, Varanasi, Uttar Pradesh,

Contact : +91 9450179177

E-mail : drmanojpathak@gmail.com

Article History:

Received: 4 December 2018

Received in revised form: 10 December 2018

Accepted on: 10 December 2018

Available online: 1 June 2019

KEYWORDS : Scanning Electron Microscope (SEM), CDRI, Myocardium, Round cell

INTRODUCTION :

Scanning electron microscope is an analytical method in the field of forensic science, employed for primary information about unknown samples and materials^[1]. Heart is an extraordinary organ situated in thoracic cavity which along with the circulatory system, works to supply blood and oxygen to all parts of the body.^[2]

Celphos is a systemic lethal protoplasmic poison which has become one of the major ways of committing suicide in Northern India. It is a solid fumigant pesticide which was earlier used for bulk preservation of wheat^[3].

Generally it is available by the names Alphas, Quickphos or Bidphos, as dark brown or greyish green tablets of 3gm of 20 mm diameter and thickness 5 mm. These tablets are composed of pure Aluminium Phosphide (the active ingredient which releases PH₃ gas), Urea and NH₄CO₃ (which in turn releases CO₂ and NH₃ and prevents self ignition of PH₃ gas). Each tablet has a capacity to liberate 1 gm Phosphine, on coming in contact with moisture^[4-6].

Phosphine is a systemic poison and affects all organs of the body. Phosphite and Hypophosphite of Aluminium Phosphide, which are non-toxic are left in the grain. Overall mortality in cases of celphos poisoning varies between 70–100%. The rate of mortality in India from poisoning varies from 15-35% where in developed countries it is 1-2%. Toxicity rating of celphos is 4 i.e. very toxic as its fatal dose is 0.5 gm.

Mortality is higher in those who consume more than two tablets and none of the patients survived who had ingested more than 3 tablets.^[6-8]

The pattern of poisoning varied in urban and rural areas, with a higher incidence of poisoning deaths in rural (64%) than in urban areas (36%). In Jamnagar,^[9] Aluminium phosphide was the most common poison consumed, being responsible for 50% of deaths, followed by insecticides 24%. Poisoning

deaths increased from 19% in 1996 to 24% in 2005. The age group most commonly affected was 16–25 years (49%). The male to female ratio was 1.9:1.0 and the rural to urban ratio was 1.5:1.0.

Although Aluminium Phosphide is a protoplasmic poison affecting all the organs of body, but the focus of present study is heart, one of the criteria of Bichat's Tripod of life. The reason behind it lies in the fact that it is the most vital organ to get affected and the cause of lethality in first 24 hours^[7-9].

CLINICAL MANIFESTATIONS :^[10]

The effect of poisoning is due to some reversible factors leading to disturbance in the permeability of Sodium, Potassium, calcium and Magnesium leading to changes in trans-membrane action potential due to focal myocardial involvement and subsequent myocardial necrosis. Direct toxic effect of celphos on myocardium is hypomagnesemia brought on by focal myocardial damage, leading to arrhythmias. Hypotension and shock is evident within 3-6 hours of ingestion of celphos. In persons, who survive the poisoning, the cardiotoxicity and hypoxia disappear within 5-7 days, due to excretion of PH₃ and restoration of normal cellular metabolism. The chemical myocarditis is responsible for varied fatal ECG changes. These symptoms are marked generally after 6-24 hours of ingestion among non-survivors. It leads to Ventricular ectopic beats, conduction disturbances, LBBB, ventricular fibrillation, aberrant conduction and idioventricular rhythm terminally leading to asystole.

Death in first 24 hours appears to be cardiogenic as evidenced by shock and ECG abnormality while survivors show complete ECG recovery.

Most of the patients who come into contact, even accidentally, succumb to its toxicity because of the gap between ingestion and initiation of treatment, because of its fatal period which is as low as 1-3 hours and range up to 3-4 days. Signs and

symptoms of mild poisoning are similar to those of upper respiratory infections. Histopathological findings can reveal the consequences of consuming the poison at tissue and cellular level & can be utilized for finding its antidote which is not available till date.

Heart shows characteristic pallor area of infarction with swelling because swollen fibres squeeze the blood. The bundles of muscles seem separated and on cutting the ventricle at autopsy table, the affected muscle shows more coarsely fibrillar arrangement than the normal area.^[1]

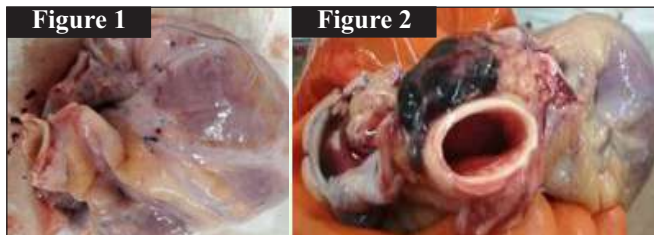
CASE DETAILS:

Dead body of a 30 year old house wife (belonging to low socio economic farmer family) was brought to the post-mortem room after 2 hours of death documented in hospital records. She was living in a joint family with two children. History revealed no previous medications but depressive personality was mentioned. Her social interactions were less with a tendency to remain aloof. The physique showed physical weakness and malnutrition.

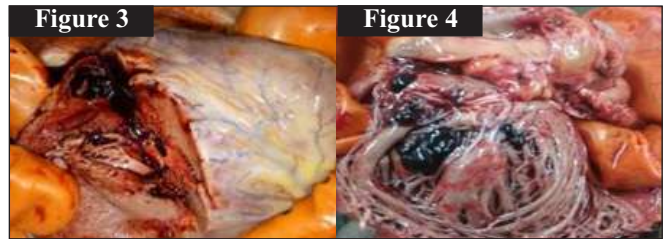
Heart was preserved in 10% formaldehyde and refrigerated at 4° C for 30 days. Fixed samples were taken for Scanning Electron Microphotograph analysis to CDRI Lucknow. Samples were treated with 1% osmium tetra oxide for 1 hour. Traces of Osmium tetra oxide were removed by washing the samples with PBS buffer, then dehydrated through graded series of Alcohol (30%, 50%, 70%, 90% and finally absolute) and later subjected to Critical Point Dehydration. Samples were mounted over Aluminium stubs and coated with film of Gold-Palladium using sputter coat unit. Processed samples were examined under SEM for imaging. 14 micrographs were taken of single heart samples from different viewing angles focusing a particular area at different magnifications.

Autopsy Findings:

Naked eye examination showed minute petechial haemorrhages on the atrial surface of heart (Figure 1) and hematoma over root of ascending aorta (Figure 2).



Further examination revealed generalised congestion of myocardium along with focal myocardial infarction patches & epicardial haemorrhagic spots over left atrial surface (Figure 3) as well as Sub-endocardial haemorrhagic area visible over right atrium along with oedema of myocardium (Figure 4).



Scanning Electron photomicrograph of the cardiac muscles cut in the longitudinal plane of heart at 6000X (Figure 5): showed rupture and separation of striated muscle fibres at places along with separation of intercalated disc. The picture also depicted the areas of focal myocardial necrosis & rhexis (can also be seen in cases of coagulative myonecrosis) occurring due to coagulation of hyper contracted sarcomeres.

Scanning Electron photo micrograph at 3000X : showed swollen myocardial fibres with separated myofibrils as well as intercalated discs, areas of focal myocardial necrosis and myocytolysis (Figure 6).

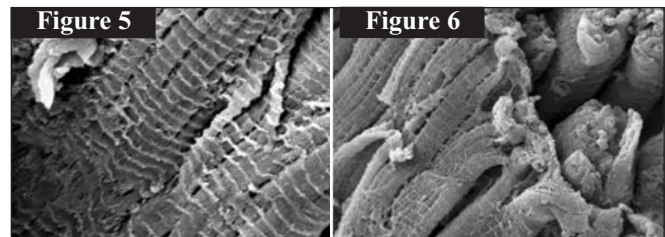


Figure 7: showed soft and flabby myocardium with cytoplasmic vacuolation (also known as round cells).

Figure 8 : showed swollen and separated (spindle shaped at few places) myocardium with round cell infiltration' separated and prominent myofibrils with focal necrosis, Beaded appearance of granulated cytoplasm, Separated myofibrils along with edema of muscle fibres and characteristic branching pattern of striated (heart) muscle fibres.

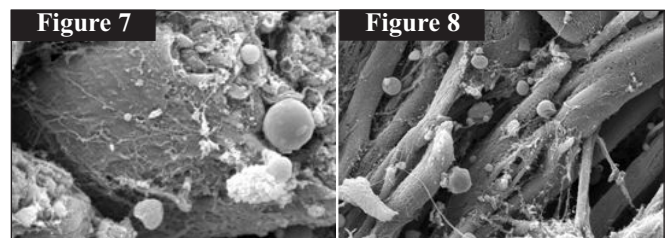
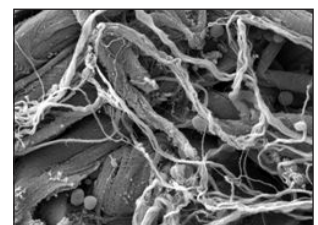


Figure 9 (In 6000X) : showed well separated myofibrils (at places branched/ accumulated and entangled striated muscle fibres), few single myofibrils along with granularity of



cytoplasm (Polymorphonuclear Cells infiltration), also showing areas of myocytolysis with vacuolation of cytoplasm. The muscles showed coarse, beaded (round cells) and areas of focal necrosis.

DISCUSSIONS : Hashemi-Domemeh et al^[12] reported cardiac failure, severe and persistent hypotension, heart congestion, sub-endocardial infarction, pericarditis, cardiac fibres detachment (due to edema & fibre destruction) in celphos poisoning.

Eroschenko Victor P^[13] has described that cardiac muscle fibres exhibit cross-striations, branching and a single central nucleus. The dark staining intercalated discs connect individual cardiac muscle fibres. Small myofibrils are visible within each cardiac muscle fibres. Delicate strands of connective tissue fibres surround the individual cardiac muscle fibres. .

Aggrawal A^[14] has described that the heart shows edema (swollen muscle fibres) with fragmentation of fibres and degeneration of myocardial muscles along with areas of myocytolysis in celphos poisoning. The features of toxic myocarditis and focal myocardial necrosis were evident in such cases. The histopathology of heart showed epicardial haemorrhages, especially in survival of more than one day. The heart was soft and flabby. The heart showed areas of focal myocardial necrosis along with areas of myocyte vacuolation (round cell infiltration) .

Vittorio Fineschi et al^[11] have observed colliquative myocytolysis, increasing edema and vacuolation (according to histology patterns of different forms of myocardial damage, contractile cycle and coronary heart disease

Ashok Kumar Jain et al^[15] on Autopsy findings of Aluminium phosphide poisoning showed Cardiac muscles with congestion, focal myocardial necrosis and round cell infiltration.

Vij K^[5], has written the side by side swelling of cardiac muscle fibres and granularity of the cytoplasm along with loss of integrity of the sarcolemma which leads to release of intracellular proteins into the extracellular spaces from the myocytes.

Siwach et al^[16-17] described that celphos contribute to granularity of the cytoplasm, more coarsely fibrillary arrangements leading to cross band formation, Coagulative myocytolysis, irreversible hyper contraction of myocardial muscle fibres (tetanic death), which occurred in early hours of myocardial damage (rhexis which means anomalous irregular cross band formation and takes place at myofibrils level)

REFERENCES:

1. Kotrly M, Turkova I, Grunwaldova V. Forensic Science Scanning Electron Microscopy and news in this field,

- Microscope Microanal15,July, 2009 Pp:798.
2. Bailey R. The heart wall. October 18,2017. [cited: December 1, 2018]. Available from: <https://www.thoughtco.com/the-heart-wall-4022792>
3. Pillay V V. Modern Medical Toxicology. 4th edition. Jaypee Brothers Medical Publishers. 2013. Pp-71
4. Vij K. Textbook of Forensic Medicine and Toxicology: principles and practice. Elsevier India; 2014. Pp-104
5. Reddy KSN. The Essentials of Forensic Medicine and Toxicology. 33rd edition. Jaypee Brothers Medical Publishers; 2014. Pp-527
6. Ranga GS, Dwivedi S, Agarwal M, Kumar D. Aluminium phosphide poisoning in a young adult: a suicidal cardiotoxin simulating myocardial ischaemia. J Ind Acad Clin Med. 2004;5:369.
7. Raman R, Dubey M. The electrocardiographic Changes in Quickphos poisoning. Ind Heart J. 37(3);1985:193–195.
8. Singh K, Oberoi S. S, Bhullar D.S.Poisoning trends in the Malwa Region of Punjab. J Punjab Acad Forensic Med Toxicology2003:26–29.
9. Gupta B.D, Hapani J.H, Shah V.N.Current trend of poisoning in Jamnagar. J Indian Acad Forensic Med;2006;28(3):90–2
10. Verma V. K, Gupta S.K, Parihar A. Aluminium Phosphide Poisoning: A Challenge for the Physician. JK Science,2001; Vol. 3:14
11. Fineschi V, Baroldi G, Silver M. D. Pathology of the Heart and Sudden Death in Forensic Medicine. CRC Press, Taylor and Francis group; 2006. Pp- 41
12. Hashemi-Domeneh B, Zamani N, Hassanian-Moghaddam H, Rahimi M, Shadnia S, Erfantalab P, et al. A Review of Aluminium Phosphide poisoning and a Flowchart to treat it. September 2016. Available from : <https://www.ncbi.nlm.nih.gov/pubmed/27749266>
13. Eroschenko VP. Defiore's Atlas of Histology with functional correlations, Indian edition. B.I. Publication Pvt. Ltd 10, (2005), Pp:116
14. Aggrawal A. Textbook of Forensic Medicine and Toxicology. Avichal Publishing Company; 2016. Pp- 607-609.
15. Jain A.K, Nigam M, Garg S.D, Dubey B.P, Arora A. Aluminium Phosphide Poisoning Autopsy Findings. JIAFM. 2005:35-38
16. Siwach SB, Yadav DR, Arora B, Dalal S, Jagdish. Acute aluminium phosphide poisoning–An epidemiological, clinical and Histo-pathological study. J Assoc Physicians India. 1988;36(10):594–596
17. Singh S, Dilwari JB, Vashisht R, Malhotra HS, Sharma BK. Aluminium phosphide ingestion. British Medical Journal. 1985;40:110–111.

A Case Report

Human Genetic Identification in Paternity testing by multiplex STR analysis using ABI 3500 xl Genetic Analyzer

1. **Monika Chakravarty**, Reporting officer, FSL Delhi
2. **Nidhi Singh**, LA Biology, FSL Delhi
3. **Damini Varshney**, SA Biology, FSL Delhi
4. **Dhruv Sharma**, AD Biology, FSL Delhi
5. **Naresh Kumar**, SSO Biology, FSL Delhi
6. **Prateek Pandya**, Assistant Professor, Amity University, Noida
7. **Anupama Raina**, Senior Scientist, All India Institute of Medical Sciences, Delhi.

ABSTRACT :

The present study explores the use of 15 STR loci D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, FGA in addition to a gender identification marker, amelogenin in forensic investigation & paternity testing.

The 15 molecular markers were analysed for human identification in a case of disputed paternity. AmpFI STR Identifier™ plus PCR amplification kit was used for DNA analysis using STRs. The amplified loci were separated through capillary electrophoresis on Applied Biosystems 3500xl Genetic Analyzer as per the manufacturer's recommended protocol. The data was analysed using Gene Mapper™ 1.4 software.

The statistical analysis was performed by Bayesian mathematics using in-house allele frequency data. The paternity index & probability of paternity were 41,431,267.4214075 & 99.9999975863640 respectively. DNA analysis conducted on the samples of mother, child and the disputed father convincingly established the paternity of the child. The Combined Paternity Index (CPI) of the molecular markers & probability of paternity in human identification are very high. The results of analysis of 15 molecular markers alongwith their biochemical characterization (including the chromosomal location of the markers & repeat unit sequence) conclusively establishes the paternity of the child & the genetic identification.

Corresponding Author

Monika Chakravarty,

Forensic Science Laboratory, Home Department, Govt. of NCT Delhi, Rohini,

Contact : +91 9868931045

E-mail : monika42@gmail.com

Article History:

Received: 28 November 2018

Received in revised form: 25 January 2019

Accepted on: 25 January 2019

Available online: 1 June 2019

KEYWORDS : DNA, STR, Paternity, PI, CPI

INTRODUCTION :

Human identity testing using DNA profiling method has been widespread since it was first described in 1985 by Jeffrey's et al.^[1]. Human genome comprise of 3 billion base pairs; Adenine A, Cytosine C, Thymine T & Guanine G. While 99.9% is same from one to the next, certain regions are highly polymorphic, comprising of repeat sequences which may be repeated in tandem or are interspersed repeats scattered throughout the genome^[2]. VNTRs could be 10-100 bases repeat microsatellites & STR are 2-6 bases repeat which are commonly used in forensics. They occur frequently in genome, are easily amplified by PCR, many markers can be multiplexed at a same time, are highly polymorphic, have low

mutation rate & are found on separate chromosomes hence are not linked. They are designated by the length of the core repeat unit & the no. of repeat units^[3]. The markers or the loci comprising of the short tandem repeat sequences are analysed in human identification. DNA analysis has undoubtedly revolutionized the field of criminal justice system. The markers are amplified through PCR by fluorescent dye labeled primers hybridizing to the forward & reverse flanking regions of these STRs. The genetic identification by molecular markers is based on the principle of Mendelian inheritance. All the alleles of the loci in an individual must be inherited from its biological parents. The genetic identification is dependent on the allele frequency of the STR loci^[4]. Statistics are required to

evaluate the strength of the inclusionary observation^[5].

MATERIAL & METHODS:

Automate Express™ Forensic DNA Extraction system was used for the extraction of genomic DNA from a paternity casework samples comprising blood samples of mothers, child & disputed father. The method uses surface chemistry and magnetic particles to provide maximum DNA recovery removing PCR inhibitors. Magnetic particles are separated at the elution step yielding purified DNA^[6]. Thermo Scientific™ Nanodrop 2000 UV- Vis spectrophotometer is used to quantify DNA^[7].

Multiplexed PCR amplification of 15 STR loci along with the sex determining amelogenin loci was performed using the Amp Fl STR Identifier plus PCR amplification kit, on Applied Biosystem proflex 96- well PCR system. The characteristics of the 15 STR loci are shown in **Table 1**.

Table 1: The Characteristics of the 15 STR Loci Examined in this Study

Locus Designation	Chromosome Location	Repeat motif	Primer lable
D8S1179	8	TCTA	6- FAM
D21S11	21q11.2-q21	TCTA	6- FAM
D7S820	7q11.21-22	GATA	6- FAM
CSF1PO	5q33.3-34	AGAT	6- FAM
D3S1358	3p	TCTA	VIC
THO1	11p15.5	AATG	VIC
D13S317	13q22-31	TATC	VIC
D16S539	16q24-qter	GATA	VIC
D2S1338	2q35-37.1	TGCC	VIC
D19S433	19q12-13.1	AAGG	NED
vWA	12p12-pter	TCTA	NED
TPOX	2p23-2per	AATG	NED
D18S51	18q21.3	AGAA	NED
D5S818	5q21-31	AGAT	PET
FGA	4q28	TTTC	PET

The PCR was carried out using 5 µl reaction mixture, 2.5 µl Primer mixture and 1ng input DNA with the amplification condition & as- an initial denaturation at 95 °C for 11 minutes, followed by 28 cycle of 94 °C for 20 seconds, 50 °C for 3 minutes and final extension of 60 °C for 10 minutes^[8].

The amplified product/amplicons were separated through capillary electrophoresis on Applied Biosystems Genetic Analyser, ABI 3500xl as per the manufacturer's protocol. 0.5

µl of each PCR reaction product was added to 10 µl Hi Di formamide & 0.3 µl of Gene Scan 500 LIZ size standard. PCR products were denatured at 95 °C for 3 minutes and chilled for 3 minutes at 4°C.

Electrophoresis was performed using the Performance optimized Polymer 4 (Applied Biosystems) with 36 cm 24 capillary lengths. The data was analysed using Gene Mapper™ IDx software v 1.4. The statistical analysis was performed by Bayesian mathematics using in house allele frequency data, constructed from 200 unrelated individuals.^[9] (**Table 2**)

Table 2: The Locus Wise Possible Number of Alleles

Locus	Possible Alleles	Total Alleles
D8S1179	8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18	11
D21S11	27, 28, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33.2, 34, 34.2	13
D7S820	7, 8, 9, 10, 11, 12, 13, 14	8
CSF1PO	7, 9, 10, 11, 12, 13, 14, 15	8
D3S1358	13, 14, 15, 16, 17, 18, 19	7
THO1	6, 7, 8, 9, 9.3, 10	6
D13S317	8, 9, 10, 11, 12, 13, 14, 15	8
D16S539	8, 9, 10, 11, 12, 13, 14	7
D2S1338	16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28	13
D19S433	10, 11, 12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17	12
vWA	13, 14, 15, 16, 17, 18, 19, 20	7
TPOX	6, 7, 8, 9, 10, 11, 12	7
D18S51	9, 10, 11, 12, 13, 14, 14.2, 15, 16, 17, 18, 19, 20, 21, 22	15
D5S818	9, 10, 11, 12, 13, 14	6
FGA	18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 32.2	11
AMELOGENIN	Amelogenin X:106 base pairs and Amelogenin Y:112 base pairs	139

RESULTS AND DISCUSSION:

The molecular markers located on different location in the chromosomes were examined. The alleles of all examined loci in the child matched completely with the mother & the disputed father. The paternity index & probability of paternity were 41,431,267.4214075 & 99.9999975863640. The results are summarized in **Table 3**. The allele frequency of in- house generated database were used for calculation. Here, the electropherogram of the mother, child & father respectively showed a non- excluded paternity case. In this case no genetic

discrepancy for the child – alleged father relationship was observed in any of the 15 STR loci. All alleles of the child at all STR loci tested were found to be present in both the mother & the alleged father.

Table 3: The Paternity Examination of Child(At each Locus, the Child Received one STR Allele from his Mother & the other from his Alleged Father)

Locus	Mother	Child	Father	PA	LOP	PI
D8S1179	15,16	14,16	14,15	14	.5	3.9215
D21S11	32,2,33,2	31,2,33,2	31,31,2	31,2	.5	4.2553
D7S820	9,12	7,12	7,10	7	.5	28.5714
CSF1PO	10,11	11,12	11,12	12	.5	1.3422
D3S1358	17,17	15,17	15,16	15	.5	1.5873
THO1	7,9,3	9,9,3	9,9	9	1	3.6697
D13S317	11,12	11,12	9,12	12	.5	1.9230
D16S539	11,12	11,13	12,13	13	.5	5.7142
D2S1338	18,23	23,23	18,23	23	.5	2.5316
D19S433	13,16	14,16	12,14	14	.5	2.0618
vWA	17,19	14,17	14,17	14	.5	3.9215
TPOX	8,8	8,11	8,11	11	.5	1.5873
D18S51	18,20	15,18	12,15	15	.5	2.7397
D5S818	10,12	11,12	11,13	11	.5	1.25
FGA	23,23	19,23	19,21	19	.5	9.0909
AMELO	XX	XY	XY			
-GENIN						

Each markers is assigned with a paternity Index (PI), which is a statistical measure of how powerful a match at a particular marker gives paternity. The PI of each markers is multiplied with each other to generate the Combined Paternity Index(CPI) which indicates the overall probability of a person being the biological father of the child in question in relation to any random man from the population. The CPI is the then converted into a probability of Paternity showing the degree of relatedness between the alleged father & the child^[10].

CONCLUSION :

In the present case 15 STR loci were multiplexed & used for paternity testing. PCR-based STR analysis has been used for paternity testing worldwide.^[11-14] The DNA profiling for human genetic identification is based on Mendelian inheritance. These STR loci are in the non- coding region of genome & are not linked. The paternity index & probability of paternity was calculated which provides with the evidence that the alleged father is the biological father of the child. The STR loci are from the mutation free zone. In paternity & identity testing the allele frequency database obtained from the population to

which the person in question belongs was used in the calculations^[15].

CPI - 41,431,267.4214075

POP - 99.9999975863640

PA : Paternal Allele

LOP : Likelihood of Paternity

PI : Paternity Index

CPI : Combined Paternity Index

POP : Probability of Paternity

REFERENCES :

1. Jeffreys AJ, Wilson V, Thein SL. Hypervariable minisatellite regions in human DNA. *Nature*.1985;314: 67-73.
2. Edward AL, Civitello A, Hammord .H.A, Caskey T, Am.J. Hum.genet.1991;49:746-756
3. H. Ellegren. *Nat. Rev. Genet.*2004;5: 435-445.
4. Singh ND, Shrivastava P, Das SP. Biochemical Characterization of Molecular Markers for Human Genetic Identification in Paternity testing by DNA profiling. *Asian Journal of Biomedical and Pharmaceutical Sciences*. 2014;04(37):52-56
5. Charabarty R, Deka R. DNA Forensics: a population genetic and biological anthropological perspective. *Physical (Biological) Anthropology. Encyclopedia of life support system(ELOSS).*2014
6. Automate Express™ Forensic DNA Extraction system, Product Bulletin Human Identification, Applied Biosystems.
7. Nanodrop 2000/2000 c spectrophotometer v 1.0 user Manual, Thermo Scientific.
8. AmpFI STR Identifier Plus PCR Amplification kit user's Guide Publication Number 444021
9. Chakravarty M, Singh N, Sharma D, Kumar N, Pandya P, Raina R. STR analysis for random Delhi-NCR population sample using AmpFI STR Identifier plus PCR amplification kit. *Anil agarwal internet journal of forensic medicine & toxicology*. 2018;july-dec;vol.19(2).
10. Subramanyam G, Damerla H, Reddy G(2012) Paternity Testing through multiplex PCR based STR Genotyping of Genomic DNA. *J.Bio, Innov* 1(2), pp: 41-47,.
11. Jacewicz R, Berent J, Prośniak A, M Kadlubek, Szram S. The evaluation of the Identifier system in paternity testing in Poland.
12. Jacewicz R, Babol K, Berent J, Prośniak A, Szram S(2005) Practical usefulness of the identifier system for paternity testing in the Central Poland population. *Arch Med Sadowej kryminol.* Apr-jun;55(2):151-3
13. Rerkmnaychoke B, Chantratita W, Jomsawat U, Thanakitgosate J(2000)Paternity testing by PCR- based STR analysis.*J Med Assoc Thai.* Mar;83 suppl 1:S55-62.
14. Chakravarty M, Singh N, Varshney D, Sharma D, Kumar N, Pandya P, Raina A (2018) Relevance of DNA Fingerprinting Technology in criminal justice system:Case study in DNA profiling.
15. El-Aly SH, Ahmed F, Abd El- Hafez. Paternity testing and Forensic DNA typing by multiplex STR analysis using ABI Prism 310 Genetic Analyser. *Journal of Genetic Engineering and Biotechnology*. 2012;10:101-12

Review Article

Application of Saliva in Forensic Odontology : An Update

1. **Abraham Johnson**, Assistant Professor (Forensic Odontology) Institute of Forensic Science, Gujarat Forensic Sciences University, Sector 9, Gandhinagar, Gujarat - 382007

ABSTRACT :

Over the last few years, investigators and researchers have been repeatedly magnetized by application of saliva in the forensics. Popularity saliva has gained amongst other body fluids owed to its multipurpose qualities that include easy and painless collection, hazard free handling, close relationship with plasma and resourceful composition. This review emphasizes on the comprehensive use of saliva in forensics including crime detection, personal identification, alcohol and drug abuse, hormone identification and serological testing.

Corresponding Author :

Dr. Abraham Johnson,

Assistant Professor (Forensic Odontology) Institute of Forensic Science, Gujarat Forensic Sciences University, Sector 9, Gandhinagar, Gujarat - 382007

Contact : +91- 8849130278

E-mail : abraham.johnson@gfsu.edu.in

Article History:

Received: 13 October 2018

Received in revised form: 12 December 2018

Accepted on: 12 December 2018

Available online: 1 June 2019

KEYWORDS : Forensics, labortary testing, investigating tool, personal identification, saliva.

INTRODUCTION:

Mandel in 1990 quoted that "saliva lacks the drama of blood, the sincerity of sweat and the emotional appeal of tears"^[1]. But now saliva is blooming as an adjunct to both blood and sweat. Saliva is evolving as a diagnostics tool as it is accessible, inexpensive, non-invasive and easy-to-use diagnostic aid. At crime scenes where oral cavity is involved along with bite marks saliva can also be traced. If obtained saliva is subjected for serological and cellular analysis, it can help in identification of the accused. In cases where bite marks of unknown animals cannot be identified saliva collected from the site helps in studying genetic profile and thus helps to spot out the particular animal in question. Recently saliva is gaining popularity in detecting drug abuse and drunk drivers. In patients of heavy metal poisoning ionic imbalance can be reflected in saliva, also some poisons excrete through oral cavity. In clinically difficult situations, where blood sampling could be a difficult act to perform such as in children, handicapped and anxious patients saliva can be used^[1,2].

In past forensic investigations have revealed saliva which is deposited in prints, bite marks, cigarette butts, marks and prints left on stamps and other objects helps in individual identification to a greater extent^[3] because saliva consists of exfoliated oral epithelial cells, microorganisms, DNA and RNA proteins^[2,3]. When saliva enters the oral cavity it makes its way through the salivary ducts and coalesces with blood cells, micro-organisms of the oral cavity, food and upper airway secretions. So this increases the chances of saliva containing vital information about the quality of life, health and general

wellbeing of a person^[4].

In forensic odontology the dearth of the contribution of saliva in literature evokes the study and compilation of previous literature to outline the interesting topic of saliva into an inclusive review. Hence, the current review article emphasize on the use of saliva in forensics.

ROLE OF SALIVA IN IDENTIFICATION

In numerous murders, attack and other criminal cases saliva is stored in nibble marks^[5]. Since saliva stays imperceptible this makes salivation stains hard to gather from skin, garments, paper or other lifeless things. In addition spit stored on substrate (fundamentally skin) can't be specifically submitted to extraction procedures^[5,6].

METHODS FOR SALIVA RECOVERY FROM SKIN

Single Swab System: This is otherwise called established technique. In this technique, single wet cotton swab or wet channel paper recoups spit from skin surface^[7].

Two-Fold Swab System: This procedure is like single swab strategy took after by second utilization of dry cotton swab. This was contemplated by Sweet et al^[8]. They discovered twofold swab procedure yields the spit better from the skin surface when contrasted with single swab system. It is trusted this is because of the way that the dampness show in the primary swab rehydrates and extricates most of the epithelial cells dried in the salivation and make them stick to the cotton strands of the swab. At the point when the second (dry) swab is connected to the site, the cells in the salivation can hold fast to the strands all the more effectively in light of the fact that they

are rehydrated after the utilization of dampness from the primary swab and the time slipped by since the principal swabbing. Subsequently, the twofold swab strategy for recuperating salivation from human skin licenses gathering of a bigger measure of DNA confirm than the established strategies considered^[7].

METHODS TO DETECT DRIED SALIVA STAINS

Chemicals: Dried spit stains can be identified from stamps and envelopes by utilizing soluble phosphatase, starch and amylase which gives a red insoluble accelerate. Nitrate and thiocyanate salts have likewise been utilized for the detection^[9].

- Lasers and bright light, quartz curve tube and argonion laser^[2].
- Fluorescent spectroscopy: Tryptophan is a sweet-smelling amino corrosive present in α -salivary amylase. It have a decent affectability in recognizing dried spit recolors on the skin in this way giving trademark emanation range on fluorescent spectroscopy^[9].
- Immunological methods: ELISA is not widely used method. Alpha -amylase activity in saliva stains is detected using a horseradish peroxidase conjugate which is combined with monoclonal antibodies^[10].

INVESTIGATION OF DRUGS OF ABUSE IN SALIVA

Urine is the most incessant and dependable organic example for medicate checking. Coordinate watched accumulation of pee is prudent to anticipate corruption however this prevents the security of individual. Because of enhanced explanatory systems vast number of medications can be distinguished in little measure of oral fluid^[11].

Medications enter the salivation by straightforward inactive dispersion which is portrayed by the exchange of medication particles down a focus inclination with no consumption of energy^[7]. Numerous medications like amphetamines, barbiturates, benzodiazepines, phencyclidine, cocaine and opioids can be distinguished in saliva^[12]. Various specialists have utilized radioimmunoassay (RIA) to identify pot, phenobarbital, amphetamine and morphine in salivation and salivary strains^[1-13].

As of late, Securetec (Ottobrunn, Germany) presented Drug wipe, which is utilized for the discovery of medications of mishandle on surfaces. It is a pen measured, immunochemical based test strip. Isolate tests are accessible for sedatives, cocaine, cannabinoids and the amphetamine gathering. The wiping part empowers the client to test medicate particles from any sort of surface^[14].

Peel et al. discovered quantifiable amounts of medications

in salivation removed with methanol and broke down by protein different immunoassay procedure and gas chromatography/mass spectrophotometry. This approach, both particular and delicate seems, by all accounts, to be a valuable assistant to serological testing in nibble marks for recognizable proof purposes. Various medications for example, phenobarbital, amphetamine and morphine have been distinguished in salivation and spit recolors by radioimmunoassay (RIA) by various agents^[15].

DEOXYRIBONUCLEIC ACID PROFILING / FINGERPRINTING :

Difficulty in analysis arises when traditional identification methods fail because of the effects of heat; trauma and autolytic processes then the techniques involving DNA analysis are of great dependency^[16]. DNA profiling is novel for each person. In spite of the fact that dirt, microscopic organisms which are wealthy in nucleases affect security of human DNA yet they didn't represent an awesome danger as the tests utilized as a part of DNA profiling are particular to person only^[17].

As saliva contains leukocytes and exfoliated epithelial cells from the host it acts as a very useful DNA source. Blood can also be a source of DNA but the difference lies in the fact that saliva is easy to access, collect and handle for diagnostic procedure since it doesn't clot. Also saliva collection is a non invasive and safer than venepuncture which leads to lesser chance of cross infection^[18].

A major drawback in using saliva is that a very small amount is available for molecular diagnostics. However, with help of Polymerase chain response (PCR) replication of thousands of duplicates of a particular DNA succession in vitro can be obtained¹⁹. The premise of every single acquired polymorphism exists in the DNA atom and they are novel and also don't change over the lifetime of a person. DNA tests are intensified by polymerase chain response for DNA writing utilizing short couple rehashes (STRs)^[8]. Short Tandem Repeat (STR) are DNA markers, also referred to as microsatellites or simple sequence repeats (SSRs), consisting of repeated DNA sequences with a core repeat of 1–5 base pairs (bp). The polymorphic repair of STR essentially in little parts additionally makes it conceivable to assess DNA from tests with a noteworthy review of degradation^[19].

Microsatellite loci are invested with a few properties that render them attractive markers for maternity/paternity assurance and scientific examination, because, in conjunction with the amplification process, they require as little as 0.5ng of DNA template^[20].

A mitochondrial DNA (mtDNA) cell is present in saliva.

The main advantage of mtDNA is that there is a high copy number in each cell caused by the high number of mitochondria present in most cells^[21]. Chromosomal DNA is inherited from both the mother and father whereas mtDNA is strictly maternally inherited^[22], thus when identifying a set of human remains, if close relatives are not available, distant maternal relatives can be used as a reference source for identification^[23]. Therefore, mtDNA testing may be successful when nuclear DNA testing fails or when genomic DNA cannot be analyzed, possibly because it is too degraded^[21].

SEX DETERMINATION FROM SALIVA IN BITEMARKS:

The likelihood of getting peeled buccal epithelial cells in salivation on nibble marks has expanded the likelihood of sex assurance of the culprit. The span of this line of request is clearly feasible for a little while, post affidavit, contingent upon the materials containing the impressions and ecological components. Thus Saliva becomes a useful DNA source because it contains leukocytes and exfoliated epithelial cells from the host; this increases the possibility of sex determination of the suspect. Two parameters can be used to identify the sex that is detection of sex chromatin. Barr bodies in case of females and F bodies in case of males can be observed and the levels of sex hormones; testosterone and 17B-estradiol can be determined by radioimmunoassay^[17].

SALIVARY BIOMARKERS:

Saliva contains DNA, RNA and proteins which carry vital information about person and these are considered to be of great value to the forensic odontologist. Saliva biomarkers have been used in diagnosis of various diseases but they have wider role in forensics. Although saliva helps in cases involving bite marks but is also useful in identification of people in situations of mass disasters. Salivary biomarkers for any particular disease can be matched and tracking that disease to hospital visits positive identification can be arrived. So in this way saliva biomarkers can be used to identify people who's DNA are not on any database^[4].

DETERMINATION OF HORMONE LEVELS:

The nearness of different hormones in saliva helps in deciding the mind of a person^[24]. Recently saliva is used in detecting steroid hormones such as cortisol, cortisone and testosterone as it is an accepted thing that salivary level of these steroids reflects the free, unbound circulating fraction^[25]. These hormones help in determining the mental state of person. Nearness of high testosterone in spit has demonstrated people to be rough, less consistent and related with individual mischief^[24]. Low morning basal salivary cortisol levels combined with a more noteworthy number of awful cerebrum

wounds and later manhandle could likewise be related with perilous fierce criminal conduct^[26].

CONCLUSION:

When most people think of saliva; which they rarely do it as a convenience, handy for licking stamps and sealing envelopes. Practicing dentists find it's a nuisance, to be sponged, evacuated or dammed. But saliva is much more than water. Saliva is a most valuable oral fluid. Over the years in forensic laboratories the need and importance of saliva as an investigative body fluid is increasing rapidly due to its wide range of use in forensics. Automated settings for saliva should be present for saliva as are routinely done for blood and urine. With constant examination and inquire about, and a communitarian work serological and odontological fields, salivation is developing as a fundamental device for scientific examination. The part of a scientific odontologist is likewise growing more. With a careful learning of a standout amongst the most powerful things that at a dental specialist faces each time he/she works in a mouth, the significance of an odontologist in a criminal examination group may create manifolds. One can dare to dream, and ponder what more is holed up behind each drop of spit one has in the mouth. More research dedicated towards this particular innocuous body fluid should be aimed at for gaining detailed information in forensic sciences.

REFERENCES:

1. Aps JK, Martens LC. Review: The physiology of saliva and transfer of drugs into saliva. *Forensic Sci Int* 2005;150:119-31.
2. Saxena S, Kumar S. Saliva in forensic odontology: A comprehensive update. *J Oral Maxillofac Pathol* 2015;19:263-5.
3. Madall VBi, Basavaraddi SM, Burde K, Horatti P. Saliva- A Diagnostic Tool. *IOSR Journal of Dental and Medical Science*.2013;11(6):96-99
4. Rajshekar M , Tennant M, Thejaswini. Salivary biomarkers and their applicability in forensic identification. *Sri Lanka Journal of Forensic Medicine, Science & Law*.May 2013;4(1):10-15.
5. Anzai-Kanto E, Hirata MH, Hirata RD, Nunes FD, Melani RF, Oliveira RN. DNA extraction from human saliva deposited on skin and its use in forensic identification procedures. *Braz Oral Res* 2005;19:216-22.
6. Sweet D, Shutler GG. Analysis of salivary DNA evidence from a bite mark on a body submerged in water. *J Forensic Sci*.1999;44:1069-72.
7. Mago J, Sidhu L, Kaur R, Kamal, Anurag T. Saliva In

- Forensics. *wjpmr*, 2016,2(4), 196-198.
8. Sweet D, Lorente M, Lorente JA, Valenzuela A, Villanueva E. An improved method to recover saliva from human skin: The double swab technique. *J Forensic Sci* 1997;42:320-2.
9. Nanda KD, Ranganathan K, Umadevi K, Joshua E. A rapid and noninvasive method to detect dried saliva stains from human skin using fluorescent spectroscopy. *J Oral Maxillofac Pathol* 2011;15:22-5.
10. Kelly Virkler, Igor K. Lednev. Analysis of body fluids for forensic purposes: From laboratory testing to non-destructive rapid confirmatory identification at a crime scene. *Forensic Science International* 2009;188:1-17
11. Vindenes V, Lund HM, Andresen W, Gjerde H, Ikdahl SE, Christophersen AS, et al. Detection of drugs of abuse in simultaneously collected oral fluid, urine and blood from Norwegian drug drivers. *Forensic Sci Int* 2012;219:165-71.
12. Cone EJ. Saliva testing for drugs of abuse. *Ann N Y Acad Sci* 1993;694:91-127.
13. Rai B. Oral fluid in toxicology. *Internet J Toxicol*. 2006;3(2):1-5.
14. Samyn N, van Haeren C. On-site testing of saliva and sweat with Drugwipe and determination of concentrations of drugs of abuse in saliva, plasma and urine of suspected users. *Int J Legal Med* 2000;113:150-4.
15. Peel HW, Perrigo BJ, Mikhael NZ. Detection of drugs in saliva of impaired drivers. *J Forensic Sci* 1984;29:185-9.
16. Silva RHA, Musse JDO, Melani RFH, Oliveira RN. Human bite mark identification and DNA technology in forensic dentistry. *Braz J Oral Sci*. 2006; 5: 1193-1197.
17. Stimson PG, Mertz CA. *Forensic Dentistry*. Florida: CRC Press LLC; 1997.
18. Streckfus CF and Bigler LR. Saliva as a diagnostic fluid. *Oral Diseases*. 2002; 8: 69-76.
19. Khare P, Chandra S, Raj V, Agarwal S. Salivary Dna For Sex Determination And Forensic Individualization. *Journal of Forensic Medicine & Toxicology*. 2012;29(1):73-78
20. Gill P. Role of Short Tandem Repeat DNA in Forensic Casework in the UK-Past, Present, and Future Perspectives. *BioTechniques*. 2002;32:366-385.
21. Pretty IA, Sweet D. A look at forensic dentistry – Part 1: The role of teeth in the determination of human identity. *Br Dent J* 2001;190:359-66.
22. Hutchison CA, Newbold JE, Potter SS, Edgell MH. Maternal inheritance of mammalian mitochondrial DNA. *Nature* 1974;251:536-8.
23. Stavrianos C, Eliades A, Kokkas A. The role of DNA in forensic odontology (Part 1) DNA analysis methods. *Res J Med Sci* 2010;4:334-9.
24. Dabbs JM, Carr TM, Frady RL, Riad JK. Testosterone, crime, and misbehaviour among 692 male prison inmates. *Personality and Individual Differences*, Volume 18, Issue 5, 1995, Pages 627-633
25. Bhandari R, Kakkar T, Bhullar RK, Bansal H, Sandhu SV, Jassar S. Saliva– An adjunct in forensic odontology. *BFUDJ* 2011;2:144-7.
26. Brewer-Smyth K, Burgess AW, Shults J. Physical and sexual abuse, salivary cortisol, and neurologic correlates of violent criminal behavior in female prison inmates. *Biological psychiatry a journal of psychiatric neuroscience and therapeutics* 2004; 1: 21-31.

Review Article

A Survey of Techniques used for Ink Examination

1. **Mandeep Kaur Purba**, Research Scholar, Amity Institute of Forensic Sciences, Amity University, Uttar Pradesh, India
2. **Shruti Gupta**, Assistant Professor, Amity Institute of Forensic Sciences, Amity University, Uttar Pradesh, India
3. **Reeta Rani Gupta**, Senior Scientific Officer, Central Forensic Science Laboratory, CBI, New Delhi-110003, India
4. **S.K. Shukla**, Professor, Amity Institute of Forensic Sciences, Amity University, Uttar Pradesh, India

ABSTRACT :

In current scenario the field of questioned documents is not only dealing with examination of handwriting but also with the comparison and analysis of writing ink. Over the past years, questioned document examiners have struggled with various documents, their examination and identification with respect to inks. Examination of ink serves as an important key in establishing the authenticity of questioned documents. This article focuses on various techniques which have been used in recent years for scientific examination of ink. The techniques included are categorized on the basis of four broad categories i.e. chromatography, electrophoresis, spectroscopy and miscellaneous. This article will help examiners to select the appropriate method and conduct examination and identification of ink with the best suited method.

Corresponding Author

Mandeep Kaur Purba,

Research Scholar,
Amity Institute of Forensic Sciences, Amity University, Uttar Pradesh, India
Contact : +91 9818431667
E-mail : mandeep.kaur0792@gmail.com

Article History:

Received: 3 December 2018
Received in revised form: 31 December 2018
Accepted on: 31 December 2018
Available online: 1 June 2019

KEYWORDS : Questioned Documents, Ink Examination, Chromatography, Electrophoresis, Spectroscopy.

INTRODUCTION :

The usage of computers, emails, digital signatures etc. has increased in recent years. In spite of increase in digital world, handwritten signatures and documents are still used as legal binder. We still use pen, pencil or marker as common writing instruments^[1].

Signature serves a mode to prove the authenticity of the signer on various legal documents such as contracts, cheques, bills, wills etc. Documents may have obliteration also known as smeared over writing done by tampering or blacking out of some parts of the document/writing by some opaque material. Apart from obliteration, addition and alteration of text are also commonly seen in fraudulent documents but in various instances, the writer himself/herself blots outs, adds or alters some portion of the writing. Unnecessary overlapping of inks also lead to suspicion. Documents having additions, alterations, obliterations etc. are collectively termed as tampered documents and the determination of their authenticity becomes very crucial and an extensive examination is demanded in terms of ink. With the same reason, tampered documents and their examination of ink with advanced techniques is being demanded on an alarming rate, making it a most thrust area for research^{[1][2]}.

Writing instrument/s used to sign or write the legal document/s

also plays a vital role in determining their authenticity. Such writing instruments contain ink which is made up of two basic components i.e. colorant and vehicle. The dyes or pigments which impart color to the ink are colorants and the medium in which colorants are dissolved is known as vehicle. Sometimes, mixing of two or more colorants is also done in order to get the required color of ink. Vehicles contain lubricants, polymers, flow control agents and various other components which improvise the properties of the ink according to the need. The deposition and flow of ink on paper is determined by the solvent present in inks^[3].

A thin film of ink is deposited on the surface of paper. Diffusion and absorption of colored component present in ink takes place at a slower rate as compared to the volatile components as colored portion is intended for permanent attachment to the paper. Since, any mass manufacturing is not flawless, manufacturing of writing instruments also comprise of some imperfections due to which the ink deposition is never reliable^{[3][4]}.

It is necessary to analyze inks both physically and chemically to establish authenticity of a fraudulent document. Comparing two or more inks can help us determine:

- Whether the inks were manufactured by the same organization or unit

- Whether the inks are from same batch of manufacturing
- The first manufacturing date of the particular ink used^[4].

The range of writing instruments with varied inks are being utilized now-a-days. It is necessary to understand the composition and nature of various types of writing inks so as to select the best suited instrumentation technique for examination accordingly. Forensic examination of any questioned document will typically include microscopic and macroscopic examinations before proceeding to any test that consumes or destroys a portion of the sample. Microscopic examinations can be conducted with a stereomicroscope and a comparison microscope. The information provided by microscopic examination of a questioned document can help quickly identify the paper and class of pen used^[1, 3]. Further techniques are used after microscopic examination which can be broadly classified into four classes: chromatography, electrophoresis, spectroscopy and miscellaneous (includes all those techniques which are not a part of first three categories).

TYPES OF INKS :

Carbon ink : Carbon ink is the one of the oldest available ink and is also known as India ink. It is a simple form of ink in which amorphous carbon in the form of a compact cake with glue is present. Water glue medium is used to dissolve the grinded cake particles. Pigmented dyes are added to this liquid medium to impart color. These liquid carbon inks are available in market. These days animal glue has been replaced by shellac and carbon along with a wetting agent to help in mixing of these two ingredients. These inks are stable as they are not soluble in water, cannot be decomposed by air, light, heat, microorganisms or moisture^[4].

Fountain pen inks : Two variants of fountain pen inks are commonly used, iron-gallotannate ink and aqueous solutions of synthetic dyes. Modern formulation of ink contains synthetic dyes which impart blue color instantly on the paper surface and turns black with time due to reaction with oxygen. This is the sole reason for its name blue-black fountain pen ink. This type of ink is also stable in nature as it is not soluble in water and is not even affected by abrasion. The aqueous solution of synthetic dye ink was developed in 1950s and has been the most popular type of fountain pen ink used. Aqueous blend of synthetic dye inks are comparatively less stable than carbon or blue-black inks but they are popular because they impart bright and attractive color. Some of these inks fade gradually and might be solvable in water. In about 1953, improvised version of this was introduced which contained pigmented dyes like copper phthalocyanine which made them more stable and permanent^[4].

Ballpoint inks : These inks were first manufactured in Europe in 1939 and reached Argentina in about 1943. Reynolds ballpoint inks were spread across United States in about 1946. These inks comprise of a mixture of synthetic dyes and several glycol solvents. Sometimes, carbon or graphite is also added to the mixture to provide stability to the ink. About 50% of the ink consist of dyes. Various other substances such as fatty acids, resins, viscosity adjusters etc. are added to the ink to provide specific features. Among fatty acids, oleic is added most commonly to provide lubrication to the ball of the pen. Ballpoints inks manufactured earlier in 1950s were oil based whereas modern day ball point inks are glycol based inks. Chelated dyes are commonly used to impart color as they are stable. In 1968, ballpoint pen inks were improvised to pressurized ballpoint inks which consisted pressurized feed system in place of flow due to gravity. The characteristics of these inks were different from glycol based inks^[4].

Rolling ball marker inks : Rolling ball marker inks are water based inks containing organic liquids. They were first manufactured in Japan. Organic liquids like glycols as well as formamide are added to these inks in order to reduce the drying of the pen. The withholding capacity of these dyes range from good to poor depending upon the dyes added to them. Some dyes added to rolling ball marker inks have affinity towards cellulose fibers which are present in paper. Some inks are water resilient and are soluble only in powerful organic solvents like pyridine.^[4]

Fiber or porous tip pen inks : Fiber tip pen inks usually contain water or xylene and were manufactured in Japan in 1962. These inks have analogous components like those present in rolling ball marker and fountain ink. Water based fiber ink is water soluble whereas xylene based fiber ink is water resilient and is soluble only in organic solvents. Glycol solvents are added to these inks to avoid drying of fiber tip^[4].

Gel pen inks : Gel pen inks were also introduced by Japanese. It is the latest advancement in ink industry. Mainly four brands of inks were brought into market i.e. Uniball Signo, Zebra J-5, Pentel Hybrid and Sakura Gelly Roll. In spite of organic dyes, these inks contain insoluble colored pigments. Gel-pen inks give similar appearance like ball point inks. In spite of being a water based ink, its consistency is gel like instead of liquid. These are not solvable in water and organic solvents. It is difficult to compare and analyze these inks due to its physical properties^[4].

EXAMINATION OF INKS :

Chromatography : Chromatography is a technique of separation of components of a sample. The separation is carried in between a stationary phase and a mobile phase. This separation takes place due to affinity of the molecules of the

substance towards the stationary phase. The comparison and examination of ink has been made possible because of chromatographic techniques which help in detection of questioned documents^[5].

LaPorte *et.al.* (2003) identified 2-Phenoxyethanol in Ballpoint Inks using gas chromatography/mass spectrometry (GC-MS). 2-Phenoxyethanol is a common volatile compound which is found in about 80% of ballpoint inks and evaporates with ageing of ink. They examined 633 ballpoint inks using GC/MS and were able to successfully identify 85% of black ink samples and 83% of blue ink samples^[6].

Weyermann *et.al.* (2007) differentiated between fresh ink (<2 weeks) and older inks under laboratory conditions using GC/MS. The purpose of their study was to analyze the drying process of ballpoint inks on the basis of disappearance of volatile solvents present in inks. The studies decrease in solvents of blue Parker® ballpoint inks using liquid extraction followed by split less gas chromatography/mass spectrometry^[7].

Causin *et.al.* (2008) analysed black and blue ballpoint inks using Ultraviolet-Visible Spectrophotometry, Thin Layer Chromatography, and Fourier Transform Infrared Spectroscopy. According to them, 100% samples of black ink and 98% samples of blue ink gave effective results with these techniques. They also concluded that TLC and UV-VIS can also be effectively used in differentiating inks on the basis of its components such as dyes, pigments etc. whereas non-colorant components can be differentiated using FT-IR^[8].

Lee *et.al.* (2014) analysed twelve types of black ballpoint pen inks using High Performance Thin Layer Chromatography (HPTLC). Extraction of inks was done using methanol and separation was done using a solvent mixture of ethyl acetate, methanol and distilled water (70: 35: 30, v/v/v). According to them, differentiation of inks between models from single brand for a few chosen pen brands was possible by direct visual comparison. Effortless sample preparation, less time consumption analysis and high differentiating power makes this method more effective and advantageous. Along with this, it was concluded that black pen ink is homogenous and HPTLC proves to be efficient and reliable for analysis of black ballpoint pen inks^[9].

Lee *et.al.* (2016) analysed 12 samples of red ballpoint pen inks using Ultra-performance liquid chromatography (UPLC). They were able to discriminate 94.45% of samples using 144 chromatograms produced by UPLC. They even concluded that UPLC is much better and effective than HPLC^[10].

Electrophoresis : Electrophoresis is a technique in which charged particles migrate in solution due to exposure to

electric field. Classification of electrophoresis is done on the basis of the solid supporting matrix by means of which the particles migrate in the electrophoretic system^[11].

Egan *et.al.* (2005) and Brewer *et.al.* (2005) prepared a database by analyzing black and blue ballpoint pen inks respectively using capillary electrophoresis with ultraviolet-visible photodiode array detection (190–600 nm) for ink analysis. Comparison of results was made with the results obtained by thin layer chromatography. It was concluded that the sample preparation and identification of dye compounds was easier with capillary electrophoresis. Identification of dye was done on the basis of electrophoretic mobility and a characteristic ultraviolet-visible spectrum^[12-13].

Spectroscopy : In spectroscopy, a beam of electromagnetic rays of selected wavelength is passed through the material. The material absorbs some of the energy rays. The residual radiations falls upon a photoelectric cell and produce changes in the current of the circuit. The changes are recorded on a graph paper. The comparison of the graphs with those of the known materials permits identification of the unknown material. In short, it is the study of interaction of electromagnetic rays with a material. This leads to either scattering, emission or absorption of electrons of the material^[14].

Mazzella *et.al.* (2003) studied 33 blue gel pen inks using filtered light examination, Raman spectroscopy and scanning electron microscopy. They carried out visual examination and classified inks into three categories; milky, metallic and normal. Further examination was carried out using these three techniques. They concluded that Raman spectroscopy and scanning electron microscope proves to be more efficient in differentiation of inks as compared to filtered light examination. A combination of Raman and SEM provided greatest degree of differentiation^[15].

Mazzella *et.al.* (2005) used non-destructive technique of Raman spectroscopy for analysis of fifty five blue gel inks of various brands and models. The inks were initially separated into two components (19 dye based and 36 pigment based gel inks) then analysed using Raman spectroscopy on specified wavelengths of 514.5 and 830nm. They could identify two major pigments i.e. violet 23 and blue 15^[16].

Jones *et.al.* (2006) used direct analysis in real time mass spectrometry for differentiating inks. A non-destructive techniques through which forty three black and blue ballpoint inks, black gel inks and black fluid inks were examined. Dyes as well as thermally unstable components of inks were efficiently analysed and contributed to the spectra obtained^[17].

Zieba-Palus *et.al.* (2006) used spectrometric methods i.e.

micro-FTIR spectroscopy, Raman spectroscopy and XRF for examination of seventy blue and black ballpoint and gel inks. They found that 90% of samples could be distinguished efficiently using these techniques on the basis of the type and color^[18].

Ifa *et.al.* (2007) conducted analysis of inks using Desorption electrospray ionization mass spectrometry (DESI-MS). They analysed samples of blue ballpoint inks used on ordinary paper without any sample preparation. In this technique a moving stage is used which produced two-dimensional molecular images. They concluded that this technique has various advantages over other extractive techniques with respect to speed and sample preservation. According to them, solubility of the analyte plays a crucial role in desorption from paper surface^[19].

Adam *et.al.* (2008) classified and individualized black ballpoint pen inks using principal component analysis of UV-Vis Absorption spectra. Analysis of the spectra from multiple measurements on the same brand of pen showed excellent reproducibility and clear discrimination between inks that was supported by statistical analysis. Indeed it was possible to discriminate between the pen ink and the ink line from all brands examined in this way, suggesting that the solvent extraction process may have an influence on these results. For the complete set of 25 pens, interpretation of the loadings for the first few principal components showed that both the pen inks and the extracted ink lines may be classified in an objective manner and in agreement with the results of parallel thin layer chromatography studies. Within each class almost all inks could be individualized^[20].

Geiman *et.al.* (2009) applied Raman spectroscopy and surface enhanced Raman scattering (SERS) to analyze synthetic dyes in ballpoint inks. They analysed 10 dyes for which spectra was obtained using a dispersive system (633 nm, 785 nm lasers) and a Fourier transform system (1064 nm laser) under different analytical conditions. According to them, Raman spectra is affected by high fluorescence background and poor spectral quality but SERS proves to be better technique. SERS is also successful in collecting data directly from TLC plate^[21].

Braz *et.al.* (2013) used Raman spectroscopy for forensic analysis of inks in questioned documents. They focused on chemistry of ink-paper interactions and the issue of intersecting lines. They demonstrated the potential of Raman-based techniques for forensic examination of inks from pen and printers^[22].

P da Silva Ferreira *et.al.* (2015) analysed ballpoint pen inks using a novel analytical technique paper spray mass spectrometry (PS-MS). They analysed inks under ambient conditions. Typical profiles were obtained on analyzing pens

from different brands, further changes in ink composition were also analysed in PS-MS. This new technique proved to be useful for analyzing documents and characterization of overlapped inks^[23].

Lian *et.al.* (2017) used Hyperspectral Imaging Technology for non-destructive and rapid analysis to reveal the original handwritings covered by the same inks. They used hyperspectral imager Nuance-Macro to analyze the reflected light signature of inks which showed the covered original handwritings. Samples were prepared using different types of blue/black ballpoint and gel pen inks. They are able to reveal overlapped handwritings in 90.5%, 69.1%, 49.5%, and 78.6% of the cases. Further, the correlation between the revealing effect and spectral characteristics of the reflected light of inks at the overlapping parts was interpreted through theoretical analysis and experimental verification. It was concluded that if the characteristics of the original writing ink and overlapped ink were same or similar, then revealing original writing was not possible whereas use of different inks in original and overlapped writing revealed original handwriting^[24].

Silva Junior *et.al.* (2017) identified dyes in pen ink by High Resolution Mass Spectrometry. They extract the dyes in ink from the paper using 1:1 solution of water and methanol and then analysed it using MALDI-QTOFMS (matrix assisted laser desorption/ionization quadrupole time-of-flight mass spectrometer). Differentiation in three commercial inks was done on the basis of presence of peaks in mass spectrum corresponding to specific dyes.^[25]

Miscellaneous : Various other research works which involve combination of the above mentioned techniques or modified versions of the following techniques are collectively listed under miscellaneous category.

Wilson *et.al.* (2004) differentiated black gel inks using various optical and chemical techniques. These techniques included microscopy, visible and near infrared reflectance, near infrared luminescence, thin-layer chromatography (TLC), spot tests, and gas chromatography-mass spectrometry (GC/MS). After analysis they prepared a flowchart showing differentiation of inks with these techniques and even concluded that other non-ball point ink lack few components which are present in gel inks^[26].

Kher *et.al.* (2006) classified ballpoint pen inks using high performance liquid chromatography and infrared spectroscopy. They obtained chromatographic data at four specific wavelengths i.e. 254nm, 279nm, 370nm, 400nm. This data was analysed independently as well as in combination with SIMCA technique using principal component analysis which help in estimation of separation between pen samples. LDA (Linear discriminant analysis) was used to determine the

probability with which observation could be allotted to a pen class. According to them, best results were obtained with HPLC combined with chemometrics as compared to IR method. They concluded that this method could prove to be valuable for non-destructive analysis of blue ballpoint ink samples^[27].

Hammond *et al.* (2007) used LAB color mode present in Adobe Photoshop for differentiation of black ballpoint pen inks. They collected 44 black ballpoint ink pens to create 990 pen-pair samples. Out of 990 samples, 44 samples which were made from known pen-pair combinations did not show any difference whereas out of remaining 946 samples, 737 pen pair samples could be differentiated using the LAB color mode and 209 samples could not be differentiated (153 similar and 56 samples were categorized as unsure). It was concluded after further examination with traditional infrared reflectance and infrared luminescence test methods that LAB color mode gave better results for about 102 samples which could not be differentiated using VSC 4C^[28].

Dunnet *et al.* (2007) used matrix-assisted laser desorption/ionization (MALDI) MS to examine pen ink dyes directly from paper. According to them, LDMS is an effective technique for questioned document examination but it has few limitations. It can detect only those dyes which are neutral or singly charged. The authors used (MALDI) MS for detection of multiple charged dyes present in modern pen inks. They were able to successfully detect >20 multiple charged dyes^[29].

Brazeau *et al.* (2007) performed quantitative analysis of ink using solid phase microextraction (SPME) followed by gas chromatography-mass spectrometry (GC-MS). They removed ink samples from paper using scalpel or paper punch in order to avoid destruction of document. They created a sampling cell through which the ink volatiles (2-phenoxyethanol) were directly adsorbed to the SPME fiber through headspace. These adsorbed volatiles were desorbed on a gas chromatograph attached with mass spectrometer. They concluded that it is possible to detect the presence of ink solvents/volatiles for a time span of 2 years.^[30]

Djozan *et al.* (2008) studied 41 ball point ink samples and were able to discriminate about 92.8% samples of various pen inks with high efficiency by developing a software for evaluating thin layer chromatograms. This software was designed to study the intensity of red, green and blue (RGB) characteristics. According to them, RGB profiles of different ballpoint inks showed differences among each other^[31].

Ismail *et al.* (2008) compared and differentiated 36 ballpoint pens commonly used in Malaysia which comprise of blue, red and black ink. 6 pens of two different brands (Papermate Kilometrico and Faber Castell) of each color were analysed

using UV-Vis spectroscopy at wavelength range 200–800 nm. The spectra obtained were evaluated visually and then by chemometric techniques i.e. principal component analysis and hierarchical cluster analysis. It was concluded that combination of UV-Vis spectroscopy and chemometric techniques proves to be an effective tool for forensic questioned document analysis^[32].

Lee *et al.* (2012) combined multivariate analysis with FTIR to differentiate between 24 black ballpoint inks from six different types of pens. ATR-FTIR spectroscopy was used to analyze the ink directly from the paper and then later the results obtained were evaluated using multivariate chemometrics. They made eight different clusters of absorbance wavenumbers 2000-675cm⁻¹ using cluster analysis. This was followed by principal component analysis to form a new variable set which was evaluated using discriminant analysis and one way-ANOVA. According to them, six black ballpoint pen inks could be classified into three main categories using discriminant analysis. Results obtained by one-way ANOVA showed that only two out of six types of pen inks cannot be discriminated at 95% confidence interval. It was concluded that combination of micro-ATR FTIR spectroscopy and chemometric techniques proves to be a successful and reliable non-destructive techniques for ink analysis on the basis of infrared spectrum^[33].

Silva *et al.* (2014) proposed a method to differentiate black pen inks of various types, models and brands for cursive handwriting. VSC 6000 was used to obtain reflectance spectra and differentiation of inks was done using partial least squares (PLS-DA). The PLS-DA models presented low root mean squared error of predictions (RMSEPs) and permitted a rapid, non-destructive and effective differentiation of all pen inks examined. They concluded that this method is useful for identification of pen type, brand and pen model in case of questioned document examination^[34].

Amador *et al.* (2017) used paper spray mass spectrometry (PS-MS) for analysis of black ball point ink writings. The inks were differentiated according to their characteristic chemical patterns obtained from six different brands. PS-MS combined with partial least squares (PLS) was used to monitor changes on the ink chemical composition after light exposure. The practicability of the developed method on forensic investigations was also demonstrated in three different applications i.e. analysis of overlapped fresh ink lines, analysis of old inks and detection of simulated forgeries^[35].

Asri *et al.* (2018) developed an objective multivariate statistical methodology (PCA and HCA) which incorporated effective data pre-preprocessing and applied the method to aged IR spectroscopic data of gel inks. According to them,

Principal Component Analysis (PCA) revealed similar observations to Hierarchical Cluster Analysis (HCA) and both techniques were more effective in distinguishing the ink samples from all others. Thus, it was concluded that use of non-destructive analysis along with chemometrics techniques for discrimination of aged inks is beneficial in forensic applications^[36].

Saini *et.al.* (2018) studied colour differentiation of ink strokes using Image Processing Techniques. According to them, use of Image processing technique is very useful as it is fast, reliable and non-destructive in nature. They studied ballpoint pen ink samples with the help of two image processing software, including Adobe Photoshop CC 2014 and Picasa 3 in order to differentiate similar colour inks of red, blue and black ballpoint pens and concluded that these techniques have allowed the separation of ballpoint pen inks^[37].

CONCLUSION :

Over the years, considerable effort has been made in identification and examination of writing inks. Various techniques have been successfully employed for ink analysis. Earlier chromatography was prominently used in spite of its destructive nature but presently use of spectroscopy has made the analysis much more reliable and authentic by a non-destructive procedure. The amount of diversity in the type of cases encountered in present scenario is vast. Usage of a single technique for examination will not give the desired results. Thus, it is important for us to combine two or more techniques in order to produce reliable and accurate results. According to the studies conducted, it can be concluded that the instead of adopting a single technique, a combination of either two or more i.e. chromatography, electrophoresis and spectroscopy proves to be highly effective in comparison and analysis of ink. A new revolution is majorly required to supersede destructive techniques with semi-destructive and/or non-destructive techniques.

REFERENCES

1. Agarwal A, Sharma N, Negi Y. Techniques for the Characterization of Inks. *IOSR Journal of Applied Chemistry* 2016; 9(10):.
2. Hilton O. *Scientific examination of questioned documents*, revised ed. : CRC Press; 1993.
3. Moody C. Black Writing Ink Analysis By Direct Infusion Electrospray Mass Spectroscopy [Masters]. University of Central Florida; 2010.
4. Ink Analysis [Internet]. whatwhenhow . [cited 2018 Jun 18]. Available from: <http://what-when-how.com/forensic-sciences/ink-analysis/>
5. Chromatographic Techniques [Internet]. nptel.ac.in. 2018 [cited 2018 Jun 18]. Available from: <http://nptel.ac.in/courses/103108100/module7/module7.pdf>
6. Laporte GM, Wilson JD, Cantu AA, Mancke SA, Fortunato SL. The Identification of 2-Phenoxyethanol in Ballpoint Inks Using Gas Chromatography/Mass Spectrometry-Relevance to Ink Dating. *Journal of Forensic Sciences*. 2004;49(1):1-5.
7. Weyermann C, Kirsch D, Vera CC, Spengler B. A GC/MS Study of the Drying of Ballpoint Pen Ink on Paper. *Forensic Science International*. 2007;168(2-3):119-27.
8. Causin V, Casamassima R, Marega C, Maida P, Schiavone S, Marigo A, Villari A. The Discrimination Potential of Ultraviolet-Visible Spectrophotometry, Thin Layer Chromatography, and Fourier Transform Infrared Spectroscopy for the Forensic Analysis of Black and Blue Ballpoint Inks. *Journal of Forensic Sciences*. 2008;1;53(6):1468-73.
9. Lee LC, Hakim MA, Ishak AA. The Analysis of Dyes in Black Ballpoint Pen Inks using High Performance Thin Layer Chromatography. *Malaysian Journal of Forensic Sciences*. 2014;5(2):22-6.
10. Ultra CB. Fast and Simple Forensic Red Pen Ink Analysis Using Ultra-Performance Liquid Chromatography (UPLC). *Malaysian Journal of Analytical Sciences*. 2016;20(2):224-30.
11. Electrophoresis [Internet]. nios.ac.in. [cited 2018 Jun 18]. Available from : <http://www.nios.ac.in/media/documents/dmlt/Biochemistry/Lesson-21.pdf>
12. Egan JM, Hagan KA, Brewer JD. Forensic Analysis of Black Ballpoint Pen Inks using Capillary Electrophoresis. *Forensic Science Communications*. 2005;1;7(3).
13. Brewer JD, Hagan KA, Egan JM. Forensic Analysis of Blue Ballpoint Pen Inks using Capillary Electrophoresis. *Research and Technology*. 2005; 1;7(3).
14. Spectroscopic Techniques [Internet]. nptel.ac.in. [cited 2018 Jun 18]. Available from : <http://nptel.ac.in/courses/102103044/pdf/mod2.pdf>
15. Mazzella WD, Khanmy-Vital A. A Study to Investigate the Evidential Value of Blue Gel Pen Inks. *Journal of Forensic Sciences*. 2003;48(2):419-24.
16. Mazzella WD, Buzzini P. Raman spectroscopy of Blue Gel Pen Inks. *Forensic Science International*. 2005;10;152(2-3):241-7.
17. Jones RW, Cody RB, McClelland JF. Differentiating Writing Inks Using Direct Analysis in Real Time Mass

- Spectrometry. *Journal of Forensic Sciences*. 2006;1;51(4):915-8.
18. Zięba-Palus J, Kunicki M. Application of the Micro-FTIR Spectroscopy, Raman Spectroscopy and XRF Method Examination of Inks. *Forensic Science International*. 2006;10;158(2-3):164-72.
19. Ifa DR, Gumaelius LM, Eberlin LS, Manicke NE, Cooks RG. Forensic Analysis of Inks by Imaging Desorption Electrospray Ionization (DESI) Mass Spectrometry. *Analyst*. 2007;132(5):461-7.
20. Adam CD, Sherratt SL, Zholobenko VL. Classification and Individualisation of Black Ballpoint Pen Inks Using Principal Component Analysis of Uv-Vis Absorption Spectra. *Forensic Science International*. 2008;15;174(1):16-25.
21. Geiman I, Leona M, Lombardi JR. Application of Raman Spectroscopy and Surface-Enhanced Raman Scattering to the Analysis of Synthetic Dyes Found in Ballpoint Pen Inks. *Journal of Forensic Sciences*. 2009;1;54(4):947-52.
22. Braz A, López-López M, García-Ruiz C. Raman Spectroscopy for Forensic Analysis of Inks in Questioned Documents. *Forensic Science International*. 2013;10;232(1-3):206-12.
23. Da Silva Ferreira P, e Silva DF, Augusti R, Piccin E. Forensic Analysis of Ballpoint Pen Inks Using Paper Spray Mass Spectrometry. *Analyst*. 2015;140(3):811-9.
24. Lian Y, Liang L, Li B. Hyperspectral Imaging Technology for Revealing The Original Handwritings Covered by the Same Inks. *Journal of Forensic Science and Medicine*. 2017;1;3(4):210.
25. Da Silva Junior CA, Segura PA. Identification of Dyes in Pen Ink by High Resolution Mass Spectrometry: A Laboratory Experiment. *Journal of Laboratory Chemical Education*. 2017;5(5):108-15.
26. Wilson JD, LaPorte GM, Cantu AA. Differentiation of Black Gel Inks Using Optical and Chemical Techniques. *Journal of Forensic Science*. 2004;19;49(2):1-7.
27. Kher A, Mulholland M, Green E, Reedy B. Forensic Classification of Ballpoint Pen Inks Using High Performance Liquid Chromatography and Infrared Spectroscopy with Principal Components Analysis and Linear Discriminant Analysis. *Vibrational Spectroscopy*. 2006;17;40(2):270-7.
28. Hammond DL. Validation of LAB Color Mode as a Nondestructive Method to Differentiate Black Ballpoint Pen Inks. *Journal of forensic sciences*. 2007;1;52(4):967-73.
29. Dunn JD, Allison J. The Detection of Multiply Charged Dyes Using Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry for the Forensic Examination of Pen Ink Dyes Directly from Paper. *Journal of Forensic Sciences*. 2007;1;52(5):1205-11.
30. Brazeau L, Gaudreau M. Ballpoint Pen Inks: The Quantitative Analysis of Ink Solvents on Paper by Solid-Phase Microextraction. *Journal of Forensic Sciences*. 2007;1;52(1):209-15.
31. Djozan D, Baheri T, Karimian G, Shahidi M. Forensic Discrimination of Blue Ballpoint Pen Inks Based on Thin Layer Chromatography and Image Analysis. *Forensic Science International*. 2008;6;179(2-3):199-205.
32. Ismail D, Austad Z, Desa WN. Ultra-violet and Visible (UV-Vis) Spectroscopy and Chemometrics Techniques for Forensic Analysis of Ballpoint Pen Inks: A Preliminary Study. *Malaysian Journal of Forensic Sciences*. 2008;5(1):47-52.
33. Lee LC, Othman MR, Pua H, Ishak AA. Application of Multivariate Chemometry for Discrimination of Black Ballpoint Pen Inks Based on the IR Spectrum. *Malaysian Journal of Forensic Sciences (MJOFS): Mission Statement*. 2012;5.
34. da Silva VA, Talhavini M, Zacca JJ, Trindade BR, Braga JW. Discrimination of Black Pen Inks on Writing Documents Using Visible Reflectance Spectroscopy and PLS-DA. *Journal of the Brazilian Chemical Society*. 2014;25(9):1552-64.
35. Amador VS, Pereira HV, Sena MM, Augusti R, Piccin E. Paper Spray Mass Spectrometry for the Forensic Analysis of Black Ballpoint Pen Inks. *Journal of the American Society for Mass Spectrometry*. 2017;28(9):1965-76.
36. Asri MNM, Desa WNSM, Ismail D. Combined Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA): an efficient chemometric approach in aged gel inks discrimination. *Australian Journal of Forensic Sciences*. 2018;:1-22.
37. Saini K, Kaur SP. Forensic Colour Differentiation of Ink Strokes using Image Processing Techniques.

Review Article

Frequently Asked Questions Regarding Court Evidence through Video-conferencing in Medicolegal Cases

1. **Vivekanshu Verma**, Associate Consultant, Emergency & Trauma Care, Medanta-The Medicity, Gurugram
2. **Santosh Kumar Verma**, Senior Advocate, Rajasthan High Court.

Corresponding Author :

Dr. Vivekanshu Verma

Associate Consultant,

Emergency & Trauma Care, Medanta-The Medicity,
Gurugram

Contact : +91 9810497871

E-mail : vivekanshu@yahoo.co.in

Article History:

Received: 10 September 2018

Received in revised form: 10 December 2018

Accepted on: 10 December 2018

Available online: 1 June 2019

KEYWORDS : Video Conference, Court Evidence, Medico Legal Cases

1. INTRODUCTION :

Video-conferencing (VC) facilities provide Courts with the capacity to receive evidence and submissions from doctors involved in court proceedings in medicolegal cases of roadside trauma, assault, sexual assault, burn, poisoning, murder and alleged medical negligence etc, where personal appearance would be expensive, inconvenient or otherwise not desirable for a treating doctor to attend a court in person. To facilitate the recording of evidence of the Medical Experts in such circumstances, via video conferencing, Medical Experts shall be able to devote their time in the hospitals in attending patients rather than commuting to Court for recording of their evidence. An over-riding factor is that, the use of video-conferencing in any particular case must be consistent with furthering the interests of justice and should cause minimal disadvantage to the parties. The Presiding Judge shall be very careful while following the procedure established by law since the force of judgment is derived from the recording of evidence. As such, the mode of taking and recording evidence is integral feature of trial. Higher Courts (Appellate/ Revisional Court) looks at the evidence through the eyes of the trial Judge. Unless a Judge is well equipped with legal knowledge and also well trained in recording evidence, it will impact on the Judgment. Even with the advancement of technology, there is a delay of milliseconds between video picture seen and sounds being heard. Allowances appropriate to this time gap need to be made to avoid one participant talking over another. Microphones set up at the bench, the bar table and at the witness box are highly sensitive. Persons during a video conferencing should assume from the time the video conference is activated until the same is disconnected that microphones are "live" and as such all remarks are audible to the court.

2. First landmark Supreme Court Judgement accepting video conferencing of doctors legal and authentic in case of alleged medical negligence:

In State of Maharashtra vs. Dr Praful B Desai (AIR 2003 SC 2053) [The question involved whether a witness can be examined by means of a video conference.] The Hon'ble Supreme Court observed that video conferencing is an advancement of science and technology which permits seeing, hearing and talking with someone who is not physically present with the same facility and ease as if they were physically present. The legal requirement for the presence of the witness does not mean actual physical presence. The court allowed the examination of a witness through video conferencing and concluded that there is no reason why the examination of a witness by video conferencing should not be an essential part of electronic evidence. Supreme Court held that a trial judge could record evidence of doctor staying abroad through video conferencing in case of alleged medical negligence. Interpreting Section 273 of the Criminal Procedure Code in the light of technological advancements, a bench comprising Justice S N Variava and Justice B N Agrawal said recording of evidence through video conferencing would be perfectly legal. The judgment relates to a case in which a US-based doctor had opined against operation of a cancer patient through video conferencing. Ignoring the advice, two Indian doctors operated on the lady, who later passed away. The patient's family went to court against the doctors. However, the US-based doctor, Ernest Greenberg, refused to come to India, but expressed willingness to give evidence through video conferencing. But the Bombay high court did not allow the trial court to go ahead citing Section 273, which lays down the procedure for recording evidence. The husband of the deceased patient, and the Maharashtra government had

appealed against the high court order in the Supreme Court. The prosecution had alleged that the two Indian doctors did not take good care of the patient after the operation as a result of which she suffered a lot before her death. In cases where the attendance of a witness cannot be procured without an amount of delay, expense or inconvenience, the court could consider issuing a commission to record evidence by way of video conferencing. Normally a commission would involve recording of evidence at the place where the witness is. However, advancement in science and technology has now made it possible to record such evidence by way of video conferencing in the town/city where the court is. Referring to the chances of witness abusing the trial judge during video conferencing, the apex court said.

As a matter of prudence, evidence by video-conferencing in open court should be accepted only if the witness is in a country which has an extradition treaty and under whose laws contempt of court and perjury are punishable. The court then directed the Mumbai court to set up a commission and take help of VSNL in recording Dr Greenberg's statement through video conferencing in the presence of the two accused doctors. It also allowed the two accused to cross-examine the US-based doctor. The court directed the Maharashtra government to bear the cost of video conferencing. Rejecting all arguments about inferior video quality, disruption of link and other technical problems, the bench said by now science and technology has progressed enough to not worry about video image/audio interruptions or disruptions. The counsel for the two doctors argued that the rights of the accused under Article 21 could not be subjected to a procedure involving 'virtual reality'. Rejecting the argument, the bench said video conferencing has nothing to do with virtual reality and gave the example of the telecast of the cricket World Cup. It could not be said that those who watched the World Cup on television were witnessing virtual reality as they were not in the stadium where the match was taking place, the court pointed out. This is not virtual reality, it is actual reality. Video conferencing is an advancement of science and technology which permits one to see, hear and talk with someone far away with the same facility as if he is present before you, that is, in your presence," the apex court said. Recording of evidence by video conferencing also satisfies the object of providing, in Section 273 CrPC, that evidence be recorded in the presence of the accused.¹

3. Video conferencing is regulated under the Indian Evidence Act & Information Technology (IT) Act:

All, relevant statutory provisions applicable to judicial proceedings including the provisions of the Information Technology Act, 2000 and the Indian Evidence Act, 1872 shall apply to the recording of evidence by video conference. In

Indian law, to incorporate the provisions on the appreciation of digital evidence, the Information Technology (IT) Act 2000, is amended to allow for the admissibility of digital evidence. Sections 65-A and 65-B provide provisions for evidences relating to electronic records and admissibility of electronic records, and that definition of electronic records includes video conferencing.

4. Minimum requisites for video conference:

- (i) A desktop or laptop with internet connectivity and printer
- (ii) Device ensuring uninterrupted power supply
- (iii) Video Camera
- (iv) Microphones and speakers
- (v) Display unit
- (vi) Document visualizer
- (vii) Comfortable sitting arrangements ensuring privacy
- (viii) Adequate lighting
- (ix) Insulations as far as possible/proper acoustics
- (x) Digital signatures from licensed certifying authorities for the coordinators at the court point and at the remote point.

5. Court permit recording of evidence through VC:

Generally, all the courts have a liberal approach towards allowing recording of evidence through video conferencing. Unless there were special reasons to deny it, courts are always willing to adopt the technology for the betterment of all. By evaluating various cases where courts admitted and denied an application for recording evidence through video conferencing, we can say that, unless it is a mandatory condition of law or a written and valid contract among parties that parties must appear physically, it must be allowed by the courts. In any other such circumstances where the court considers that if the recording of evidence through video conferencing is allowed, it will defeat the purpose of the law or procedure established by law, the court can deny it.

6. Doctor can appear in court by video conference:

A Court may either "suo moto" or on application of a party or an expert medical witness, direct by a reasoned order, that any doctor shall appear before it or give evidence or make submissions to the Court through video conference. In criminal medico legal cases, where the Doctor to be examined is a prosecution witness or court witness in medicolegal cases of roadside trauma or assault, the prosecution counsel and where doctor to be examined is a defence witness in alleged medical negligence, the defence counsel will confirm to the Court his location, willingness to be examined by video

conferencing, place and facility of such video conferencing. In case person to be examined is an accused in medical negligence suit, prosecution will confirm his location at a remote point.

7. Timings of VC for doctors in medico legal cases:

Video conference shall ordinarily take place during the court hours. A time slot between “11.00 AM to 1.00 PM” on working days should be kept fixed for Video Conferencing. However, in case of witness living out of India with different time zone (USA, UK) the Court may pass suitable directions with regard to timings of the video conferencing as the circumstances may dictate.

8. Cost Bearing of video conferencing:

In criminal cases, the expenses of the video conference facility including expenses of preparing soft copies/certified copies of the Court record for sending to the co-ordinator at the remote point and fee payable to translator/interpreter/special educator, as the case may be, and to the co-ordinator at the remote point shall be borne by such party as the Court directs taking into account the Delhi Criminal Courts (Payment of Expenses to Complainant and Witnesses) Rules, 2015.

In civil cases, as a general rule, the party making the request for recording evidence by video conference shall bear the expenses. In other cases, the court may make an order as to expenses as it considers appropriate taking into account rules/instructions regarding payment of expenses to complainant and witnesses as may be prevalent from time to time.

9. High court Guidelines for lawyers conducting Video conferencing :

Delhi High Court issued the following directions for conducting Video conferencing:²

- i) The identity of the person to be examined shall be confirmed by the court, with the assistance of the co-ordinator at remote point at the time of recording of the evidence.
- ii) Reference to the 'court point' means the Courtroom or other place where the Court is sitting or the place where Commissioner appointed by the Court to record the evidence by video conference is sitting and the 'remote, point' is the place where the person to be examined via video conference is located, for example, a prison.
- iii) Person to be examined includes a person whose deposition or statement is required to be recorded or in whose presence certain proceedings are to be recorded.
- iv) Wherever possible, proceedings by way of video conference shall be conducted as judicial proceedings and the same courtesies and protocols will be observed.
- v) Where the person to be examined is in another State or Union Territory, a judicial Magistrate or any other responsible official as may be deputed by the District Judge concerned or Sub-Divisional Magistrate or any other responsible official as, may be deputed by the District Collector concerned,
- vi) Where the person to be examined is in custody, the concerned Jail Superintendent or any other responsible official deputed by him,
- vii) Where the person to be examined is in a hospital, public or private, whether run by the Central Government, the State Government, local bodies or any other person, the Medical Superintendent or In-charge of the said hospital or any other responsible official deputed by him,
- viii) Where the person to be examined is a juvenile or a child who is an inmate of an Observation Home/Special Home/Children's Home/ Shelter Home, the Superintendent/Officer In-charge of that Home or any other responsible official deputed by him.
- ix) Where the person to be examined is in Nirmal Chhaya, the Superintendent/Officer In-Charge of the Nirmal Chhaya or any other responsible official deputed by him,
- x) Video conferencing facilities can be used in all matters including remands, bail applications and in civil and criminal trials where a witness is located intrastate, interstate, or overseas. However, these guidelines will not apply to proceedings under section 164 of Cr.P.C.
- xi) The guidelines applicable to a Court will mutatis mutandis apply to a Local Commissioner appointed by the Court to record the evidence. “Mutatis mutandis” means making necessary alterations while not affecting the main point at issue.
- xii) Role of Interpreter/ translator: A translator can be appointed by court in case the person to be examined is not conversant with Court language; an expert in sign language in case the person to be examined is speech and/or hearing impaired; For reading of documents in case the person to be examined is visually challenged; an interpreter or special educator, as the case may be, in case the person to be examined is temporarily or permanently mentally or

physically disabled.

- xiii) Privacy of witness: The court may, at the 'request of a person to' be examined, or on its own motion, taking into account the best interests of the person to be examined, direct appropriate measures to protect his privacy keeping in mind his age, gender and physical condition. Where a party or a lawyer requests that in the course of video-conferencing some privileged communication may have to take place, Court will pass appropriate directions in that regard.
- xiv) Reviewing recorded audiovisual of VC: The audio-visual shall be recorded at the court point. An encrypted master copy with hash value shall be retained in the court as part of the record. Another copy shall also be stored at any other safe location for backup in the event of any emergency Transcript of the evidence recorded by the Court shall be given to the parties as per applicable rules. A party may be allowed to view the master copy of the audio video recording retained in the court on application which shall be decided by-the Court consistent with furthering the interests of justice.
- xv) Marking of exhibits:- Exhibits admitted in evidence during VC shall be marked as follows:-
 - (a) if filed by the prosecution with the capital letter 'P' followed by a numeral, P1, P2, P3 and the like;
 - (b) if filed by defence with the capital letter 'D' followed by a numeral, D1, D2, D3 and the like;
 - (c) in case of Court exhibits with the capital letter 'D' followed by a numeral C1, C2, C3 and the like.

10. Video conferencing – is it oral evidence or it is documentary evidence :

All statements which the court require and permit to be made before it by the witnesses, regarding the matter of fact under inquiry are oral evidence. In video conferencing, if the court is just permitting to make these statements through electronic means, it is not covered in the definition of documentary evidence. It is purely a oral evidence in electronic form.

But, if any documents are being admitted as evidence then they may be treated as documentary evidence depending on the facts and circumstances and discretion of the court.

11. Guidelines which a trial court must keep in mind while recording evidence through video conferencing:-

Kolkata High court gave some guidelines, which a trial court

must keep in mind while recording evidence through video conferencing:³

- i. The court must satisfy itself regarding the identity of the witness.
- ii. The oath must be administered to witness before recording his evidence.
- iii. The witness can only be examined during the working hours of Indian courts.
- iv. Copies of the documents to be proved must be provided to witnesses well in advance.
- v. It must be ensured by the court that the witness is alone in the room of Indian embassy from where he is giving evidence through video conferencing.
- vi. The demeanor of the witness must be recorded by the court which is relevant for the purpose of evaluation of the evidence.
- vii. Once the recording of the evidence is started through video conferencing, it must be continued on day by day basis till evidence of such witness is recorded completely.
- viii. Other conditions can be imposed by the court to ensure smooth recording of evidence through VC.

12. High court Guidelines for Doctors attending court evidence via Video conferencing:⁴

Punjab & Haryana High Court issued the following directions in exercise of the inherent powers conferred on this Court under Section 482 Cr.P.C. and/ or Section 151 CPC and Under Article 226 of the Constitution:⁴

- (i) The State Government shall set up Video Conferencing Rooms in the Civil Hospitals, if not already available, to facilitate recording of evidence of medical experts through Video Conferencing. The Court shall satisfy itself that the doctor to be examined at the remote point can be seen and heard clearly and similarly that the doctor to be examined at the remote point can clearly see and hear the Court.
- (ii) The Public Prosecutor in criminal cases or Advocate for the claimant in accident cases or any other Advocate representing any party, who wishes to examine a Medical Expert is required to disclose the place of posting of the concerned Doctor along with his e-mail address and/or the contact phone number.
- (iii) The photocopies/ soft copies of the documents to be proved by examining the Medical Expert, shall be forwarded to the concerned Expert by either the Public Prosecutor in criminal cases or Advocate for the Claimant in accident cases or any other Advocate representing any party requiring evidence of the medical experts. The Court will allow the Public Prosecutor or Advocates for preparation of the

photocopies of the relevant documents, if the same are not available with them as per rules.

(iv) If the documents to be proved by the Medical expert are in possession of a third person or party, a simultaneous direction would be issued by the Court requiring that person to make available the documents in the Court at the time of recording of evidence of the medical expert through Video Conferencing.

(v) The concerned Court will fix a date, before which the examination-in-chief will be furnished by the Medical Expert concerned to the Court.

(vi) The concerned Court will also fix a date for the purposes of cross-examination giving tentative time slot for Video Conferencing until mechanism of booking of slots is put in place. The Doctor will confirm his availability in the said slot along with his statement to be sent to the Court.

(vii) On the given time, the Court will organize two way or three-way Video Conferencing i.e. between Court, Medical Expert and the Central/District Jail, if the accused is in custody and not in Court to facilitate recording of the statement of the medical experts.

(viii) The Registrar (Computerization) of this Court will be coordinator to facilitate the mechanism of recording of such evidence including development of module for fixation of time slot. In case of any difficulty in implementing the directions, the State Governments and/or the Officers including Medical Experts and the Judicial Officers may give their suggestions, which shall be given effect to as far as possible.

(ix) Until rooms/studios are established in Civil Hospitals, the medical experts may go to such rooms/studios available in the District Court, DC office or the NIC office. In-charge Officers of such institutions would facilitate recording of evidence of medical experts by permitting them access to the VC rooms/studios. The State Government shall issue appropriate directions in this regard.

(x) The above directions will be applicable to the Medical Experts posted outside the District of the place of Court sitting and shall not preclude the Court in appropriate cases to record evidence of the Medical Experts in Court.

(xi) The record of proceedings including transcription of statement shall be prepared at the court point under supervision of the Court and accordingly authenticated. The soft copy of the transcript digitally signed by the co-ordinator at the court point shall be sent by e-mail through NIC or any other Indian service provider to the remote point where printout of the same will be taken and signed by the deponent. 'A scanned copy of the statement digitally signed by co-ordinator at the remote point would be sent by e-mail through NIC or any other Indian service provider to the court point. The hard copy would also

be sent subsequently, preferably within' three days of the recording, by the co-ordinator at the remote point to the court point by courier/mail.

(xii) Putting medical records/ MLR/PMR/ documents to a doctor at remote point: If in the course of examination of a doctor at a remote point by video conference, it is necessity to put a document to him, the Court may permit the document to be put in the following manner:

(a) if the document is at the court point, by transmitting a copy of it to the remote point electronically including through a document visualizer and the copy so transmitted being then put to the person.

(b) if the document is at the remote point, by putting it to the person and transmitting a copy of it to the court point electronically including through a document visualizer. The hard copy would also be sent subsequently to the court point by courier/mail.

13. Affidavit is required from doctor submitting his evidence in medicolegal cases:

A Division Bench of Punjab & Haryana Court, While deciding Murder case Reference No.8 of 2007 titled "State of Punjab Vs. Mohinder Singh", noticing spelling mistakes in the medical evidence given by the Doctors, gave the following directions: "In view of the aforesaid, in order to ensure that medical evidence is placed on Court records in correct and clear terms and also that Presiding Officers of Courts do not take it lightly, we direct the Registrar (Judicial) to issue instructions to Secretaries (Health) and Director General/Directors (Health Services), and Presiding Officers of Courts in Punjab, Haryana and U.T. Chandigarh, that at the time of recording of evidence of Doctors, who are produced as expert witnesses, Courts shall ask them (Medical Doctors etc.) to submit medical reports on affidavit in clear terms with correct spelling.⁴ In pursuance of above directions, the Registrar (Rules) has issued a communication dated 28.08.2008 to the effect that requisite affidavits are required to be prepared by the Doctors, who appear as expert witnesses, as the original records always remain with them, which they can use/refer to, while preparing their affidavits.

14. In some medicolegal cases doctor's VC is not preferred by courts. Courts still prefer doctors (who have prepared medicolegal reports of the victim/accused) to visit the court in criminal cases of sexual assault of minors under POCSO (Protection of child against sexual offences) act. The courts, to protect child's privacy, keeping in mind his/her tender age, gender and physical condition. In these cases, in the course of video-conferencing, some privileged communication may take place during internet transmission, so Courts avoid VC

and summons the doctors to visit personally.

15. Can evidence be recorded in legal cases through VC:

By amendment in Cr.P.C in 2009, a provision was added to subsection (1) of section 275 Cr.P.C. which states as follows:

“Provided that evidence of a witness under this subsection may also be recorded by audio-video electronic means in the presence of the advocate of the person accused of the offense”.

By analyzing this provision, we can say that taking evidence of a witness through video conferencing is permissible.

By observing various guidelines of various high courts and supreme court, it is clear that evidence can be taken through video conferencing.

Here it is worth mentioning that as per Section 273 Cr.P.C., evidence must be recorded in the presence of the accused. But, here presence does not mean the actual physical presence of the accused. Evidence may also be taken in presence of the pleader of accused if attendance of accused is dispensed with. Presence of pleader is deemed to be the presence of accused as provided by section 273 and 275 (1) proviso.

In case of **State of Maharashtra vs Dr. Praful B. Desai and Another**: AIR 2003 SC 2053, the Apex Court interpreted the meaning of the term 'presence of the accused' in the aforesaid manner.

15. Apex court and various other court allowed recording of evidence through video conferencing in following cases:

In **State of Maharashtra vs Praful B Desai (Dr.)** Supreme court permitted recording of evidence of witnesses staying abroad through video conferencing.

In **Md.AjmalMd.AmirKasab @Abu ... vs State Of Maharashtra**:The court permitted Kasab to appear through video conferencing.

Delhi High Court in International Planned Parenthood Federation (IPPF) vsMadhuBalaNath directed courts to have a liberal and pragmatic approach in allowing the witnesses to depose through Video conferencing.

Gujarat High Court Ketan @ ArcitPravinbhai Patel vs State of Gujarat gave directions to the State to consider the production of undertrial person in concerned courts through video conferencing.

The Bombay High Court taking suo-motu cognizance of a letter written by Shaikh Abdul Naeem, who was one of the accused in the Aurangabad Arms Haul case has directed the Maharashtra government to install video conferencing facilities in all courts in the state by the end of March 2017.

In February 2018, it was reported that Jammu bench of Jammu and Kashmir High court for the first time heard 11

cases listed before Srinagar wing of High court through video conferencing. The Chief Justice of J & K High court, Justice BadarDurrez Ahmed had himself heard these cases.

Apex court in **AshaRanjanvs State of Bihar and others**, transferred Mohammad Shahabuddin from Siwan Jail, district Siwan in Bihar, to Tihar Jail, Delhi directing that pending trials shall be conducted by video conferencing by the trial court.

The Division Bench of Madras High Court created history when it conducted the court proceedings over Skype from Chennai for the first time in a case related to 89 inmates of an unauthorized private children's Home for girls run by Mose Ministries in Turuchi. In this case, girls rescued from the brothels in Delhi were repatriated and rehabilitated in their hometowns in several parts of India. To avoid any discomfort to them the court decided to take evidence through video conferencing.

SIL Import, USA vs Exim Aides Exporters, Bangalore (1999) 4 SCC 567. In this decision also use of available technology was given a boost. But, it was also held that technologies like internet, email etc. were being used swiftly even before the bill was discussed in parliament. So, if Parliament has decided that notice is to be given in writing, the court cannot ignore this fact. The court assumes that Parliament was well aware of the modern technology available.

Grid Corpn.Of Orissa Ltd. vs AES Corpn. 2002 AIR (SC) 3435. Here supreme court held that it is not necessary for two parties to sit together at one place where an effective consultation can be done through electronic means and remote conferencing, unless, law or any condition specifically mentioned in the contract has binding on them to do so.

Hon'ble Karnataka High court in Twentieth Century Fox Film Corporation Vs NRI Film Production Associates (p) Ltd. (AIR 2003 Kant 148) held that a witness must file an affidavit or undertaking duly verified by a notary or a judge before he is examined, that the person who is going to depose on screen is the same person who is shown as the witness on the records.

16. Apex court denied recording of evidence through video conferencing: In **SanthinivsVijayaVenketesh**, the apex court held that Evidence via video conference not permissible in matrimonial cases. If both parties are not present in court, then there is less possibility of emotional bond. It can create a dent in the process of settlement. The family court judge should never be the slave of the concept of a speedy trial. Reconciliation requires the presence of both parties at the same time and same place. This if permitted, can defeat the purpose of the whole act.

17. Technical difficulties faced by doctors, lawyers and

judges during VC:

- i. Lack of infrastructure.
- ii. Still, in many parts of the country, courts do not have basic infrastructure like computer systems, uninterrupted supply of electricity and high-speed internet connections to facilitate recording of evidence through video conferencing.
- iii. Lack of technical knowledge required for video conferencing among some judges, doctors, lawyers and no availability of technical assistance to them.
- iv. No mechanism to compel witnesses in countries other than India, to be present at the time of the conference.
- v. People are still not as acquainted with the technology, as required, and hence, they are reluctant to adopt it. They still prefer the conventional methods.
- vi. Some Judges do not have enough time to record evidence through video conferencing and they have to appoint commissioners for this purpose. But commissioners are not well acquainted with technology and guidelines for recording evidence through video conferencing.
- vii. The speed of the internet is not world class.
- viii. Some governments has not supported the efforts of courts and has not provided with the appropriate funds required for making this possible.
- ix. Advocates have a tendency to linger on the cases by taking adjournments during the trials. Parties in default also want to take advantage of the loopholes in the legal system as delays irritate opposite parties and sometimes may even change the fate of matter in their favor. Generally, only one party to the suit is interested in it. Party in default and his counsels do not agree to adopt this procedure as it may result in early disposal of a matter which will probably be decided against them.

18. CONCLUSION :

The courts have generally observed that, for speedy and effective disposal of cases, there is a requirement to avail technologies and innovations in the justice delivery system by including video conferencing, but, with necessary safeguards and precautions. In our technological age justice can be awarded by recognizing latest discoveries promoting forensic efficiency by liberal use of scientific aids of audiovisual aids to prove guilt of the accused by conducting timely court evidence of treating doctors from their Hospitals, in medicolegal cases pending in trial courts, because justice delayed is justice denied for the victim.

REFERENCES :

1. Video Conferencing Guidelines issued by Supreme court in State of Maharashtra vs. Dr Praful B Desai (AIR 2003 SC 2053)
<https://indiankanoon.org / doc / 560367 /> Last accessed on 9/9/18.
2. Video Conferencing Guidelines issued by Delhi High Court. http://www.nja.nic.in / CJ - CM_Resolution / Delhi_HC / Video Conferencing Guidelines issued by DHC. PDF Last accessed on 9/9/18.
3. Video Conferencing Guidelines issued by Kolkata High Court in case of SujayMitravs State of West Bengal CRR No.1285 of 2015.
4. Video Conferencing Guidelines issued by Punjab & Haryana High Court in State of Punjab Vs. Mohinder Singh in MRC No.8 of 2007, CRM No.18934 of 2013. <https://indiankanoon.org / doc / 138702728 /> Last accessed on 9/9/18.
5. Mode of treating and Recording evidence, including recording of evidence through Video Conferencing by Vijaywada court.
[http://ecourtd.gov.in / sites / default / files / Topic -2 \(Mode of treating and Recording evidence, including recording of evidence through VC\) pdf](http://ecourtd.gov.in / sites / default / files / Topic -2 (Mode of treating and Recording evidence, including recording of evidence through VC) pdf). Last accessed on 9/9/18.

Review Article

Systematic Review of Causes of Head Trauma in Age Groups 0-14 Years Versus 0-20 Years in Iran

1. **Siamak Soltani**, Assistant Professor, Forensic and Legal Medicine, Iran University of Medical Sciences, Tehran, Iran
2. **Kamran Aghakhani**, Professor, Forensic Medicine, Iran University of Medical Sciences, Tehran, Iran
3. **Saeed Mohammadi**, Forensic Medicine Specialist, Forensic Center of Hormozgan Province, Bandar Abbas, Iran
4. **Shahrokh Mehrpisheh**, Assistant Professor, Neonatology, Mazandaran, University of Medical Sciences, Mazandaran, Iran

ABSTRACT :

Introduction : Trauma is the main cause of mortality in children, with different patterns in different countries. Head trauma is a major part of pediatric trauma in Iran. It has different effects for children compared to adults and is a major cause of mortality, disability, and psychological disorders later in life.

Objectives: The aim of this study was to examine the causes of pediatric head trauma in Iran in order to decrease its incidence and economic-social burden in the society by creating awareness among families and organizations.

Methods: All studies on pediatric head trauma in Iran conducted between 2000 and 2018 were retrieved by searching Indexed/PubMed.

Conclusions: Analysis was performed on the cause of 968 cases of head trauma in children aging 0-14 years (of which 32.3% was caused by motor vehicle accidents, 48.2% by falling, and 9.2% by being hit by objects). In the age group of 0-20 years (13004 cases), the main cause was motor vehicle accidents in 43.2%, falling in 32.1% and being hit by objects in 3.3%.

Corresponding Author :

Shahrokh Mehrpisheh,

Assistant Professor of Neonatology,

Mazandaran, University of Medical Science, Mazandaran, Iran

Contact : 009809118023357

E-mail : s.mehrpisheh@yahoo.com

Article History:

Received: 6 November 2018

Received in revised form: 6 December 2018

Accepted on: 6 December 2018

Available online: 1 June 2019

KEYWORDS : Iran, Head Trauma, Head Injury, Epidemiology, Pediatric, Prevention, Causes of Head Trauma

INTRODUCTION :

Trauma is defined as any injury caused inadvertently or purposely by an external force (which includes motor vehicle accidents, falling, poisoning, drowning, hit by objects, or fighting other people)^[1]. One out of every 10 cases of mortality worldwide is caused by trauma. In developing countries, the main cause of disabilities is traumas in age group of 1-29 years. As per the World Health Organization (WHO), the burden of diseases caused by trauma will increase from 14% to 20% by 2020 and one-fourth of all cases of mortality caused by external factors will belong to children aged below 15 years^[1-3].

Injuries caused by head trauma comprise a main part of mortality and disability throughout life, especially in children and are a major cause of disability in them. In the US, over 45% of mortality in children aged 1-14 years occurs as a result of head trauma. About 70-80% of mortality caused by accidents in children is due to injuries to their central nervous system (CNS)^[4]. A study conducted in Tehran from 1999 to 2000 showed that head trauma is the main cause of mortality in children admitted to the trauma ward of six studied hospitals^[5]. Brain traumas often occur due to neglecting safety principles by motorcycle riders, cyclists, construction and industrial

workers, and automobile drivers^[2].

Pediatric head trauma has different outcomes compared to adult head trauma, because the skull is thinner and more flexible in children; the head-to-body ratio is higher in children and they have fewer protective muscles. Neck ligaments and joints are more flexible in children. Moreover, blood circulation in the brain and synaptogenesis are higher in the development period compared to maturity. Therefore, brain trauma in this period may lead to deficits in the development of the CNS and consequently behavioral and cognitive impairment^[6].

The present study aimed to examine the causes of brain trauma in Iranian children and variables affecting these accidents so that its incidence and thus its economic-social burden can be decreased educating families and organizations in charge.

MATERIALS AND METHODS :

Data for present study has been collected from studies conducted in Iran during the years 2000-2018 and published in indexed/ PubMed journals. The following keywords were used when searching for articles: trauma[ti] AND ("craniocerebral trauma"[MeSH Terms] OR "craniocerebral trauma"[All

Fields] OR "head trauma"[All Fields]), Pediatric[ti/ab] AND head AND trauma.

In cases where percentages were reported instead of the number for cause of trauma (e.g. References 7 and 8) the number for each cause was calculated by multiplying the percentages by the total number of patients. In case of Reference 9 which reported the number for each cause of trauma for all patients, the cause of head trauma of the age group of interest was calculated based on the ratio of the number of cases in the noted age group. Of course, this may not be an accurate estimation in a small population. Analysis was conducted in Office Excel 2013 and results are presented in numbers (%).

RESULTS:

Of all the retrieved articles on causes of head trauma in Iran, three reported on age 0-14 years and seven reported on age 0-20 years (**Table 1**). In other articles, either age groups or types of trauma were not specified. In the present study, analysis was conducted on 968 cases of head trauma in children aged 0-14 years, of which 32.3% were caused by motor vehicle accidents, 48.2% by falling, and 9.2% of being hit by objects. Furthermore, 13004 cases of head trauma in the age group of 0-20 years were examined, of which 43.2% were due to motor vehicle accidents, 32.1% due to falling, and 3.3% due to being hit by objects. The most common external cause of head

trauma in the age group of 0-20 years was motor vehicle accidents; however, in the age group of 0-14 years, it was falling (**Table 2**).

DISCUSSION AND CONCLUSION:

The most frequent external cause of head trauma in the age range of 0-14 was falling, which is similar to the results reported by studies in other countries^[14]. Study on 304 children visiting Taleqani Hospital, revealed that children aged 0-2 years had the largest number of cases of head and shoulder injury. Falling and road accidents had an increasing trend among the age group of 3-6 compared to 0-2 years, but it had a decreasing trend in the age group of 7-10 years^[1].

The most frequent cause of head trauma in the age group of 0-20 years was motor vehicle accidents. Studies on more age groups reported results similar to those of the age group of 0-20 years^[8,9,15-17]. In Iran, one can drive motor vehicles after turning 18 and upon receiving a driver's license. Therefore, the risk of incidence of accidents is increased in them, which may increase the rate of head trauma caused by motor vehicle accidents in adults.

Head trauma, in children or adults, occurs more in men and boys than women and girls, by 2-, 4-, or 6-fold or a number between these, as reported by some studies^[2, 5, 8-10, 12, 16-20]. The reasons for this difference may be the more freedom enjoyed by boys and their tendency to play more high-risk games

Table 1 : Data on Cause of Head Trauma

Reference	City	Hospital	Year	0-20 year old patients	0-14 year old patients	Motor vehicle accident	Falling	Being hit by objects	Fight	Sport	Other
(10)	Gilan	Poursina	2010-2011	----	588	248	234	43	10	39	14
(8)	Tehran	Imam Hossein	2006-2008	----	76	20	52	4	----	----	----
(1)	Kermanshah	Taleqani	2008	----	304	50	199	27	----	1	27
(2)	Hamadan	Be'sat	2013-2016	9077	----	3759	2702	432	714	----	----
(11)	Tehran	Imam Hossein	2006-2007	368	----	184	150	----	4	26	4
(9)	Tehran	Imam Hossein & ShohadayeTajrish	2012-2014	62	----	36	18	----	----	----	8
(12)	Mashhad	Shahid Kamyab (Emdadi)	2012	95	----	61	20	----	----	6	8
(5)	Tehran	Six university-affiliated hospitals	1999-2000	2354	----	921	1074	----	----	----	----
(13)	Gilan	Poursina	2009-2010	668	----	441	167	----	23	----	----
(7)	Rafsanjan	Ali IbnAbitaleb	2009	380	----	274	42	----	----	----	64

Table 2 : Comparison of Causes of Head Trauma in Patients Aged 0-20 and 0-14 years

Age Range	Total Number	By Motor Vehicle Accidents	By Falling	Hit By Objects	By Fights	By Sports	By Other Factors
0-14 Years	968 (100%)	313 (32.3%)	467 (48.2%)	89 (9.2%)	10 (1.0%)	40 (4.1%)	22 (2.3%)
0-20 Years	13004 (100%)	5615 (43.2%)	4173 (32.1%)	432 (3.3%)	741 (5.7%)	32 (0.2%)	84 (0.6%)

compared to girls. Similar results were reported by studies in Sweden and Uganda^[21,22]. In addition, head trauma is higher in rural and urban regions^[13], probably indicating the role of the environment on the formation of high-risk habits. On the other hand, the tools and the environment are sometimes less safe in rural than urban areas.

The incidence of trauma is higher in the middle months of the year, especially from late-May to late-December^[2,5]. Nevertheless, other studies have reported that the highest number of accidents occur at the beginning of winter and mid-summer^[15]. Another retrospective study conducted in two successive years in the trauma emergency department of Alzahra Hospital, Isfahan, Iran on trauma in 2300 children reports that the most common season for trauma is summer. It reported that trauma occurs twice in boys than girls. Contrary to the present study, the noted study showed that 32% of pediatric trauma occurred by falling, and 46.7% by motor vehicle accidents. Most common type of trauma was head trauma^[23].

Although the calculations in this study indicate that the most frequent cause of pediatric head trauma is falling yet one must keep in mind that these statistics can not be used because many studies conducted in Iran have not reported the statistics on head trauma or children aged 0-14 separately. It is noteworthy that, in this study, the trauma caused by the 2003 Bam earthquake and the Iran-Iraq War were excluded^[24,25].

The brain of children has a larger water content, larger ratio compared to the entire body a more vulnerable vascular structure, and a different degree of myelination compared to adults. Therefore, epidural and subdural hematoma and its secondary syndrome are more common in children^[26]. A study on average head injury in France indicates that 24.5% of those aged 13-20 years suffer from disability up to 10 month after injury. In some cases, recovery takes up to 3 months, and 5.9% of cases have symptoms up to 6 months later and symptoms persist up to 10 year in some cases^[26].

Some studies in Iran have reported that trauma in children has the higher percentage compared to other age groups^[8]. Thus, it is necessary to equip all hospitals with pediatric trauma wards, and perform planning and decision-making in order to prevent accidents to children^[27]. Studies such as the present one can

assist in this regard. A study conducted in Scoland showed that, over the course of 39 years (from 1975 to 2012), the annual child mortality has considerably decreased following training and preventive measures. Mortality caused by accidents had the highest reduction, i.e. 80%, with the highest reduction in men^[28].

As the most frequent cause of pediatric head trauma was falling and motor vehicle accidents, it is recommended that the floors of houses be completely covered by carpet and rugs and the playgrounds be covered by sponges or natural/artificial lawn. This can significantly reduce the force exerted to the head upon hitting the ground^[29]. In games in which falling is possible, children must wear simple and light safety helmets^[30]. At home, kindergartens and schools, children must be educated using picture books that falling may have negative consequences for them. In urban trips, children must not ride motorcycles and use child safety seats in cars. Although the use of a child safety seat may prevent trauma, patents must keep in mind that it cannot be effective in high-speed accidents^[31]. Children's playground must be as far away as possible from motor vehicles. Parents and care givers must receive the necessary training^[32, 33]. In cases of deliberate trauma, the wrongdoers must be introduced to the judiciary to prevent these accidents in future^[34].

REFERENCES:

1. Jalalvandi F, Arasteh P, Safari Faramani R, Esmailivand M. Epidemiology of Pediatric Trauma and Its Patterns in Western Iran: A Hospital Based Experience. *Global journal of health science*. 2015;8(6):139-46.
2. Saatian M, Ahmadpoor J, Mohammadi Y, Mazloumi E. Epidemiology and Pattern of Traumatic Brain Injury in a Developing Country Regional Trauma Center. *Bulletin of emergency and trauma*. 2018;6(1):45-53.
3. Chong SL, Khan UR, Santhanam I, Seo JS, Wang Q, Jamaluddin SF, et al. A retrospective review of paediatric head injuries in Asia - a Pan Asian Trauma Outcomes Study (PATOS) collaboration. *BMJ open*. 2017;7(8):e015759.
4. Garg K, Sharma R, Gupta D, Sinha S, Satyarthee GD, Agarwal D, et al. Outcome Predictors in Pediatric Head Trauma: A Study of Clinicoradiological Factors. *Journal of pediatric neurosciences*. 2017;12(2):149-53.
5. Zargar M, Sayyar Roudsari B, Shadman M, Kaviani A, Tarighi P.

- Pediatric transport related injuries in Tehran: the necessity of implementation of injury prevention protocols. *Injury*. 2003;34(11):820-4.
6. Sarioglu FC, Sahin H, Pekcevik Y, Sarioglu O, Oztekin O. Pediatric head trauma: an extensive review on imaging requisites and unique imaging findings. *European journal of trauma and emergency surgery* 2017.
7. Iranmanesh F. Outcome of head trauma. *Indian journal of pediatrics*. 2009;76(9):929-31.
8. Nayeabghayee H, Afsharian T. Correlation between Glasgow Coma Scale and brain computed tomography-scan findings in head trauma patients. *Asian journal of neurosurgery*. 2016;11(1):46-9.
9. Hashemi B, Amanat M, Baratloo A, Forouzanfar MM, Rahmati F, Motamedi M, et al. Validation of CRASH Model in Prediction of 14-day Mortality and 6-month Unfavorable Outcome of Head Trauma Patients. *Emergency*. 2016;4(4):196-201.
10. Yousefzadeh-Chabok S, Kazemnejad-Leili E, Kouchakinejad-Eramsadati L, Hosseinpour M, Ranjbar F, Malekpouri R et al. Comparing Pediatric Trauma, Glasgow Coma Scale and Injury Severity scores for mortality prediction in traumatic children. *Ulus Travma Acil Cerrahi Derg*. 2016;22(4):328-32.
11. Masoumi B, Heydari F, Hatamabadi H, Azizkhani R, Yoosefian Z, Zamani M. The Relationship between Risk Factors of Head Trauma with CT Scan Findings in Children with Minor Head Trauma Admitted to Hospital. *Open access Macedonian journal of medical sciences*. 2017;5(3):319-23.
12. Ehsaei MR, Heidari M, Karimiani EG, Ehsaei A. Ophthalmic complications among cases of head trauma in north-eastern Iran. *International ophthalmology*. 2014;34(4):825-9.
13. Yousefzadeh Chabok S, Ramezani S, Kouchakinejad L, Saneei Z. Epidemiology of pediatric head trauma in guilan. *Archives of trauma research*. 2012;1(1):19-22.
14. Marx J, Walls R, Hockberger R. *Rosen's Emergency Medicine - Concepts and Clinical Practice*: Elsevier Health Sciences; 2013.
15. Aghakhani K, Heidari M, Ameri M, Mehrpisheh S, Memarian A. Characteristics of Traumatic Brain Injury among Accident and Falling Down Cases. *Acta medica Iranica*. 2015;53(10):652-5.
16. Saadat S, Rashidi-Ranjbar N, Rasouli MR, Rahimi-Movaghar V. Pattern of skull fracture in Iran: report of the Iran National Trauma Project. *Ulus Travma Acil Cerrahi Derg*. 2011;17(2):149-51.
17. Zamani M, Esmailian M, Mirazimi M, Ebrahimian M, Golshani K. Cause and final outcome of trauma in patients referred to the emergency department: a cross sectional study. *Iranian journal of emergency medicine*. 2014;1(1):22-7.
18. Aghakhani K, Heidari M, Yousefinejad V, Okazi A. Frequency of intracranial injury in cadavers with head trauma with and without scalp injury in Tehran. *Journal of forensic and legal medicine*. 2014;28:36-8.
19. Zandi M, Seyed Hoseini SR. The relationship between head injury and facial trauma: a case-control study. *Oral and maxillofacial surgery*. 2013;17(3):201-7.
20. Moini M, Rezaishiraz H, Zafarghandi MR. Characteristics and outcome of injured patients treated in urban trauma centers in Iran. *The Journal of trauma*. 2000;48(3):503-7.
21. Mutto M, Lawoko S, Nansamba C, Ovuga E, Svanstrom L. Unintentional childhood injury patterns, odds, and outcomes in Kampala City: an analysis of surveillance data from the National Pediatric Emergency Unit. *Journal of injury & violence research*. 2011;3(1):13-8.
22. Kobusingye O, Guwatudde D, Lett R. Injury patterns in rural and urban Uganda. *Injury prevention : journal of the International Society for Child and Adolescent Injury Prevention*. 2001;7(1):46-50.
23. Memarzadeh M, Hoseinpour M, Sanjary N, Karimi Z. [A study on trauma epidemiology in children referred to Isfahan Alzahra Hospital during 2004-7]. *Feyz, Journal of Kashan University of Medical Sciences*. 2011;14(5):488-93.
24. Sabzehchian M, Abolghasemi H, Radfar MH, Jonaidi-Jafari N, Ghasemzadeh H, Burkle FM, Jr. Pediatric trauma at tertiary-level hospitals in the aftermath of the Bam, Iran Earthquake. *Prehospital and disaster medicine*. 2006;21(5):336-9.
25. Taher AA. Pediatric facial injuries in Tehran: a review of 87 patients. *The Journal of craniofacial surgery*. 1993;4(1):21-7.
26. Garcia-Rodriguez JA, Thomas RE. Office management of mild head injury in children and adolescents. *Canadian family physician Medecin de famille canadien*. 2014;60(6):523-31, e294-303.
27. Mihic J, Rotim K, Marcikic M, Smiljanic D. Head injury in children. *Acta clinica Croatica*. 2011;50(4):539-48.
28. Hamill V, Barry SJ, McConnachie A, McMillan TM, Teasdale GM. Mortality from Head Injury over Four Decades in Scotland. *Journal of neurotrauma*. 2015;32(10):689-703.
29. Murayama H, Hitosugi M, Motozawa Y, Ogino M, Koyama K. Simple strategy to prevent severe head trauma in Judo. *Neurologia medico-chirurgica*. 2013;53(9):580-4.
30. Persaud N, Coleman E, Zwolakowski D, Lauwers B, Cass D. Nonuse of bicycle helmets and risk of fatal head injury: a proportional mortality, case-control study. *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne*. 2012;184(17):E921-3.
31. Stewart CL, Moscariello MA, Hansen KW, Moulton SL. Infant car safety seats and risk of head injury. *Journal of pediatric surgery*. 2014;49(1):193-6; discussion 6-7.
32. Allen KA. The neonatal nurse's role in preventing abusive head trauma. *Advances in neonatal care : official journal of the National Association of Neonatal Nurses*. 2014;14(5):336-42.
33. Altman RL, Canter J, Patrick PA, Daley N, Butt NK, Brand DA. Parent education by maternity nurses and prevention of abusive head trauma. *Pediatrics*. 2011;128(5):e1164-72.
34. Roujeau T, Mireau E, Bourgeois M. Nonaccidental head injury. *Handbook of clinical neurology*. 2013;112:905-12.

Review Article

The role of Insects in Forensic investigation: A review on modern techniques

1. **Hussein R. Mahmood**, Lecturer, University of AL-Qadisiyah, Faculty of sciences, Iraq
2. **Taif Mothhar Al-Khalidi**, Lecturer, University of AL-Qadisiyah, Faculty of sciences, Iraq
3. **Abbas H. Burhan**, Assist lecturer, AL-Diwaneyah teaching hospital, AL-Qadisiyah province, Iraq
4. **Mohammed M. Alkhuzai**, Assist lecturer, University of AL-Qadisiyah, Faculty of sciences

ABSTRACT :

In the current scientific review .we discussed the benefits of insects in criminal investigations and the latest methods and techniques have been developed to gather evidences .The attraction of insects to the decomposed bodies and laying eggs provide a very useful idea for scientists to recognize the postmortem index or interval(PMI).The study of insect populations and the stages of larval development has a significant importance role in recognition whether the location of the corpses has been changed or not and the cause of death. The scientists of Criminal medicine must cooperate with entomologists in the field of forensic investigation, where they have to understand the potential importance of Entomology in criminal sciences.

Corresponding Author :

Dr. Hussein R. Mahmood

Lecturer, University of AL-Qadisiyah, Faculty of sciences,
Iraq

Contact : +

E-mail : hussein.mahmood@qu.edu.iq

Article History:

Received: 14 January 2019

Received in revised form: 8 February 2019

Accepted on: 8 February 2019

Available online: 1 June 2019

Keywords: Forensic sciences, PMI, larvae, insects

INTRODUCTION

Forensic entomology is the identification of insect's applications and arthropods in the field of criminal research^[1].A large number of insects and arthropods have been diagnosed for decomposing of vertebrate's bodies^[2].A lot of scientists pointed out that the period between committing a crime and discovering a body is very useful to determine the death time (PMI), the movement of the body, the method and cause of death and the association of suspects at the place of death^[3].Forensic insect sciences have developed very rapidly over the past few years, and courts have begun to accept insects as a criminal guide. On the level of the Arab world there is interest in forensic sciences, but it shows that this science is not applied at the official level, In any of the Arab countries so far. The aim of this review was to provide the required information for criminal doctors to use insect data, larval shape, dates of growth, the spreading of toxic contents in their tissues and newly developed techniques in the field of criminal research.

HISTORY OF CRIMINAL ENTOMOLOGY:

It is noteworthy that the first use of insects in criminal investigations was in China in 1235, when the mystery of killing of a Chinese farmer was exposed to a deep pruning scissors strike, the village leader asked the farmers to bring their pruning scissors, and put them on the ground, and do not work any movement, The flies collected on one of these machetes and revealed the killer; because of the traces of blood

and flesh on his pruning scissors which he used for the crime, despite his diligence in cleaning it to hide his crime; the flies attracted the smell of blood even after washing the pruning scissors^[4].In 1855, Dr. Bergeretd' Arbois applied the forensic entomology "criminal insects" to a criminal case and was published as a Case Report. Subsequently, research in the field of Entomology and the applications of its criminal branch was intensified until criminal forensic scientists became an integral part of criminal investigations in many countries of the world today^[5].

THE ESTIMATION OF POSTMORTEM INDEX(PMI)

The larvae of insect feeding on the carcasses give a good method to assess PMI for about one month, where the most important and first step is the correct identification of species that differ in maturity and growth rates. The measurement of the oldest larvae length and dry weight and compare them to the source data is used to estimate the age of larva at PMI, where the age of larvae and environmental temperatures assessment constitute an important role in the development of larva. Each larval stage has a specified temperature degrees and days to reach its growth and when we obtain the history of larvae and compared it with the temperatures of the crime site ,PMI would be estimated. When Researchers want to determine the age of larvae, They depend on the adult insects first generation, where small belly in pale gray and wavy wings

are used for the identification^[9].

THE RELATIONSHIP BETWEEN ARTHROPODS AND POSTMORTEM CHANGES

Auto-decomposition or autolysis is a process that happens once the occurrence of death, and at that time, cells died and digested by enzymes and this brings about to the decomposition of body. Bacteria present in gastrointestinal tract (GIT) start to destroy the fluids of soft tissues producing gases such as H₂S, CO₂, CH₄, NH₃ and SO₂. Apneumones is a term for Volatile particles released from the decomposing corpses attract insects. The Volatile chemicals released at different stages of decomposition can be isolated by investigators and these Volatile particles are able to modify the behavior of insect^[6]. Sulfur-based compounds are responsible for attracting insects to bodies, but egg laying or oviposition place by ammonium-rich compounds exist in bodies^[7].

There are four categories of insects on the decomposed carrion Smith (1986):

- (1) Necrophagous are the species that feed on the carcasses and corpses.
- (2) Predators and parasites that feed on decomposing species: schizophagous.
- (3) Ants, wasps and some beetles are the species that feed on the carrion and other arthropods.
- (4) Omnivorous species such as springtails and spiders.

Both group 1 and 2 are the most important groups for the main aims of forensic sciences. They include flies and beetles^[2].

Diptera are insects that are mostly involved in legitimate investigations. Calliphoridae, Sarcophagidae and Muscidae include The predominant species. Sarcophagidae and Calliphoridae may reach within minutes after death, While the attraction of the house fly after the arrival of the body to the stage of bloating of decomposition. Wounds and body openings are usual areas of oviposition. When hatch, they produce a moving larva is said to be maggot, that grow quickly through the three instars, to reach the full size. Maggot migrates to the driest areas and begins to form pupa (forming a pupa), where the adult fly emerged^[8].

THE STAGES OF THE DECOMPOSITION PROCESS

There are five stages of decomposition depending on the physical appearance of the bodies and the internal temperature as well as the gathering insects^[9].

The first stage or fresh stage :it takes 1-2 days and extends from death until the body swells and characterized by the following:

-No significant morphological changes occur at this stage.

- Insects attract to the corpses within 10 minutes once death occurs without oviposition.

-Cellular breakdown leads to releasing chemicals attract insects and no morphological modifications^[6]

The second Stage or bloated stage (it takes 2-7days): In this stage Diptera adults were brought to the bodies in large numbers. The gases produced by anaerobic bacteria metabolic activities cause the abdomen swelling and the latter part of the body forms balloon-like appearance. The activities of arthropods associated with rotting causes elevated internal temperatures of the body.

The third stage and is also known as the stage of decay (5-13 days):

-The broken of abdominal wall, carcass shrinkage, and bloated stage ended.

-The internal temperatures heighten to 14° C and as a result the odor of degradation is increasing as the temperature increases and low with low temperature.

- The weight of carcass is decreasing on the tenth day and increasing the biomass of fly larvae and flies larvae away from the corpse to turn into a pupa.

The fourth stage (Post-Decomposition) : it takes 10-23 this stage starts with the leaving of dipteran larvae carcasses, behind them secondary corrosion products (BOD). BOD includes the hair, cartilage, bones, tissue with small parts, wet and sticky substances. BOD is the main site, where the activity of arthropod through this stage.

The fifth stage(The Remains stage) :average 18-90 + days

- Bones with a small amount of cartilage.

- Secondary corrosion products (BOD) are dried.

- Diptera adults and larvae are decline^[9].

INSECT DATA TO DETERMINE THE CRIME SITE

A lot of differences were reported in insect species involved with decomposed bodies in different environments and habitats. An accurate examination can reveal the variability of species because species associated with one species of habitat on the body are different with those where the body is transferred after the days of death.

MODERN TECHNIQUES IN FORENSIC ENTOMOLOGY

Bimolecular techniques include:

DNA analysis : One of the basic steps in estimating the larva age is the identification of the fly species correctly. Morphological comparisons are required a time-consuming experience and to get rid of this issue, the species of target

insects can be identified by amplifying polymerase chain reactions for appropriate regions of larval genomes and then reference data are used for comparisons^[10]. In 2001, Jeffrey Wells and Felix Spirling developed a method in which the DNA of mitochondria was used to recognize between Chrysomyinae species. This type of techniques is very useful when we work on identifying samples that do not have clear morphological characteristics at certain stages of life^[11].

Gene expression studies : Despite the use of physical properties and the sizes of various instars to estimate the age of the fly, a recent study was conducted to determine the age of eggs based on the expression of certain genes. This is especially useful to determine the developmental stages that are not evident through size change, like as eggs or pupa, where the general interval can only be estimated according to the particular developmental stage periods. This is usually done by smashing the stages into small units separated by the expected change in gene expression^[12].

Techniques used in Entomotoxicology : The drugs consumed by the dead person can accumulate in the flies larvae that feed on the carcasses. Mass spectrometry, gas chromatography (GC) and thin layer chromatography (TLC), are used to investigate the presence of toxic substances during the advances of decomposition stages. In the same time, toxins can affect the stages of larval development. A lot of drugs such as heroin and Cocaine can accelerate larval growth in the case of existence in the carcasses and Poisons such as malthione can retard the colonization of insects when it exist in the carrion^[10].

Potassium permanganate staining

The absence of a scanning electron microscope, a faster and less expensive technique is staining by KMnO₄ (potassium permanganate). Normal saline solution is used to rinse eggs and put them in a glass petri dish, then eggs are soaked in 1% KMnO₄ for one minute, dehydrated, then examine under light microscope after mounting them on a slide. The different morphological characteristics are compared. The features used to identify eggs such as the plastron length, size width and morphology. The results are compared with standards to determine the species that belong to the eggs^[13].

Mock crime scenes : The use of fake crime scenes using animal carcasses such as pigs and rabbits has become very important tool and very common when forensic scientists are subjected to training. These animal carcasses represent a human victim bodies and could be used to interpret the different environmental impacts on arthropods and the post-mortem estimates^[14].

CONCLUSION :

Forensic insect sciences have developed very rapidly over the past few years, and courts have begun to accept insects as a criminal guide. On the level of the Arab world there is interest in forensic sciences, but it shows that this science is not applied at the official level. In any of the Arab countries so far. This review provides the required information for persons who work in forensic medicine to use insect data, larval shape, dates of growth, the spreading of toxic contents in their tissues and newly developed techniques in the field of criminal research.

REFERENCES :

1. Catts EP, Goff ML. Forensic entomology in criminal investigations. *Annu Rev Entomol.* 1992;37:253–72.
2. Amendt J, Krettek R, Zehner R. Forensic entomology. *Naturwissenschaften.* 2004;91:51–65.
3. Sukontason K, Narongchai P, Kanchai C, Vichairat K, Sribanditmongkol P, Bhoopat T, et al. Forensic entomology cases in Thailand: a review of cases from 2000 to 2006. *Parasitol Res.* 2007;101:1417–23.
4. Benecke M. A brief history of forensic entomology. *Forensic Science International.* 2001; 214.
5. Gennard D. *Forensic Entomology An Introduction*, Wiley 2007
6. LeBlanc HN, Logan JG. Exploiting Insect Olfaction in Forensic Entomology. In: Amendt J, Goff ML, Campobasso CP, Grassberger M, editors. *Current Concepts in Forensic Entomology.* 2010; 205–21.
7. Ashworth JR, Wall R. Responses of the sheep blowflies *Lucilia sericata* and *L. cuprina* to odour and the development of semiochemical baits. *Med Vet Entomol.* 1994;8:303–9.
8. Goff ML, Lord WD. Entomotoxicology; a new area of forensic investigation. *Amer J foren med pathol.* 1994;15:51–7.
9. Tullis K, Goff ML. Arthropod succession in exposed carrion in a tropical rainforest on O'ahu Island, Hawaii. *J. Med. Entomol.* 1987;24:332–9.
10. Chen WY, Hung TH, Shiao SF. Molecular identification of forensically important blow fly species (Diptera: Calliphoridae) in Taiwan. *J Med Entomol.* 2004;41:47–57.
11. Wells D, Spirling AH. DNA-based identification of forensically important Chrysomyinae (Diptera: Calliphoridae). *Forensic Science International.* 2001;120:110–115.
12. Tarone AM, Jennings K C, Foran, David R. Aging Blow Fly Eggs Using Gene Expression : A Feasibility Study. *Journal of Forensic Sciences.* 2007;52 (6): 1350–1354.
13. Sukontason K, Sukontason K L, Piangjai S, Boonchu N, Kurahashi H, Hope M, Olson JK. Identification of forensically important fly eggs using a potassium permanganate staining technique. *Micron.* 2004;35: 391–395.
14. Schoenly KG. . *American Biology Teacher* v68 n7 September. 2006; 402–410.

Significance of Accreditation of Forensic Science Laboratories in India

1. **Madhavi Jog**, Assistant Professor*
2. **Astha Pandey**, Associate Professor*

*Institute of Forensic Science, Gujarat Forensic Sciences University, Gandhinagar, Gujarat, India.

Corresponding Author

Dr. Astha Pandey,

Associate Professor,

Institute of Forensic Science, Gujarat Forensic Sciences University, Gandhinagar, Gujarat, India.

Contact : +91 9601996653

E-mail : aasthapande@gmail.com

Article History:

Received: 17 November 2018

Received in revised form: 12 December 2018

Accepted on: 12 December 2018

Available online: 30 April 2019

KEYWORDS : Accreditation, Forensic Science Laboratory, ISO/IEC 17025, Certification.

DEVELOPMENT OF FORENSIC SCIENCE LABORATORY IN INDIA :

In India, application of science and technology for the investigation and detection of crime holds a long back history since our ancestors' time. Though, they were unknown to forensic science in the form it exists today, but scientific techniques and methods were commonly used in India since olden times¹. With increase in rate of crime, a need of separate department was felt that is equipped with all modern instruments and separate divisions that can handle variety of criminal and civil cases occurring in India.

With this view, the first state forensic science laboratory in India was established in the year 1952 at Calcutta^[2] and became the first multidisciplinary forensic science laboratory in the country by the year 1958. After that, the first Central Forensic Science Laboratory was established at Calcutta during 1957. During the year 1965, the second Central Forensic Science Laboratory was established at Hyderabad. The Central Forensic Science Laboratory, Chandigarh, established as FSL in Lahore (established in year 1933) was shifted to Chandigarh during 1961. Over the years many full-fledged forensic science laboratories were established in various states. By mid 1990's, most of the States had established their own forensic science laboratories.

Forensic Science management involves examination of crime scene, recovery of evidence, laboratory examination, interpretation of findings and presentation of the conclusions necessary for investigation and intelligence purpose and above all the reconstruction of the crime scene. This field involves examination of wide range of substances.

Forensic science laboratory is indispensable, one of the three fundamental pillars of criminal judicial system and is a connecting link between the police and judiciary. FSL is a multidisciplinary institution, in which through modern & up-

to-date sophisticated instrumentation and verified scientific methodology, the physical exhibits pertaining to crime are scientifically analyzed. Examination report of forensic science lab is established as strong evidence in establishing the involvement of accused under Indian evidence act and criminal procedure code 293, which is given by the Director, Deputy Director or Assistant Director of the concerned division.

Continuous research and development work is also done in forensic science lab to solve the puzzle of complicated cases, which is totally different from the R&D work of the other academic & scientific institutions due to connection with criminal judicial system.

Forensic science lab also provides the forensic help to investigation officers in crime scene investigation through mobile forensic units along with the analytical work of examining the crime exhibits. Mobile forensic units provide the forensic help in identification and collection of proper physical exhibits from the scene of crime³.

With the development of all this and its importance, the need for standardisation of various aspects- their analytical processes, reporting methods, etc. by way of 'Accreditation' was increasingly perceived.

Accreditation is the independent evaluation of conformity assessment bodies against recognised standards to carry out specific activities to ensure their impartiality and competence. Through the application of national and international standards, government, procurers and consumers can have confidence in the calibration and test results, inspection reports and certifications provided. Accreditation bodies are established in many countries with the primary purpose of ensuring that conformity assessment bodies are subject to oversight by an authoritative body. Accreditation bodies, which have been evaluated by peers as competent, sign

arrangements that enhance the acceptance of products and services across national borders, thereby creating a framework to support international trade through the removal of technical barriers^[4]. Some might ask, “Why are we worried about international standards for forensic science? There are no global issues^[5]...”

ACCREDITATION AND FORENSIC SCIENCE

First of all, there are indeed global issues, and increasingly so. Cross-border crimes such as money laundering, the cultivation, manufacture, and distribution of drugs, and human trafficking require harmonization of approaches and mutual confidence in results. Identification of mass-disaster victims from medical or dental records and from DNA analysis is also a place where consistency and reliability of international approaches matters, whether the disaster is caused by a terrorist incident, by an accident, or by a natural event such as tsunami.

Of more immediate concern is the fact that forensic testing is under attack from defence attorneys. The response is made more difficult by the knowledge that no test is infallible, and there are bound to be failures in forensic-evidence testing just like there are failures in tests outside forensics. The critics will hunt down any actual or perceived errors in cases and use them to support their stance on lack of overall reliability. They may also suggest that examiners may be biased, with a reasonable justification in a few headline-grabbing cases, such as the 2004 Madrid train-bombing investigation where a fingerprint was erroneously ascribed to an Oregon attorney, Brandon Mayfield, and not to the actual bomber^[5].

It can be argued that any testing laboratory in any industry or government regulatory sector should be committed to achieving the best possible quality of testing, and even without the need to respond to the above concerns; forensic service providers should embrace accreditation as an integral part of their operations. This is happening and has passed the critical mass in regard to the number of accredited laboratories such that users—attorneys and courts—will now question why a crime laboratory is not accredited. In some sectors, for example, accreditation is mandatory for DNA testing where the results are entered into the national DNA database^[5].

ISO/IEC 17025

The standard that applies to accreditation of testing laboratories is ISO/IEC 17025. The content of the International Standard covers a number of factors including:

- The qualifications, training and experience of the staff;
- The right equipment—which has been properly calibrated and maintained;
- Adequate quality-assurance procedures;

- Proper sampling practices;
- Appropriate testing procedures;
- Valid test methods;
- Traceability of measurements to national standards;
- Accurate recording and reporting procedures;
- Suitable testing facilities.

All these factors contribute to a laboratory being technically competent to perform testing and are as relevant to forensic science as they are to international trade.

ISO (the International Organization for Standardization) was established in 1947, with its headquarters in Geneva, Switzerland. ISO is not an acronym for the name of the organization (that would be IOS), but it is instead a play on the Greek word ISOS, meaning **equal or the same**.

The mission of ISO is the development of standards to facilitate international exchange of goods and services. It does this by making use of a network of national standards institutes from more than 163 countries, with ANSI being representative in the United States. For some standards, an ISO partners with IEC (the International Electrotechnical Commission) which is the world's leading organization for the preparation and publication of International Standards for all electrical, electronic, and related technologies.

ACCREDITATION VERSUS CERTIFICATION

Accreditation of forensic science laboratories refers to a recognition process for laboratories that meet certain standards for management, operations, equipment, and security. Four major accrediting bodies offer accreditation programs for forensic labs in the United States (ASCLD/LAB, FQS, A2LA, and ABFT). Laboratory accreditation is the process of an independent competent authority inspecting policies, practices and procedures for compliance with a credible set of standards. It provides the public and the users of the services with an objective measure of reliability of the laboratory operations. On the other hand, certification refers to a credentialing process for individual analysts and technicians who meet certain standards for education, training, and experience. Innumerable organizations offer certification for forensic analysts in fields ranging from toxicology to forensic art^[6].

ACCREDITATION OF FSL IN INDIA

The accreditation of crime laboratories or crime scene investigation units is achieved through assessment by an accrediting body, but the question of who accredits the accreditors is legitimate. The answer is that ISO/IEC 17011 is the accrediting body's equivalent of ISO/IEC 17025 and 17020 for the testing and scene-examination bodies. The

conformity audit—called an evaluation—is conducted by the International Laboratory Accreditation Cooperation (ILAC) or one of the regional accreditation cooperations that are recognized by it. These are the Inter American Accreditation Cooperation (IAAC) and the Asia Pacific Laboratory Cooperation (APLAC). APLAC groups organisations in the Asia Pacific region are responsible for accrediting, testing and inspection facilities. Members are nationally recognized accreditation bodies and are usually owned or endorsed by government.⁷ "In India, Laboratory Accreditation activities are administered under the direction of the Board for National Accreditation Board for Testing and Calibration Laboratories (NABL), by involving Technical Committees and Evaluation Panels as the recommending bodies. NABL system is based on the ISO/ IEC 17011 for Accreditation Bodies, ISO/ IEC 17025:2005 "General Requirements for the Competence of Testing and Calibration Laboratories" for laboratories. The work in Forensic Science Laboratories being interdisciplinary in nature, there is a need to develop and supplement the "General Criteria for Laboratory Accreditation" for the purpose of accrediting Forensic Science Laboratories. The document "Specific Criteria for Accreditation of Forensic Science Laboratories" has been evolved by a Technical Committee specifically constituted for the purpose. It supplements the document ISO/ IEC 17025:2005 "General Requirements for the Competence of Testing and Calibration Laboratories" and provides specific guidance on the accreditation of Forensic Science Laboratories for assessors as well as the laboratories who are preparing for accreditation. This Specific Criteria is in compliance with ISO/ IEC 17025:2005.

NABL has adopted the following four objectives which define the purpose and nature of the program :^[8]

- To improve the quality of laboratory services provided to criminal justice system.
- To develop and maintain criteria which can be used by a laboratory to assess its level of performance and to strengthen its operations.
- To provide an independent, impartial and objective system by which laboratories can be benefited through a total operational review.
- To offer to the general public and users of the laboratory services a means of identifying those laboratories which have demonstrated that they meet established standards.

NABL issues Guidelines for Forensic Science Laboratory Assessment under following headings:^[8]

- | | |
|---------------------------|-----------------------------|
| 1. Scope of Accreditation | 2. Quality Policy Statement |
| 3. Organization and | 4. Quality Management |

- | | |
|-------------------------------|-----------------------------|
| Management | system |
| 5. Records | 6. Personnel |
| 7. Training & Development | 8. Evidence Management |
| 9. Methods and Procedures | 10. Quality Assurance |
| 11. Proficiency Testing | 12. Case Record Review |
| 13. Court Testimony | 14. Corrective and |
| Monitoring | Preventive Actions |
| 15. Instruments and Equipment | 16. Calibration |
| 17. Reports | 18. Procurement of Services |
| | and Supplies |
| 19. Accommodation and | 20. Subcontracting of |
| Safety | Technical Work |

1. Scope of Accreditation : The scope of accreditation shows the activities for which the body in question has requested accreditation and in which the body has been deemed to be competent, following an assessment. The scope of accreditation defines which services can be offered as accredited. It also includes the fields of accreditation as well as the testing methods, types of inspections and certification categories in which the body is found competent.

As a rule, scopes of accreditation are fixed scopes described in detail. An accredited body may also have a flexible scope, where the scope is presented at a more general level. Predominantly, the flexible scope has been developed to meet the needs of bodies that work in research and/or development, for situations where the properties of an object to be tested, calibrated, inspected or certified cannot always be predicted in advance. A fixed scope might also contain flexibility.

2. Quality Policy Statement: Being clear about an organization's intentions and goals is the first step toward effectiveness and results. This information, if it is well communicated and monitored in the organization, forms the basis for directed progress.

The ISO9000 standard identifies the techniques of policies (for stating intentions for how we will operate) and objectives (for stating goals for what we want to achieve) as primary tools of top management to direct the organization toward its quality goals for the products and processes of the company.

One specific policy required by the standard is called the quality policy. The quality policy is a general statement made by top management that it intends to achieve quality as a result of the company's operation. The quality policy is more than a "slogan" in that its effectiveness is measurable through the performance of the quality management system. By developing, communicating and maintaining a quality policy, the organization is held accountable to achieve the

expectations for quality stated in the policy.

Third-party auditors will look for objective evidence that the policy is effectively communicated and implemented throughout the organization. It will not be uncommon for an auditor to ask several employees to state the company's quality policy. While the expected response does not have to be a "word-for-word" recitation of the policy, every employee must be able to demonstrate knowledge of the policy and how it applies to their day-to-day job. Thus, any preparation plan for an ISO9000 registration audit will include training and/or communication of the quality policy.

3. Organization and Management: The laboratory shall be legally identifiable. It shall be organized and shall operate in such a way that its permanent, temporary and mobile facilities meet the requirements of the ISO criteria.

The laboratory shall:

- a) Have managerial staff with the authority and resources needed to discharge their duties;
- b) Have arrangements to ensure that its personnel are free from any commercial, financial and other pressures which might adversely affect the quality of their work;
- c) Be organized in such a way that confidence in its independence of judgement and integrity is maintained at all times;
- d) Specify and document the responsibility, authority and interrelation of all personnel who manage, perform, or verify work affecting the quality of calibrations and tests;
- e) Provide supervision by persons familiar with the calibration or test methods and procedures, the objective of the calibration or test and the assessment of results. The ratio of supervisory to non-supervisory personnel shall be such as to ensure adequate supervision;
- f) Have a technical manager (however named) who has overall responsibility for the technical operations;
- g) Have a quality manager (however named) who has responsibility for the quality system and its implementation. The quality manager shall have direct access to the highest level of management at which decisions are taken on laboratory policy or resources, and to the technical manager. In some laboratories, the quality manager may also be the technical manager or deputy technical manager;
- h) Nominate deputies in case of absence of the absence of the technical or quality manager;

- i) Where relevant, have documented policy and procedures to ensure the protection of clients' confidential information and proprietary rights;
- j) Where appropriate, participate in interlaboratory comparisons and proficiency testing programs.

4. Quality Management System: A quality management system (QMS) is a formalized system that documents processes, procedures, and responsibilities for achieving quality policies and objectives. A QMS helps coordinate and direct an organization's activities to meet customer and regulatory requirements and improve its effectiveness and efficiency on a continuous basis.

ISO 9001:2015, the international standard specifying requirements for quality management systems, is the most prominent approach to quality management systems.

Quality management systems serve many purposes, including:

- Improving processes
- Reducing waste
- Lowering costs
- Facilitating and identifying training opportunities
- Engaging staff
- Setting organization-wide direction

5. Records: The laboratory shall establish and maintain documented procedures for identification, collection, indexing, access, filling, storage, maintenance and disposition of quality records.

Quality records shall be maintained to demonstrate conformance to specified requirements and the effective operation of the quality system. Pertinent quality records from the subcontractor shall be an element of these data.

All quality records shall be legible and shall be stored and retained in such a way that they are readily retrievable in facilities that provide a suitable environment to prevent damage or deterioration and to prevent loss. Retention times of quality records shall be established and recorded. Where agreed contractually, quality records shall be made available for evaluation by the customer or the customer's representative for an agreed period.

NOTE: Records may be in the form of any type of media, such as hard copy or electronic media.

6. Personnel: Scientific Management of Forensic Science laboratories must have personnels with pertinent scientific qualifications and suitable forensic experience. It includes-

6.1 Director :

The Director of the laboratory should be a person having direct operational and administrative control of the laboratory, must

have minimum qualification of M.Sc. degree. However, it is desirable to have Ph.D. degree and additional qualifications in management and administration with sufficient experience. Director must have sufficient knowledge about all the related fields; to the scientific functions and forensic aspects of the laboratory's work, preferably through an experience as a forensic scientist.

6.2 Reporting Officers and Supporting Staffs

Good communication skill, presence of mind and according to NABL, the reporting officer shall have minimum qualifications and experience as given by NABL for each sub-disciplines of Forensic Science like Chemistry and Explosives, Toxicology, Biology and Serology, DNA Fingerprinting, Physics, Ballistics, Questioned Documents, Forensic Psychology, Fingerprints, Crime Scene Investigation.

Beside them, few supporting staffs or non-testifying staffs independent of previous experience, qualifications, must have satisfactorily completed laboratory's training program before being authorized to work independently.

7. Training and Development: A training programme must be designed and documented for each functional area of the laboratory. The training programme must include:

- ☐ the performance of competency test(s) in all related areas and

- ☐ Where relevant, the presentation of evidence in the court

Competency testing required to include:

- ☐ an evaluation of acquaintance with the existing literature and

- ☐ the analysis and identification of known and unknown materials

New members of staff, whatever be their qualifications or previous experience, must have satisfactorily completed the laboratory's training programme before being authorised to work independently by the director of the laboratory.

8. Evidence Management:

8.1 Evidence Control

The forensic science laboratory must have a well-documented evidence control system. The control system is effectively designed in a way it ensures and documents the integrity of physical evidences received. A chain of custody record (e.g. Signature of the person, date, time, description of evidence) must be maintained so that it provides a comprehensive history of each evidence transfer, over which the laboratory has control.

Each individual item of evidence must be marked with unique case no. designator for identification. Should the item not lend itself to marking, its proximal container must be marked.

Labelling on caps/ lids alone is not acceptable because of the risk of wrongly replacing the lids during batch testing of similar samples. The identification should be retained throughout the life of the item in the laboratory. Evidence must be protected from loss, cross transfer, contamination and/or deleterious changes. When destructive tests are necessary, procedures must ensure that as much material as possible is retained for re-analysis, if required. The laboratory must establish and document its policy and procedures for the retention and disposal of exhibits following the completion of examinations and/or testing.

9. Methods and Procedures: The methods and procedures of various types of analysis in different divisions is to be prepared in the systematic manner as standard operating procedure for instruments and tests to be carried out for various types of evidences received in the laboratory.

10. Quality Assurance: It means all the planned and systematic activities implemented within the quality system and demonstrated as needed, to provide adequate confidence that an entity will fulfil, requirements for quality.

Analytical performance must be monitored by operating quality controls which are appropriate to the type and frequency of testing undertaken by a laboratory. The range of quality control activities available to laboratories includes the use of following:

- Certified reference materials and internally generated reference Materials;
- Statistical tables;
- Positive and negative controls;
- Control charts;
- Alternative methods;
- Repeat testing;
- Spiked samples,
- Standard additions and internal standards;
- Independent checks (verification) by other authorized personnel.

11. Proficiency Testing: The aim of proficiency testing is to compare a laboratory's performance against that of similar laboratories. This helps the laboratory to monitor its performance and diagnose problems before they become expensive in terms of time and money. In other words, proficiency testing supplements the laboratory's internal quality control and complements, where utilized, its accreditation by NABL.

Proficiency testing can assist with method evaluation, precision estimation and the availability of reference material

for future testing. If the laboratory performs well, proficiency results can also be used as a marketing tool which both reassures and builds customer confidence.

12. Case Record Review: A critical component of any forensic science laboratory's continuous quality improvement (CQI) system and finding flaws, is the ongoing, periodic review of case files taken from any **case sampling**. These quality assurance (QA) case reviews should be performed by skilled **QA case reviewers** who collect information to assess practice, services, and outcomes and to determine whether specific requirements have been met.

Pivotal to gaining a complete picture of the case is conducting case-related interviews, or interviewing various parties involved in the cases. QA case reviews employing a comprehensive **case review instrument** as well as **case-related interviews** will yield meaningful data that can be used to make individual, unit, division, regional and state-wide practice improvements.

Through these thorough case records file reviews and interviews, the court can better understand how the agency's policies, procedures, and practices are affecting justice. Assessment of this detailed case-level data helps in evaluating the quality of services being delivered, and how the agency can better ensure ongoing safety and care, permanency, and well-being. The laboratory's policies and manuals should provide clear guidance for carrying out and completing case reviews.

13. Court Testimony Monitoring: Court testimony monitoring is necessary to evaluate the delivery of the analyst's testimony and to ensure that it is scientifically consistent with the findings documented in the case file. If an analyst testifies during the calendar year, they must be monitored at least once.

Two methods of testimony monitoring are employed:

- ☐ Court testimony observation.
- ☐ Court officer evaluation.

14. Corrective and Preventative Action: The laboratory shall establish and maintain documented procedures for implementing corrective and preventative action. Any corrective or preventative action taken to eliminate the causes of actual or potential nonconformities shall be to a degree appropriate to the magnitude of problem and commensurate with the risks encountered. The laboratory shall implement and record any changes to the documented procedures resulting from corrective or preventative action.

14.1 Corrective action

The procedures for corrective action shall include:

- (a) The effective handling of customer complaints and reports of product nonconformities;

- (b) Investigation of the cause of nonconformities relating to product, process and quality system and recording the results of the investigation;

- (c) Determination of the corrective action needed to eliminate the cause of nonconformities;

- (d) Application of controls to ensure that corrective actions are taken and that it is effective.

14.2 Preventative Action

The procedures for preventative action shall include:

- (a) The use of appropriate sources of information such as processes and work operations which affect product quality, concessions, audit results, quality records, service reports and complaints to detect, analyse and eliminate potential causes of nonconformities;

- (b) Determination of the steps needed to deal with any problems requiring preventative actions;

- (c) Initiation of preventative action and application of controls to ensure that it is effective;

- (d) Ensuring that relevant information on actions taken is submitted for management Review.

15. Instruments and Equipment: Each major instrument/equipment must be recorded in a register and the following details have to be included:

- ☐ Name of the instrument/equipment,
- ☐ The manufacturer's name, type, identification and serial number or other unique identification,
- ☐ Date received and date placed in service, where appropriate
- ☐ Current location, where appropriate
- ☐ Condition when received (for example, new, reconditioned), where appropriate.

16. Calibration:

16.1 Calibration - Instruments/ Equipment

All the Instruments/ equipment should to be properly calibrated. Where equipment used for tests, including equipment used for subsidiary measurements, have a significant effect on the accuracy or validity of the test results, that equipment shall be calibrated or otherwise verified before being put into service and shall be subjected to a programme of re-calibration and/ or re-verification.

16.2 Calibration – Chemical Analysis

Quality testing in a testing laboratory, particularly in the cases of its assessment, high lights the need to consider the accuracy of its measurements and analytical results and to ensure that the principles necessary to establish accuracy have not been omitted. So the calibration of the parameters associated with

chemical analyses and material tests warrants particular attention.

17. Reports: The results of each test or series of tests carried out by the laboratory shall be reported accurately, clearly, unambiguously and objectively, in accordance with any instructions in the test methods. The results should normally be reported in a certificate, test report or test certificate and should include all the information necessary for the interpretation of the test results and all information required by the method used. Besides this, the following information must be included in the reports of tests/examinations conducted by the laboratory as per the guidelines of NABL:

- A title (e.g. test certificate, test report)
- The date of issue of the report
- The name and address of the laboratory and if different from the address, the location where tests were performed
- Unique identification number of the report (e.g. by report number) on each page
- The page number and the total number of pages (i.e. page 'x' of 'y') on each page
- The name and address of the client
- Description, unambiguous identification and date of receipt of the item(s) tested or examined
- Date of performance of the test and or examination
- Identification of the test/examination method or procedure
- Test/examination result
- Reference to sampling procedure used by the laboratory where these are relevant to the validity or application results
- Reference to other information where this may be relevant to the validity or application of results
- The name, title and signature or equivalent identification of the person authorised to release the report.

18. Procurement of Services and Supplies:

19. Accommodation and Safety: Laboratory accommodation, calibration and test areas, energy sources, lighting, heating and ventilation shall be such as to facilitate proper performance of calibrations or tests. The environment in which these activities are undertaken shall not invalidate the results or adversely affect the required accuracy of measurement. Particular case shall be taken when such activities are undertaken at sites other than the permanent laboratory premises.

The laboratory shall provide facilities for the effective monitoring, control and recording of environmental conditions as appropriate. Due attention shall be paid, for example, to biological sterility, dust, electromagnetic interference,

humidity, mains voltage, temperature and, sound and vibration levels, as appropriate to the calibrations or tests concerned.

There shall be effective separation between neighbouring areas when the activities therein are incompatible. Access to and use of all areas affecting the quality of these activities shall be defined and controlled. Adequate measures shall be taken to ensure good housekeeping in the laboratory.

It is also the laboratory's responsibility to comply with the relevant health and safety requirements.

20. Subcontracting of Technical Work: Where a laboratory sub-contracts any part of the calibration or testing, this work shall be placed with a laboratory complying with these requirements. The laboratory shall ensure and be able to demonstrate that its sub-contractor is competent to perform the activities in question and complies with the same criteria of competence as the laboratory in respect of the work being sub-contracted. The laboratory shall advise the client in writing of its intention to sub-contract any portion of the testing to another party.

The laboratory shall record and retain details of its investigation of the competence and compliance of its subcontractors and maintain a register of all subcontracting.

LIST OF ACCREDITED INDIAN FORENSIC SCIENCE LABORATORIES

(ISO/IEC 17025: 2005)

As of now, there are 4 Central Forensic Science Laboratories (CFSLS), 28 State / Union Territory Forensic Science Laboratories (State / UT FSLs) along with their Regional FSLs (32 RFSLs) and Mobile FSLs (144 MFSLs). It must be noted that out of total 210 forensic science laboratories, only following 7 have been accredited so far. **(Table 1)**

INTERNATIONAL SCENARIO :

In the United States, there are two main programs applicable to forensic laboratories. The first is one provided by the American Society of Crime Laboratory Director Laboratory Accreditation Board (ASCLD/LAB). This has been in operation since 1982, and at the start of 2003, 237 of approximately 350 crime laboratories in the United States were ASCLD/LAB accredited.

There are also laboratories in Australia, Canada, Hong Kong, New Zealand, and Singapore that are ASCLD/LAB accredited. However, the main accreditation programs for testing laboratories worldwide are based on ISO Standard 17025. This has been used as the basis of forensic programs in Canada, Australia, England, and Holland.

The ASCLD/LAB program recognizes controlled substances, biology (including DNA), traces evidence, toxicology, latent

Table 1 : Name & Contact Details of The Laboratory

Sr. No.	Name & Contact Details of The Laboratory	Certificate No.	Date of Issue	Date of Expiry
1	Andhra Pradesh Forensic Science Laboratory, Red Hills, Hyderabad Andhra Pradesh Forensic Science Laboratory ^[2]	T-0756	27.11.2011	26.11.2013
2	Central Forensic Science Laboratory Directorate of Forensic Science, MHA,GOI, Ramanthapur Hyderabad- 500013	T-0289	15.03.2011	14.03.2013
3	Central Forensic Science Laboratory Directorate of Forensic Science, Railway Board Building, Shimla	T-0992	16.05.2011	15.05.2013
4	Central Forensic Science Laboratory, 30 Gorachand Road, Kolkata- 700 014	T-1576	25.08.2011	24.08.2013
5	Central Forensic Science Laboratory, Central Bureau of Investigation Block-4, CGO Complex, Lodhi Road, New Delhi - 110003	T-1464	04.10.2010	03.10.2012
6	Forensic Science Laboratory, Govt. of NCT Delhi, Madhuban Chowk, Sector-14, Delhi- 110085	T-1705	15.06.2012	14.06.2014
7	Regional Forensic Science Laboratory Falsawadi Police Line, Surat, Gujarat- 395 003	T-1882	08.12.2010	07.12.2012

print development and comparison, questioned documents, firearms and tool marks, and crime scene examination as areas of testing or disciplines in forensic science. In addition to crime scene examination, a laboratory must submit itself for evaluation in all disciplines in which it is active and cannot elect to become accredited in selected subset of a discipline. The evaluation consists of a compliance audit against a published set of standards, expressed as criteria. The criteria are classified as "essential", "important", and "desirable". To become accredited, a laboratory must meet all essential criteria that apply, 75% of important criteria, and 50% of desirable criteria.

The ISO- based programs differ somewhat in operation. The main international standard provides a set of clauses describing quality management and technical requirements that must be met. These same standards apply to all testing laboratories seeking ISO accreditation, no matter what the testing area is. The program is tailored to specific testing areas, such as forensic science, by means of amplification documents that provide guidance on acceptable interpretations of clauses in the field of application. There is no list of disciplines, and laboratories are not required to seek accreditation in every area in which they conduct testing. However, consensus operation of ISO program requires that the laboratory and its accrediting body develop and publish a scope of accreditation that

describes the tests and materials covered by the accreditation. Forensic Quality Services (FQS) was formed by the National Forensic Science Technology Centre (NFSTC) and provides ISO- based accreditations to forensic science laboratories in the United States.

There is, de facto, a third accreditation program in forensic testing in the United States. This is for forensic DNA- testing and DNA – data basing laboratories. Laboratories seeking to produce data for inclusion in the national DNA database (Combined DNA Index System- CODIS) or to receive federal government financial support for such work are required to show compliance with national quality assurance standards. Such compliance can be demonstrated by accreditation through the ASCLD/LAB or FQS accreditation programs that use the national standards as field specific guidelines. Direct accreditation can also be obtained through FQS.

There is no program that establishes compulsory certification of forensic laboratory testing personnel. However, all three accreditation programs contain requirements for establishing and maintaining the competency of personnel. Typically, these are requirements for training, professional development, and proficiency testing.

Training requirements establish the knowledge base and practical skills required before being permitted to conduct

unsupervised casework. Professional development establishes pathways whereby analysts continue to improve their theoretical and practical abilities. Proficiency testing provides a process for monitoring the overall competency of systems and individuals by presenting them with samples the composition of which is known (or can be determined) by testing agency but not by the analyst.

CONCLUSION :

A lot has happened since Dr. Edmond Locard laid the foundations for forensics as a scientific discipline in the first decade of the 20th Century: Colonel Calvin Goddard set up the country's first independent crime laboratory at North-western University in the early 1930s, and Dr. Alec Jeffreys introduced the world to forensic DNA testing in the mid 1980s. The power of forensic science to identify the guilty and exonerate the innocent is far beyond what the pioneers could have dreamt of. At the same time, the science and legal communities have become more and more willing and able to scrutinize what forensic laboratories do in minute detail.

The law and science demand that what the forensic laboratories do must be 'fit for purpose' and that's why accreditation is needed to improve the quality of laboratory services provided to criminal justice system, to develop and maintain criteria which can be used by a laboratory to assess its level of performance and to strengthen its operations, to provide an independent, impartial and objective system by which laboratories can be benefited through a total operational review, and to offer to the general public and users of the laboratory services a means of identifying those laboratories which have demonstrated that they meet established standards.

REFERENCES :

1. Tewari RK, Ravikumar KV. History and development of forensic science in India. J Postgrad Med 2000;46:303
2. AP Forensic Science laboratories. Available from: www.apfsl.gov.in. Accessed on Dec. 11th, 2018.
3. Directorate of state forensic science laboratory, Govt. of Rajasthan. Available from: www.home.rajasthan.gov.in/sfsl. Accessed on Dec. 11th, 2018.
4. ILAC. Available from: <https://ilac.org/>. Accessed on Dec. 11th, 2018.
5. Evidence Technology Magazine – Home. Available from: www.evidencemagazine.com. Accessed on Dec. 11th, 2018.
6. Tilstone WJ, Savage KA, Clark LA. Forensic Science: An Encyclopedia of History, Methods, and Techniques. Santa Barbara, Calif.: ABC-CLIO;2006.307 p.
7. APLAC: Home Page. Available from: <https://aplac.org/>. Accessed on Dec. 11th, 2018.
8. NABL Available from: <https://www.nabl-india.org/>. Accessed on Dec. 11th, 2018.

Commentary (Scientific Correspondence)

The Protection of Children from Sexual Offences Act, 2012

1. **Kunal Khanna**, Assistant Professor, Deptt. of Forensic Medicine, Kalpana Chawla Govt. Medical College, Karnal (Haryana)
2. **Vijay Pal**, Medicolegal Advisor to Govt. of Haryana, Professor & Head, Deptt. of Forensic Medicine, Kalpana Chawla Govt. Medical College, Karnal (Haryana)

Corresponding Author :

Dr. Vijay Pal

Medicolegal Advisor to Govt. of Haryana
Professor & Head, Deptt. of Forensic Medicine
Kalpana Chawla Govt. Medical College, Karnal (Haryana)
Contact : +91-94164-78078, 94163-12472
E-mail : drvijaykhanagwal@yahoo.co.in

Article History:

Received: 11 March 2019
Received in revised form: 20 March 2019
Accepted on: 20 March 2019
Available online: 1 June 2019

Keywords: Pcoso, Children, Sexual Assault, Penetrative Assault

INTRODUCTION :

The incidence of sexual offences is increasing day by day in India despite stringent laws against the guilty for such offences. The children are even more vulnerable for various sexual offences on account of their innocence, fear of injury or harm by the hands of perpetrator and ultimately non-resistance offered by them against the accused. According to a study by Govt. of India, every 2nd child was sexually abused at one or the other stage in our country. Before the Criminal Law Amendment Act, 2013 and Protection of children from sexual Offences Act, 2012, there were two punishable sexual offences in the Indian Penal Code, viz. rape, that is, peno-vaginal penetration and outraging the modesty of a woman, which referred to actions such as groping or taking indecent photographs. Earlier, the law did not distinguish between adult and child survivors. In view of the rise in sexual offences against the children of tender age, the Parliament thought it necessary to formulate the Protection of children from sexual Offences (POCSO) Act in the year 2012 so that the heinous crimes of sexual abuse and sexual exploitation of children could be addressed effectively. Consequently, in the POCSO Act, all types of sexual offences against children as well as their corresponding punishments have been listed in detail.

BACKGROUND :

National Crime Record Bureau data showed increased cases of sexual offences against the children which warranted to undertake appropriate national, bilateral & multilateral measures to prevent:

- inducement/coercion of a child to engage in unlawful sexual activity
- Exploitative use of children in prostitution/other unlawful sexual practices
- Exploitative use of children in pornographic performances & materials

ROLE OF STATE GOVERNMENTS

- Designation of Sessions Court in each district as Special Court under the Act
- Appointment of Special Public Prosecutor
- Establishment of Special Juvenile Police Units, Child Welfare Committees and District Child Protection Unit
- Formulate scheme for payment of compensation
- Preparation of guidelines for support professionals like doctors, NGOs, etc.
- Publicity and awareness creation
- Training of police officers and other concerned persons
- Set up of State Commission for the Protection of Child Rights (SCPCR) for monitoring implementation of the Act

SALIENT FEATURES:

1. Child was defined as any person below the age of 18 years. Child offenders not punishable under the Act; instead, to be dealt with under the Juvenile Justice (Care and Protection of Children) Act, 2000.
 - Child-friendly provisions for reporting, recording of evidence, investigation and trial.
 - Special Courts for speedy trial
2. **Followings offenses are clearly defined :**
 - Penetrative Sexual Assault (S.3)
 - Aggravated Penetrative Sexual Assault (S.5)
 - Sexual Assault (S.7)
 - Aggravated Sexual Assault (S.9)
 - Sexual Harassment (S.11)
 - Using child for Pornographic Purposes (S.13)
 - Corresponding punishments for above sections are

provided in the even Sections 4, 6, 8, 10, 12 and 14, respectively. Additionally, storage of child porn is punishable under Section 15.

- Abetment and attempt to commit offences are defined under the Sec.16.
- Punishment for Abetment (Sec.17)
- Attempt to commit an offence (half the punishment) is defined under Sec.18.

3. PENETRATIVE SEXUAL ASSAULT (S.3)

A person is said to commit "penetrative sexual assault" if-

- a. he penetrates his penis, to any extent, into the vagina, mouth, urethra or anus of a child or makes the child to do so with him or any other person; or
- b. he inserts, to any extent, any object or a part of the body, not being the penis, into the vagina, the urethra or anus of the child or makes the child to do so with him or any other person; or
- c. he manipulates any part of the body of the child so as to cause penetration into the vagina, urethra, anus or any part of body of the child or makes the child to do so with him or any other person; or
- d. he applies his mouth to the penis, vagina, anus, urethra of the child or makes the child to do so to such person or any other person.

4. PUNISHMENT FOR PENETRATIVE SEXUAL ASSAULT (S.4)

Imprisonment of either description for a term which shall not be less than seven years but which may extend to imprisonment for life, and shall also be liable to fine.

5. AGGRAVATED PENETRATIVE SEXUAL ASSAULT (S.5)

- a) Whoever, being a police officer, commits penetrative sexual assault on a child
- b) whoever being a member of the armed forces or security forces commits penetrative sexual assault on a child.
- c) whoever being a public servant commits penetrative sexual assault on a child; or
- d) whoever being on the management or on the staff of a jail, remand home, protection home, observation home, or other place of custody or care and protection established by or under any law for the time being in force, commits penetrative sexual assault on a child, being inmate of such jail, remand home, protection home, observation home, or other place of custody or care and protection; or

- e) whoever being on the management or staff of a hospital, whether Government or private, commits penetrative sexual assault on a child in that hospital; or
- f) whoever being on the management or staff of an educational institution or religious institution, commits penetrative sexual assault on a child in that institution; or
- g) whoever commits gang penetrative sexual assault on a child.: When a child is subjected to sexual assault by one or more persons of a group in furtherance of their common intention, each of such persons shall be deemed to have committed gang penetrative sexual assault within the meaning of this clause and each of such person shall be liable for that act in the same manner as if it were done by him alone; or
- h) whoever commits penetrative sexual assault on a child using deadly weapons, fire, heated substance or corrosive substance; or
- i) whoever commits penetrative sexual assault causing grievous hurt or causing bodily harm and injury or injury to the sexual organs of the child; or
- j) whoever commits penetrative sexual assault on a child, which
 - i. physically incapacitates the child or causes the child to become mentally ill as defined under clause (b) of section 2 of the Mental Health Act, 1987 or causes impairment of any kind so as to render the child unable to perform regular tasks, temporarily or permanently; or
 - ii. in the case of female child, makes the child pregnant as a consequence of sexual assault;
 - iii. inflicts the child with Human Immunodeficiency Virus or any other life threatening disease or infection which may either temporarily or permanently impair the child by rendering him physically incapacitated, or mentally ill to perform regular tasks; or
- k) whoever, taking advantage of a child's mental or physical disability, commits penetrative sexual assault on the child; or
- l) whoever commits penetrative sexual assault on the child more than once or repeatedly; or
- m) whoever commits penetrative sexual assault on a child below twelve years; or
- n) whoever being a relative of the child through blood or adoption or marriage or guardianship or in foster care

or having a domestic relationship with a parent of the child or who is living in the same or shared household with the child, commits penetrative sexual assault on such child; or

- o) whoever being, in the ownership, or management, or staff, of any institution providing services to the child, commits penetrative sexual assault on the child; or
- p) whoever being in a position of trust or authority of a child commits penetrative sexual assault on the child in an institution or home of the child or anywhere else; or
- q) whoever commits penetrative sexual assault on a child knowing the child is pregnant; or
- r) whoever commits penetrative sexual assault on a child and attempts to murder the child; or
- s) whoever commits penetrative sexual assault on a child in the course of communal violence; or
- t) whoever commits penetrative sexual assault on a child and who has been previously convicted of having committed any offence under this Act or any sexual offence punishable under any other law for the time being in force; or
- u) whoever commits penetrative sexual assault on a child and makes the child to strip or parade naked in public,

is said to commit aggravated penetrative sexual assault.

6. PUNISHMENT FOR AGGRAVATED PENETRATED SEXUAL ASSAULT (S.6)

Whoever, commits aggravated penetrative sexual assault, shall be punished with rigorous imprisonment for a term which shall not be less than ten years but which may extend to imprisonment for life and shall also be liable to fine.

7. SEXUAL ASSAULT

Whoever, with sexual intent touches the vagina, penis, anus or breast of the child or makes the child touch the vagina, penis, anus or breast of such person or any other person, or does any other act with sexual intent which involves physical contact without penetration is said to commit sexual assault.

8. PUNISHMENT FOR SEXUAL ASSAULT

Whoever, commits sexual assault, shall be punished with imprisonment of either description for a term which shall not be less than three years but which may extend to five years, and shall also be liable to fine.

9. AGGRAVATED SEXUAL ASSAULT (S.9)

10. PUNISHMENT FOR AGGRAVATED SEXUAL ASSAULT (S.10)

Whoever, commits aggravated sexual assault shall be punished with imprisonment of either description for a term which shall not be less than five years but which may extend to seven years, and shall also be liable to fine.

11. SEXUAL HARASSMENT (S.11)

A person is said to commit sexual harassment upon a child when such person with sexual intent, —

- i. utters any word or makes any sound, or makes any gesture or exhibits any object or part of body with the intention that such word or sound shall be heard, or such gesture or object or part of body shall be seen by the child; or
- ii. makes a child exhibit his body or any part of his body so as it is seen by such person or any other person; or
- iii. shows any object to a child in any form or media for pornographic purposes; or
- iv. repeatedly or constantly follows or watches or contacts a child either directly or through electronic, digital or any other means; or
- v. threatens to use, in any form of media, a real or fabricated depiction through electronic, film or digital or any other mode, of any part of the body of the child or the involvement of the child in a sexual act; or
- vi. entices a child for pornographic purposes or gives gratification therefor.

Explanation- Any question which involves “sexual intent” shall be a question of fact.

12. PUNISHMENT FOR SEXUAL HARASSMENT (S.12)

Whoever, commits sexual harassment upon a child shall be punished with imprisonment of either description for a term which may extend to three years and shall also be liable to fine.

13. USE OF CHILD FOR PORNOGRAPHIC PURPOSES

Whoever, uses a child in any form of media (including programme or advertisement telecast by television channels or internet or any other electronic form or printed form, whether or not such programme or advertisement is intended for personal use or for distribution), for the purposes of sexual gratification, which includes-

- a) representation of the sexual organs of a child;

- b) usage of a child engaged in real or simulated sexual acts (with or without penetration)
 - c) the indecent or obscene representation of a child,
- shall be guilty of the offence of using a child for pornographic purposes.

14. PUNISHMENT FOR USING CHILD FOR PORNOGRAPHIC PURPOSES (S.14)

- 1) Whoever, uses a child or children for pornographic purposes shall be punished with imprisonment of either description which may extend to five years and shall also be liable to fine and in the event of second or subsequent conviction with imprisonment of either description for a term which may extend to seven years and also be liable to fine.

If the person using the child for pornographic purposes commits an offence referred to in section 3, by directly participating in pornographic acts, he shall be punished with imprisonment of either description for a term which shall not be less than ten years but which may extend to imprisonment for life, and shall also be liable to fine.

- 2) If the person using the child for pornographic purposes commits an offence referred to in section 5, by directly participating in pornographic acts, he shall be punished with rigorous imprisonment for life and shall also be liable to fine.
- 3) If the person using the child for pornographic purposes commits an offence referred to in section 7, by directly participating in pornographic acts, he shall be punished with imprisonment of either description for a term which shall not be less than six years but which may extend to eight years, and shall also be liable to fine.
- 4) If the person using the child for pornographic purposes commits an offence referred to in section 9, by directly participating in pornographic acts, he shall be punished with imprisonment of either description for a term which shall not be less than eight years but which may extend to ten years, and shall also be liable to fine.

15. PUNISHMENT FOR STORAGE OF PORNOGRAPHIC MATERIAL INVOLVING CHILD (S.15)

Any person, who stores, for commercial purposes any pornographic material in any form involving a child shall be punished with imprisonment of either description which may extend to three years or with fine or with both.

16. ABETMENT OF AN OFFENCE (S.16)

A person abets an offence, who-

First.- Instigates any person to do that offence; or

Secondly.- Engages with one or more other person or persons in any conspiracy for the doing of that offence, if an act or illegal omission takes place in pursuance of that conspiracy, and in order to the doing of that offence; or

Thirdly.- Intentionally aids, by any act or illegal omission, the doing of that offence.

17. PUNISHMENT FOR ABETMENT (S. 17)

Whoever abets any offence under this Act, if the act abetted is committed in consequence of the abetment, shall be punished with punishment provided for that offence.

Explanation.- An act or offence is said to be committed in consequence of abetment, when it is committed in consequence of the instigation, or in pursuance of the conspiracy or with the aid, which constitutes the abetment.

18. PUNISHMENT FOR ATTEMPT TO COMMIT AN OFFENCE (S. 18)

Whoever attempts to commit any offence punishable under this Act or to cause such an offence to be committed, and in such attempt, does any act towards the commission of the offence, shall be punished with imprisonment of any description provided for the offence, for a term which may extend to one-half of the imprisonment for life or, as the case may be, one-half of the longest term of imprisonment provided for that offence or with fine or with both.

19. REPORTING OF OFFENCES (S.19)

1) Notwithstanding anything contained in the Code of Criminal Procedure, 1973, any person (including the child), who has apprehension that an offence under this Act is likely to be committed or has knowledge that such an offence has been committed, he shall provide such information to,

- (a) the Special Juvenile Police Unit; or
- (b) the local police.

2) Every report given under sub-section (1) shall be-

- a) ascribed an entry number and recorded in writing;
- b) be read over to the informant;
- c) shall be entered in a book to be kept by the Police Unit.

3) Where the report under sub-section (1) is given by a

child, the same shall be recorded under sub-section (2) in a simple language so that the child understands contents being recorded.

- 4) In case contents are being recorded in the language not understood by the child or wherever it is deemed necessary, a translator or an interpreter, having such qualifications, experience and on payment of such fees as may be prescribed, shall be provided to the child if he fails to understand the same.
- 5) Where the Special Juvenile Police Unit or local police is satisfied that the child against whom an offence has been committed is in need of care and protection, then, it shall, after recording the reasons in writing, make immediate arrangement to give him such care and protection (including admitting the child into shelter home or to the nearest hospital) within twenty-four hours of the report, as may be prescribed.
- 6) The Special Juvenile Police Unit or local police shall, without unnecessary delay but within a period of twenty-four hours, report the matter to the Child Welfare Committee and the Special Court or where no Special Court has been designated, to the Court of Session, including need of the child for care and protection and steps taken in this regard.
- 7) No person shall incur any liability, whether civil or criminal, for giving the information in good faith for the purpose of sub-section (7).

20. OBLIGATION OF MEDIA (S. 20)

Any personnel of the media or hotel or lodge or hospital or club or studio or photographic facilities, by whatever name called, irrespective of the number of persons employed therein, shall, on coming across any material or object which is sexually exploitative of the child (including pornographic, sexually-related or making obscene representation of a child or children) through the use of any medium, shall provide such information to the Special Juvenile Police Unit, or to the local police, as the case may be.

21. PUNISHMENTS FOR REPORTING IN VIOLATION (S. 21)

- 1) Any person, who fails to report the commission of an offence under subsection (1) of section 19 or section 20 or who fails to record such offence under sub-section (2) of section 19 shall be punished with imprisonment of either description which may extend to six months or with fine or with both.
- 2) Any person, being in-charge of any company or an institution (by whatever name called) who fails to report

the commission of an offence under sub-section (/) of section 19 in respect of a subordinate under his control, shall be punished with imprisonment for a term which may extend to one year and with fine. The provisions of sub-section (/) shall not apply to a child under this Act.

22. PUNISHMENT FOR FALSE COMPLAINT OR FALSE INFORMATION (S.22)

- 1) Any person, who makes false complaint or provides false information against any person, in respect of an offence committed under sections 3, 5, 7 and section 9, solely with the intention to humiliate, extort or threaten or defame him, shall be punished with imprisonment for a term which may extend to six months or with fine or with both.
- 2) Where a false complaint has been made or false information has been provided by a child, no punishment shall be imposed on such child.
- 3) Whoever, not being a child, makes a false complaint or provides false information against a child, knowing it to be false, thereby victimizing such child in any of the offences under this Act, shall be punished with imprisonment which may extend to one year or with fine or with both.

RECORDING OF STATEMENT OF A CHILD (S. 24)

The statement of the child shall be recorded at the residence of the child or at a place where he usually resides or at the place of his choice and as far as practicable by a woman police officer not below the rank of sub-inspector.

ADDITIONAL PROVISIONS REGARDING STATEMENT TO BE RECORDED

The Magistrate or the police officer, as the case may be, shall record the statement as spoken by the child in the presence of the parents of the child or any other person in whom the child has trust or confidence.

MEDICAL EXAMINATION OF A CHILD (S.27)

- 1) The medical examination of a child in respect of whom any offence has been committed under this Act, shall, notwithstanding that a First Information Report or complaint has not been registered for the offences under this Act, be conducted in accordance with section 164-A of the Code of Criminal Procedure, 1973.
- 2) In case the victim is a girl child, the medical examination shall be conducted by a woman doctor.
- 3) The medical examination shall be conducted in the presence of the parent of the child or any other person in whom the child reposes trust or confidence.
- 4) Where, in case the parent of the child or other person

referred to in sub-section (3) cannot be present, for any reason, during the medical examination of the child, the medical examination shall be conducted in the presence of a woman nominated by the head of the medical institution.

PROCEDURE IN CASE OF COMMISSION OF OFFENCE BY CHILD AND DETERMINATION OF AGE BY SPECIAL COURT

Where any offence under this Act is committed by a child, such child shall be dealt with under the provisions of the Juvenile Justice (Care and Protection of Children) Act, 2000.

PERIOD FOR RECODING OF EVIDENCE OF CHILD AND DISPOSAL OF CASE (S.35)

(1) The evidence of the child shall be recorded within a period of thirty days of the Special Court taking cognizance of the offence and reasons for delay, if any, shall be recorded by the Special Court.

(2) The Special Court shall complete the trial, as far as possible, within a period of one year from the date of taking cognizance of the offence.

CHILD NOT TO SEE ACCUSED AT THE TIME OF TESTIFYING. (S.36)

(1) The Special Court shall ensure that the child is not exposed in any way to the accused at the time of recording of the evidence, while at the same time ensuring that the accused is in a position to hear the statement of the child and communicate with his advocate.

(2) For the purposes of sub-section (7), the Special Court may record the statement of a child through video conferencing or by utilising single visibility mirrors or curtains or any other device.

TRIALS TO BE CONDUCTED IN CAMERA (S.37)

The Special Court shall try cases *in camera* and in the presence of the parents of the child or any other person in whom the child has trust or confidence:

THE POCSO RULES REGARDING COMPENSATION

- To be paid by Central or State Govt. on recommendation of Special Court or application of victim/parent/ guardian
- Interim compensation ordered by Special Court to meet immediate needs of relief/ rehabilitation at any stage after registration of FIR- to adjusted against final compensation
- Compensation may be recommended for award on conviction or discharge or acquittal of accused/accused not traced or identified, when

Special Court opines that child has suffered loss/ injury due to offence

FACTORS FOR CONSIDERATION FOR COMPENSATION

- type & gravity of abuse and harm/ injury suffered
- Expenditure likely/ incurred on medical treatment
- Loss of educational opportunity/ employment suffered
- Relationship of child to offender
- Single instance of abuse/ abuse over a period of time
- Whether child contracted STD/HIV/ AIDS due to offence
- Disability suffered due to offence
- Financial condition of victim/ victim's family (to determine rehabilitation
- Any other relevant factor

PUBLIC AWARENESS ABOUT ACT.

The Central Government and every State Government, shall take all measures to ensure that-

- a) the provisions of this Act are given wide publicity through media including the television, radio and the print media at regular intervals to make the general public, children as well as their parents and guardians aware of the provisions of this Act;
- b) the officers of the Central Government and the State Governments and other concerned persons (including the police officers) are imparted periodic training on the matters relating to the implementation of the provisions of the Act.

MONITORING OF IMPLEMENTATION OF ACT.

The National Commission for Protection of Child Rights constituted under section 3, or as the case may be, the State Commission for Protection of Child Rights constituted under section 17, of the Commissions for Protection of Child Rights Act, 2005, shall, in addition to the functions assigned to them under that Act, also monitor the implementation of the provisions of this Act in such manner as may be prescribed.

INSTRUCTIONS TO AUTHORS

•Unpublished, Ethical, Un-Plagiarised original manuscript written in English should be sent to: Dr. Parmod Kumar Goyal, Editor-in-Chief, Journal of Punjab Academy of Forensic Medicine and Toxicology by email at: drparmodgoyal@gmail.com

•Images (Good quality) should be sent separately in JPEG format.

•References should be in Vancouver Style only.

The Publication Particulars

The JPAFMAT is the official publication of the Punjab Academy of Forensic Medicine & Toxicology, published since 2001.

The Contents of the Journal

The journal accepts a range of articles of interest, under several feature sections as follows:

Original Papers: Includes conventional observational and experimental research.

Commentary: Intended for Reviews, Case Reports, Preliminary Report and Scientific Correspondences.

Letter to the Editor

Designed to be an avenue for dialogue between the authors of the papers published in the journal and the readers restricted to the options expressing reviews, criticisms etc. It could also publish letters on behalf of the current affairs in the field of Forensic Medicine in the country.

Editorial

Intended as a platform, for the Editor-in-Chief and for others with a keen interest in forensic medicine that wished to comment on the current affairs.

Special Features

In the History of Indian Forensic Medicine, Book Review, Abstracts, Announcement etc, which appear frequently, but not necessarily in every issue.

News and Notes

Intended for providing information of members and activities of the Academy and other such other organizations affiliated to the Academy may appear frequently and not in every issue.

General Principles

The text of observational and experimental articles is usually (but not necessarily) divided into the following sections: Introduction, Methods, Results, and Discussion. This so-called "IMRAD" structure is not an arbitrary publication format but rather a direct reflection of the process of scientific discovery. Long articles may need subheadings within some sections (especially Results and Discussion) to clarify their content. Other types of articles, such as case reports, reviews, and editorials, probably need to be formatted differently. Electronic formats have created opportunities for adding details or whole sections, layering information, cross linking or extracting portions of articles, and the like only in the electronic version. Double spacing all portions of the manuscript—including the title page, abstract, text, acknowledgments, references, individual tables, and legends—and generous margins make it possible for editors and reviewers to edit the text line by line and add comments and queries directly on the paper copy. If manuscripts are submitted electronically, the files should be double-spaced to facilitate printing for reviewing and editing. Authors should number all of the pages of the manuscript consecutively, beginning with the title page, to facilitate the editorial process.

International Uniform Requirements

Please visit <http://www.icmje.org/> for detailed instructions for manuscript submission.

Note : Manuscript handling charges Rs. 1500/- to be paid after acceptance for Indian Authors.

Format of Application for Membership

To

The General Secretary

Punjab Academy of Forensic Medicine & Toxicology (PAFMAT)

Dear Sir,

I wish to become a Life Member / Annual Member of PAFMAT. I am furnishing the required particulars below with a request to enrol me in the academy. The fee of Rs. / Rs. ---- for Life Membership / Annul Membership is enclosed as a Demand Draft with No _____ of _____ Bank, in the name of PAFMAT along with my two passport size photographs.

I have gone / will go through the rules and regulations of the academy and I agree to abide by the same.

PARTICULARS

1. Full Name (in block letters)
2. Father's / Husband's name
3. Date of Birth
4. Qualification (with name of university & date of passing)
5. Official Designation & Place of Posting
6. Permanent Address
7. Address for Correspondence (subsequent change of address to be intimated)
8. Phone No. & Email

Place

Yours Sincerely

Date

(Signature)

FOR USE OF PAFMAT

Membership Accepted with Life / Annual membership No. _____ / PAFMAT / _____ /

Dated

Treasurer

Secretary

President

Note : Payment can be made by NEFT in the account of academy (A/c No. 2082101026802, IFSC CNRB0002082, CANARA BANK, Gopal Nagar, Amritsar) after telephonic talk with President/Secretary/Treasurer. Scanned copy of the filled form can be sent by email to Secretary.

Life Members (PAFMAT)

No.	Name	No.	Name	No.	Name
1	Dr. R.K. Gorea	43	Dr. Mian Abdur Rashid	85	Dr Iram Khan
2	Late Dr. Sat Pal Garg	44	Dr. Shilekh Mittal	86	Dr Charanpreet K. Pawar
3	Dr. D.S. Bhullar	45	Dr. B.R. Sharma	87	Dr Mukul Chopra
4	Late Dr. A.S. Thind	46	Dr. D.Harish	88	Dr Mohit Gupta
5	Dr. Hakumat Rai	47	Dr. Krishna D. Chavali	89	Dr Rahul Chawla
6	Dr. K.K. Aggarwal	48	Dr. Ashwani Kumar	90	Dr Maneel Grover
7	Dr. Jaswinder Singh	49	Dr. Vikram Bains	91	Dr Y.S. Bansal
8	Dr. Karamjit Singh	50	Late Dr. Kirpal Singh	92	Dr C.S. Gautam
9	Dr. Harjinder Singh	51	Dr. Gurbachan Singh	93	Dr S.P. Mandal
10	Dr. Virender Pal Singh	52	Dr. Sangeet Dhillon	94	Dr Murli . G
11	Dr. Ashok Chanana	53	Dr. Sukhbir Singh Chauhan	95	Dr Anil Kumar Mittal
12	Dr. J.S. Dalal	54	Dr. Parminder Singh Bhatti	96	Dr G.A. Sunil Kumar Sharma
13	Dr. Jagdish Gargi	55	Dr. Rakesh Kumar	97	Dr Abhishek Yadav
14	Dr. R.S. Parsad	56	Dr. Jagbir Singh	98	Dr Jagdev Kullar
15	Late Dr. Ajit Singh	57	Dr. Karnveer Singh	99	Dr Gurpreet Kaur Randhawa
16	Dr. Harish Tuli	58	Dr. Rajiv K. Chowdhary	100	Dr Gursirat Singh Khokhar
17	Dr. S.K. Bal	59	Dr. Parmod Kumar Goyal	101	Dr Satinder Pal Singh
18	Dr. S.S. Sandhu	60	Dr. Ajay Kumar	102	Dr Saginder Samaraj
19	Dr. Akashdeep Aggarwal	61	Dr RK Sharma	103	Dr Neha Sharma
20	Dr. Kuldeep Singh	62	Dr Brij M. Gupta	104	Dr Sunil Mahajan
21	Dr. Vishal Garg	63	Dr Sunil Gambhi	105	Dr Harshdeep Kashyap
22	Dr. S.S. Oberoi	64	Dr Vijal Pal Khangwal	106	Dr Kiran Kumar
23	Late Dr. Ram Lubhaya	65	Dr Rajiv Joshi	107	Dr Swati Tyagi
24	Dr. Amandeep Singh	66	Dr Manpreet Kaul	108	Dr Mini
25	Dr. Harkirat Singh	67	Dr Sheikh AnayatUllah	109	Dr Mandeep Kaur
26	Dr. I.S. Bagga	68	Dr Satinder Pal Singh	110	Dr Gurinder Singh
27	Dr. Harpreet Singh	69	Dr Preetinder Singh Chahal	111	Dr Minal
28	Dr. Parminder Singh	70	Dr Kulbhushan Garg	112	Dr Kanchan Jyoti Heera
29	Dr. Anil Garg	71	Dr Imran Sabri	113	Dr Manpinder Kaur Bhullar
30	Dr. O.P. Aggarwal	72	Dr Bindu Aggarwal	114	Dr Arashdeep Singh
31	Dr. Gaurav Sharma	73	Dr Adish Goyal	115	Dr Chamandeep Singh Bains
32	Late Dr. Madhur Tayal	74	Dr Charak Sangwan	116	Dr Maninder Singh
33	Dr. Gurmanjit Rai Mann	75	Dr Pardeep Singh	117	Dr Akhilesh Agarwal
34	Dr. Didar Singh	76	Dr Ishwer Tayal	118	Dr Guneet
35	Dr. Kuldeep Singh	77	Dr Ripan Chanana	119	Dr Hitesh Bhatia
36	Dr. Pankaj Gupta	78	Dr Gurvinder Singh Kakkar	120	Dr. Deep Rattan Mittal
37	Dr. Karam Singh	79	Dr Ravdeep Singh	121	Dr. Arun Kumar Maria
38	Dr. Baljit Singh	80	Dr Rohit Kumar Singal	122	Dr. S Valliappan
39	Dr. Puneet Khurana	81	Dr Prabhdeep Singh	123	Dr. Preet Inder Singh
40	Dr. Puneet Arora	82	Dr Jasbir Singh	124	Dr. Harvinder Singh Chhabra
41	Dr. Prabhsharan Singh	83	Dr Jatinder Pal Singh	125	Dr. Bhoj Kumar Sahu
42	Dr. Dildar Singh	84	Dr Alok Kandpal	126	Dr. Amarnath Mishra

Life Members (PAFMAT)

No.	Name	No.	Name
127	Dr. Shekhar Chumber	165	Dr. Vivekanshu Verma
128	Dr. Navroz Goyal	166	Dr. B.V. Naga Mohan Rao
129	Dr. Gurjeet Singh	167	Dr. Anju Gupta
130	Dr. Deepika Kanwar	168	Dr. Guriqbal Singh
131	Dr. Mohd. Amjad Bhatt	169	Dr. Prashanthi Krishna Dharma
132	Dr. Nikhil Mehta	170	Dr. Amarjit Singh
133	Dr. Charan Kamal	171	Dr. Keshav Soni
134	Dr. Amit Singla	172	Dr. Faisal Nasim Gilani
135	Dr. Sukhdeep Singh	173	Dr. Ashok Sagar
136	Dr. Munish Kumar	174	Dr. Suresh Chand
137	Dr. Arindam Chatterjee	175	Dr. Jaswinder Singh
138	Dr. Ranjodh Jeet Singh	176	Dr. Prateek Rastogi
139	Dr. Kanika Kohli	177	Dr. Varun Garg
140	Dr. Vinka Maini	178	Dr. Hitesh Chawla
141	Dr. Ravi Tejpal	179	Dr. Sanjeev Buri
142	Dr. Preet Mohinder Singh	180	Dr. Sunil M Doshi
143	Dr. Satbir Singh	181	Dr. Sanjay Kumar
144	Dr. Kamaljit Singh	182	Dr. Akhilesh Pathak
145	Dr. Mrinal Kanti Jha	183	Dr. Raghvendra Kumar Vidua
146	Dr. Vijay Arora	184	Dr. Jitendra Kumar Gupta
147	Dr. Vivek Srivastava	185	Dr. Manoj Kumar Pathak
148	Dr. Pankaj Chhikara	186	Dr. Surendra Kumar Pandey
149	Dr. Lalit Kumar	187	Dr. Mayank Gupta
150	Dr. Prem Chandra Srivastava	188	Dr. Amandeep Kaur
151	Dr. Niraj Kumar		
152	Dr. Om Parkash Saini		
153	Dr. Rajesh Kumar Verma		
154	Dr. Shailender Kumar		
155	Dr. B.L. Chaudhary		
156	Dr. Parmod Kumar Saini		
157	Dr. Rajendra Singh Kulhari		
158	Dr. Nidhi Sachdeva Agarwal		
159	Dr. Rajeev Varma		
160	Dr. Pragnesh Bhartkumar Parmar		
161	Dr. Yatiraj Singi		
162	Dr. Navpreet Kaur		
163	Dr. Kunal Khanna		
164	Dr. Smitha Rani Shetty		

Punjab Academy of Forensic Medicine & Toxicology

Undertaking format for organizing PAFMATCON/Any Other CME/Workshop under the banner of PAFMAT

To

The President / General Secretary

Punjab Academy of Forensic Medicine & Toxicology

Subject : Consent for Holding the Conference.

Dear Sir

As discussed and decided in the general / executive body meeting of the academy dated at(name the venue), I give my consent to hold the annual conference of Punjab Academy of Forensic Medicine & Toxicology on(Tentative date) in (Name of the medical college / venue)

Subject to the following:-

- a. The conference and / or the CME programme shall be under the auspices of Punjab Academy of Forensic Medicine & Toxicology. The banner showing the same will be displayed at a suitable area on the main venue.
- b. The President and the General Secretary of the Academy will be suitably seated on the dais during the inaugural programme. The President will address the gathering about the policies, programs or other relevant aspects of the Academy. The General Secretary will read out the annual report.
- c. The registration of the President, General Secretary, Secretary Finance and the Editor-in-Chief of the Academy will be complimentary.
- d. The conference will get accredited with at least 4 CME Credit hours from Punjab Medical Council.
- e. The President and / or General Secretary of the Academy along with one member of Punjab Medical Council will be the signatory to the certificate issued to delegate attending the conference / CME / workshop.
- f. The organizing committee will send formal invitation to all the office bearers of the academy.
- g. The Journal of the Academy will be released during the inaugural programme. The Editor-in-Chief and the Joint Editor will be invited to the dais for the release ceremony.
- h. The Organizing Secretary of the programme will hand over the list of the delegates to the General Secretary of the Academy at the end of the conference.
- i. The Organizing Committee will collect Rs. 100/- (Rupees one hundred only) per delegate of the programme and will deposit the collected amount in the account of the Journal of PAFMAT / hand over the Cheque for the collected amount favoring Journal of Pb. Aca. Of Forensic Med. & Toxicology to the Editor-in-Chief after the conference.

Sd/-

Organizing Chairman / Secretary

Name:

Format of Application for Subscription of Journal

To

The Editor-in-Chief

Journal of Punjab Academy of Forensic Medicine & Toxicology (JPAFMAT)

Dear Sir,

I wish to Subscribe the Journal of Punjab Academy of Forensic Medicine & Toxicology. I am furnishing the required particulars below with a request to subscribe the Journal. The fee of Rs. 1000/4000 for Journal of Punjab Academy of Forensic Medicine is enclosed.

PARTICULARS

1. Full Name (in block letters)
2. Father's / Husband's name
3. Date of Birth
4. Qualification (with name of university & date of passing)
5. Official Designation & Place of Posting
6. Permanent Address
7. Address for Correspondence (subsequent change of address to be intimated)
8. Phone No. & Email
9. Photo
10. Copy of Medical Council Registration Certificate

Place

Yours Sincerely

Date

(Signature)

- Note:**
1. Payment can be made through online transfer in the Journal Account. For any query related to subscription of the Journal, feel free to talk/whatsapp on mobile no. 98760-05211
 2. The above information can be sent by email to editor-in-chief
 3. Subscribers of the Journal are entitled for Five (5) CME credit hours per year as per CME Accreditation Guidelines issued by Punjab Medical Council.

Glimpse of PAFMATCON-2018



Editorial Board



Editor-in-Chief

Dr Parmod Kumar Goyal



Joint Editor

Dr Amandeep Singh



Assistant Editor

Dr Satinder Pal Singh



Web Master

Dr Dildar Singh

National Advisory Board

Dr T.D. Dogra
Dr J.S. Dalal
Dr R.K. Gorea
Dr O P Aggarwal
Dr K K Aggarwal
Dr Balbir Kaur
Dr K. Vij
Dr Dalbir Singh
Dr Sanjoy Dass
Dr E.J. Rodrigueus
Dr Gurudatta Pawar
Dr D.S. Badkur
Dr Jagadeesh. N.
Dr Pramod Kumar

Editorial Committee:

Dr S. S. Oberoi
Dr Sangeet Dhillon
Dr Ishwar Tayal
Dr Kuldeep Singh
Dr Harjinder Singh
Dr Harpreet Singh
Dr Parul Kher
Dr Antara DebBarman
Dr Gurpreet S. Sandhu
Dr S K Dhatarwal
Dr Jaskaran Singh
Dr Manoj Pathak
Dr Jaswinder Singh
Dr PC Srivastava
Dr Om Parkash Saini

Manuscript Review Committee

Dr OP Murti
Dr V. V. Pillay
Dr Shiv Kochhar
Dr R.K. Sharma
Dr N K Aggarwal
Dr Amitabh Shrivastva
Dr Shankar M. Bakkannavar
Dr Mukesh Yadav
Dr C.B. Jani
Dr. Vijal Pal Khanagwal
Dr. Samita Sinha
Dr. Manjot Kaur
Dr. Karan Maheshwari
Mrs. Ritika Gupta, FSL Bathinda
Dr. Nidhi Sachdeva
Dr. Vivek Srivastava
Dr. Pardeep Singh
Dr. Monika Gupta
Dr. Navita Aggarwal
Dr. Tanvir Kaur Sidhu
Dr. Sandeep Kaur
Dr. Priti Chaudhary
Dr. Vijay Suri
Dr. Saranpal Singh
Dr. Lovleen Marwaha
Dr. Tanuj Kanchan
Dr. Raghuvendra K. Vidua
Dr. Vivek Kumar (Siliguri)
Dr. Pragnesh Kumar
Dr. Imran Sabri

Subscription Information

Members of PAFMAT will receive the journal free of cost.

Subscription rates for Non-Members and Institutions for Year 2019

Personal: Rs. 1,000/- Institutions: Rs. 4,000/-
Abroad : Postal Charges Extra

- Subscription request be sent on email to drparmodgoyal@gmail.com
- Payments be made in Bank Account of Journal of Punjab Academy of Forensic Medicine (A/c No. 65075504236, State Bank of India, Branch Rajindra Hospital Patiala, IFS Code SBIN0050263)

Printed & Published by:

Dr Parmod Kumar Goyal
Professor & Head,
Department of Forensic Medicine & Toxicology
Adesh Institute of Medical Sciences & Research,
Bathinda (Pb.) India M. 9876005211, 0164-5055073
E-mail: drparmodgoyal@gmail.com

Claims for Missing Issue

A copy will be sent to the member / subscriber provided the claim is made within 2 months of the publication of the issue and Rs. 50/- deposited in the Journal account as postal Charges.

Printed at:

Subhash Mittal Printing Press
Hospital Bazar, Bathinda
M. 99880-11022
e-mail: mittalpress@gmail.com